“STUDIES ON COLLAR ROT *(SCLEROTIUM ROLFSII* SACC.) OF LENTIL AND IT’S MANAGEMENT.”

Ph. D. Thesis

by

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
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"STUDIES ON COLLAR ROT *(SCLEROTIUM ROLFSII* SACC.) OF LENTIL AND IT'S MANAGEMENT."

Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)

by

Ashok Kumar Koshariya

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

Agriculture
(Plant Pathology)

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July, 2020

CERTIFICATE -I

This is to certify that the thesis entitled **"Studies on collar rot *(Sclerotium rolfsii* sacc.) of lentil and it's management."** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Ashok Kumar Koshariya** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or certificate course. All the assistance and help received during the course of the investigation have been duly acknowledged.

Date: 04/08/2020

Chairman
Advisory Committee

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

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Member	(Dr.N. Lakpale)	----------
Member	(Dr. Deepak Chandrakar)	----------
Member	(Dr. R.R. Saxena)	----------
Member	(Head of the Department)	----------

CERTIFICATE -II

This is to certify that the thesis entitled **"Studies on collar rot *(Sclerotium rolfsii* sacc.) of lentil and it's management."** submitted by **Ashok Kumar Koshariya** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** in the **Department of Plant Pathology** has been approved by the external examiner and Student's Advisory Committee after oral examination in collaboration with the external examiner.

Signature External Examiner

(Name S.K. SRIVASTAVA)

Date: 17/03/2021

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Faculty Dean _____

Approved / Not approved

Director of Instructions _____

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I start in the name of God and Universe -who has bestowed upon me all the physical and mental attributes that I posses and skill to cut through and heal a fellow human.

Destiny is not a matter of chance; it is a matter of choice. It is not a thing to be waited for; it is a thing to be achieved. Any specific endeavour is not a result of individual's effort. It would certainly be wrong on my part if I claim I have carried entire research on my own without any body's help. The gratitude has been a courtesy of rich culture towards all who shares burden of my work and help to succeed in my endeavour. Hence, it is a matter of pleasure and a sincere duty to acknowledge all the persons and spirits in the form of teachers, friends, near or dear ones who directly or indirectly gave their supporting hands while doing the research or preparation of this present manuscript. It is the most difficult task to accomplish and to express all the feelings and sense of gratitude in words as
"Gratitude being the memory of mind and feeling of heart"

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I express my sincere gratitude to Indira Gandhi Krishi Vishwavidyalaya, Raipur for all the facility and it makes me immensely proud and honored to be a student of this legendary and prestigious university. Finally, I offer my thanks to any other source of contribution that might have inadvertently have been left out.

I frankly admit that it is not possible to remember all the faces that stood behind the facade at this juncture and omission of any name does not mean lack of gratitude. Finally I wish to thank all the teaching and nonteaching staff of department of plant pathology, College of Agriculture, Raipur for their encouragement and help during my study.

The completion of this thesis could not have been possible without the blessing and encouragement from my respected ***"Parents" Shri Dali Chand Koshariya, Smt. Nirmala Devi,*** *and my dear nephew* ***Chahat and Shivam*** *for which I have no word to express.*

Date: 04/08/2020
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LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
BOD	Biological oxygen demand
B.C.	Before christ
cm	Centimeter
mm	Milimeter
et al	Co worker
CD	Critical difference
CFU	Colony forming unit
C.G.	Chhattisgarh
cv	Cultivar
DAI	Days after inoculation
DAS	Days after sowing
^{0}C	Degree Celsius
Fig	Figure
G	Gram
Ha	Hectare
Kg	Kilogram
L	Liter
Mt	Metric tonne
Ml	Milliliter
m ha	Million hectare
viz.	Namely
No.	Number
ppm	Part per million
%	Per cent
/	Per, also means and or
S.No.	Serial number
SEm±	Standard error of means
i.e.	That is

THESIS ABSTRACT

a)	Title of the Thesis	:	**"Studies on collar rot *(Sclerotium rolfsii* Sacc.) of lentil and it's management."**
b)	Full Name of the Student	:	Ashok Kumar Koshariya
c)	Major Subject	:	Plant Pathology
d)	Name and Address of the Major Advisor	:	Dr. N. Khare, Principal Scientist Department of Plant Pathology, College of Agriculture, IGKV, Raipur, Chhattisgarh
e)	Degree to be Awarded	:	Doctor of Philosophy in Agriculture (Plant Pathology)

Signature of the Student

Signature of the Major Advisor

Date: 04/08/2020

Signature of Head of the Department

ABSTRACT

Present investigation on **"Studies on Collar Rot *(Sclerotium rolfsii* Sacc.) of Lentil and it's Management."** was carried out in the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Collar rot of lentil (*Sclerotium rolfsii* Sacc.) is caused by the ubiquitous soil-born pathogen *Sclerotium rolfsii* and reported almost all over the world wherever lentil is grown. The survey of fourteen locations in seven lentil growing district of Chhattisgarh state(Raipur, Mungeli, Bemetara, Bilaspur, Kawardha, Baloda bazaar and Dhamtari) was made for the occurrence and severity of lentil collar rot. The maximum mean disease incidence was observed in Mungeli districtand lowest in Dhamtri district. Forteen isolates of *S. rolfsii* were Isolated from disease samples collected from different locations. The isolates differed markedly in terms of mycelial growth rate, hyphal width, distance between septa , mycelia growth pattern, Sclerotial number, Sclerotial weight, size, shape, pigmentation and arrangement of sclerotia in culture medium . Pathogenicity of these isolates was proved in artificially inoculated condition. *In vivo*(sick soil method) for aggressiveness test, isolate SR4 obtained from Temari, Mungeli, found to be most virulent. Therefore, isolate SR4 was used as inoculums (mass multiplied on wheat grain

medium) to make sick soil for further screening studies. Soil factor (soil type, soil texture and soil pH) strongly influenced the collar rot disease development in lentil. In case of soil type least mortality per cent (19.17 %) was observedin Matasi. However, highest mortality was observed in Kanhar 57.41 %.The infected soil of different texture, showed least mortality per cent (23.14%) in sandy loam texture followed by sandy texture with mortality per cent of 26.82 % and sandy loam texture (30.83 %). However, highest mortality was observed in clay texture soil 62.96 %, followed by Clay loam 46.59 %. Significantly least mortality per cent (20.97%) was observed at pH 8.5 followed by pH 8 (24.02 %) and pH 7.5 (39.81 %), However, highest mortality was observed at pH 6.0 it was 81.25%.

Effect of different date of sowing on Collar rot disease development showed that incidence of wilt was recorded higher in early sown crop that is 20th Oct. in both the year of testing. Maximum mean disease incidence 62.90 per cent was recorded in 20th Oct sown crop followed by 30th Oct sown crop (54.67%). The minimum collardisease incidence was recorded 23.25 per cent in the late sown crop 30November in both years.Effect of Tillage practices for disease control was showed that incidence of collar rotwas recorded higher in zero tillage in both the year of testing. Maximum mean disease incidence 37.12 per cent was recorded in zero tillage lentil crop followed by Conventional tillage (31.60%). The minimum collar rot disease incidence was recorded 16.73 per cent in the minimum tillage in both years. Effect of seed priming for disease control showed that incidence of collar rot was recorded higher in no seed priming in both the year of testing. Maximum mean disease incidence 14.40 per cent was recorded in lentil crop. The minimum collar rot disease incidence was recorded 12.68 per cent in the seed priming in both years.

Biocontrol agents, *Trichoderma* spp. (40 isolate), fluorescent *Pseudomonas* (30 isolates) and *Bacillus* spp . (22 isolates) were isolated from collar rot infected lentil area in Chhattisgarh and screened against *S. rolfsii* under *in vitro* dual culture and *in vivo* pot experiment. Among 40 isolates of *Trichoderma* spp., Isolate T32, T7 and T3 showed maximum mycelial growth inhibition of *S. rolfsii* by 91.69, 91.16 per cent and 90.88% respectively over control, while least inhibition was observed in isolate T16 (50.26 %). *In vivo* pot experiment, *Trichoderma* spp., Isolate T23 and T40 were found significantly. Among Thirty fluorescent *Pseudomonas* isolates were evaluated for their efficacy against *S. rolfsii.* Isolate P26 was found significantly efficient in reducing the mycelial growth of *S. rolfsii* by 79.01 % over control, followed by P7 (78.42 %), P13 (77.78 %), P22 (73.09 %), P3 (73.80 %), P16 (72.81 %), P29 (68.61%), P21 (67.98%), P12 (64.27%), P5 (61.90 %) which were statistically at par with each other. Minimum inhibition was recorded in P20 (15.53 %) followed by P28 (17.07 %), P2 (32.09 %) and

P18 (34.99%) which were at par with each other. In relation to other factor, fluorescent *Pseudomonas* isolate also inhibit sclerotia formation in *S. rolfsii*. All the treatments were significantly superior in decreasing the incidence of collar rot and enhancing the growth of lentil, over control under *in vivo* pot experiment. Isolate Isolate P13 exhibited significant minimum mortality 15.24 per cent followed by P26 (15.57 per cent) and P22 (15.73 per cent) which were statistically at par with each other. Maximum mortality was observed in P8 (60.79 per cent). *Bacillus* spp. inhibited the mycelial growth by the antagonistic activity across *S. rofsii* under *in vitro* dual culture . Isolate B21 was found significantly efficient in reducing the mycelial growth of *S .rofsii* by 72.33 per cent over control followed by B19 (70.39 per cent1). Minimum inhibition was recorded in B14 (18.73 per cent).*Invivo* pot experiment, Isolate B21exhibited significant minimum mortality 17.35 per centfollowed by B19 (mortality 22.64 per cent) and B13 (mortality 26.63 per cent). Maximum mortality was observed in B9 (69.54 per cent) as compared to control (81.95 per cent). Talc based formulations were prepared from most effective isolate of biocontrol agent *Trichoderma* spp. (*Tricho*-32), fluorescent *Pseudomonas* (*Pf* -26) and *Bacillus* spp . (*Bs* -21) and used for seed treatment and soil application against collar rot disease in lentil. Seed treatment was done with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) of biocontrol agent to find out effective dose for control of lentil collar rot and it is concluded that increase in dose more than the recommended can efficiently controldisease and also increase plant growth. Seed treatment with biocontrol agent alone waseffective in controlling collar rot of lentil as compared to control but combined seed treatment and soil application most significantly increased the plant vigour index and reduced the incidence of collar rot under greenhouse conditions .

Among the botanicals evaluated, *Agave* recorded maximum mycelial inhibition of 100 per cent at all the concentrations tested, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively with a mean of 65.25 %.

The efficacy of fungicides (7 systemic, 4 non-systemic and 6 combo) was tested at different concentrations of 20, 50, 100, 200 and 500 ppm against *S. rolfsii* on PDA by poisoned food technique. It was concluded that systemic fungicides like, Hexaconazole 5% EC, Propiconazole 25% EC and combo products Tubaconzole 50% + Trifloxystrobin 25% WG, Captan 70% + Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% showed complete inhibition of the pathogen at all the concentrations tested. Whereas, the non-systemic fungicide Mancozeb 75%WP, Thiram 75% WS and Propineb 70% WP was found inhibitive only at higher concentrations (100 ppm) against *S. rolfsii* under *in vitro* condition. Similar results was found in case of seed treatment with fungicide under pot experiment. Propineb 70% WP was found to be highly effective

fungicide against *S. rolfsii* under *in vitro* dual culture and *in vivo* pot experiment and also showed compatibility with biocontrol agents (*Trichoderma* spp. (*Tricho* -32), fluorescent *Pseudomonas* (*Pf* -26) and *Bacillus* spp. (*Bs* -21)) Therefore, Propineb 70% WP fungicide can be used in combined application with biocontrol agents for integrated management of lentil collar rot. The combination of seed dressing formulation *Trichoderma* spp. (*Tricho* -32), fluorescent *Pseudomonas* (*Pf* -26) and *Bacillus* spp. (*Bs* -21) and Propineb 70%WP provided maximum protection to emerging seedling. The seed treated with *Pf* - 26 + *Bs*-21 + Propineb 70%WP provided the highest germination, effectively help to increased plant growth and minimum per cent disease incidence of collar rot of lentil in pot experiment .

Most of the strain of *Trichoderma* along with combination of fungicide showed positive response for plant growth promoting activity. Seeds treated with *Trichoderma* isolates, showed significant increase in plant hight (53.62cm in T5 to 58.28cm in T7;53.57cm in control treatment). Among all the treatment of *Trichoderma* along with combination of fungicide the treatment of combination of *Trichoderma harzianum* + Propineb and *Trichoderma* mutant formulation significantly reduced lentil plant mortality (and was superior to other *Trichoderma* based formulations. Combination of *Trichoderma harzianum* + Propineb and *Trichoderma* mutant was evaluated and compared with a few other *Trichoderma* strains, fungicides, and combination treatment, at field conditionover 2 years. Combination of *Trichoderma harzianum* + Propineb and *Trichoderma* mutant treatment of seeds significantly improved seedling emergence, reduced disease incidence, and improved yield" over both years

Total 271 lentil entries were screened against collar rot disease, out of which139 entries were screened during 2018-2019 while, 132 entries/ germplasm were screened in 2019-2020. During first*rabi*season 2019-2020, all lentil entries/ germplasm were susceptible to highly susceptible to collar rot. While, during second *rabi* season 2018-19, Only3 germplasm, DPL-62, VL-1 and VL-4, were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirseyfokar and Dehati Masoor were identified as resistant. Rest of entries/ germplasm lines were found susceptible tohighly susceptible to thedisease.

शोधग्रंथ सारांश

अ) शोधग्रंथ का शीर्षक : मसूर की पद गलन (*स्क्लोरासियम रोल्फसाई*) पर अध्ययन और इसका प्रबंधन।

ब) छात्र का नाम : अशोक कुमार कोशरिया

स) प्रमुख विषय : पादप रोग विज्ञान

द) प्रमुख सलाहकार का नाम और पता : डॉ. एन. खरे,

प्रमुख वैज्ञानिक पादप रोग विज्ञान, इंदिरा गांधी कृषि विश्वविद्यायल, रायपुर, 492012 छत्तीसगढ़

प्रदान की जाने वाली उपाधि : पी.एच.डी. पादप रोग विज्ञान

छात्र का हस्ताक्षर

मुख्य सलाहकार के हस्ताक्षर

दिनांक : 4/8/2020

विभाग अध्यक्ष का हस्ताक्षर

सारांश

वर्तमान शोद्य कार्य पादप रोग विभाग कृषि महाविद्यालय इंदिरा गांधी कृषि विश्वविद्यायल, रायपुर में मसूर की पद गलन (*स्क्लोरासियम रोल्फसाई*) पर अध्ययन और इसका प्रबंधन पर किया गया । मसूर की पद गलन (*स्क्लोरासियम रोल्फसाई सेक.*) सर्वव्यापी मिट्टी जनित रो जनक के कारण पैदा होती है, जो पैथोजेन स्क्लेरोशियम रोल्फसाई में पैदा होती है जो पूरी दुनिया में जहाँ भी मसुर उगाइ जाती है। छत्तीसगढ़ राज्य के सात मसूर उगाने वाले जिले (रायपुर, मुंगेली, बेमेतरा, बिलासपुर, कवर्धा, बलौदा बाजार और धमतरी) से चौदह स्थानों का सर्वेक्षण मसूर की पद गलन की उपस्थिति और उग्रता के लिए सर्वेक्षण किया गया था। सबसे अधिक औसत बीमारी की उपस्थिति मुंगेली जिले में और सबसे कम धमतरी जिले में देखा गई। विभिन्नि स्थानो में से एकत्र किए गए रोग के नमूनों से *स्क्लेरोसियम रोल्फसाई* के चौदह नमुनो को पृथक किया गया। ये पृथक्कृत कवकजाल वृद्वि दर, कवकतन्तु चौडाई पटों के बीच दूरी, कवकजाल वृद्वि दर, स्क्लेरोशियम संख्या, स्क्लेरोशिया भार, नाप, आकार, रंजकता और संवर्धन माध्यम में स्क्लेरोशिया का विन्यास माप दण्डों पर उल्लेखिनीय ढंग से भिन्न थे । इन पृथक्कृतो का रोगजनक क्षमता कृत्रिम रूप से नेवाशित दशा में साबित किया गया *इन विवो* (बीमार मिट्टी विधि) उग्रता परिक्षण में तेमरी, (मुंगेली) से प्राप्त पृथक्कृत *एसआर* 4 सबसे अधिक उग्र पाया गया। इसलिए, आगे मूल्याकन अध्ययन के लिए पृथक्कृत *एसआर* 4 का उपयोग निवेशद्रव्य के रूप को बीमार मिट्टी को बिमार बनाने के लिये किया गया। मृदा का प्रकार, मृदा कवाकार एवं मृदा pH प्रभावशाली ढ़ंग से मसूर में पद गलन रोग के विकास को प्रभावति किया। मृदा प्रकार के सदर्भ में सबसे कम मृत्यु संख्या मटासि मृदा (19.17 प्रतिशत)में देखा गया जबकि सबसे अधिक (57.41 प्रतिशत) कान्हार मृदा में देखा गया। विभिन्न कणाकार वाले संकमित मृदा के संदर्भ में सबसे कम मृत्यु संख्या (23.14 प्रतिशत) रेतीली दोमट कणाकार प्रदर्शित किया, तत्पाशचात रेतीली (26.82 प्रतिशत) ने किया। हांलाकि अधिकतम मृत्यु संख्या (62.96 प्रतिशत) चिकनी कणाकार वाली मृदा में देखा गया तत्पश्चात चिकनी दोमट (46.59 प्रतिशत) में देखा गया। सार्थकरूप से न्युनतम मृत्यु संख्या (20.57 प्रतिशत) pH 8.5 पर देखा गया तत्पश्चात pH 8 (24.02) प्रतिशत) pH 6 में देखा गया

कॉलर रॉट का रोग विकास पर, बुवाई की अलग–अलग तारीखों का प्रभाव यह दर्शाता है कि शुरुआती बोई गई फसल में पद गलन की घटना अधिक दर्ज की गई जो कि परीक्षण के दोनों वर्षों में 20 अक्टूबर है। अधिकतम औसत रोग की घटना 20 अक्टूबर मे बोई गई फसल में 62.90 प्रतिशत दर्ज की गई, इसके बाद 30 अक्टूबर को बोई गई फसल (54.67 प्रतिशत) थी। दोनों वर्षों में देर से बोई गई फसल (30 अक्टूबर में) न्यूनतम कॉलर रोग की घटना 23.25 प्रतिशत दर्ज की गई। रोग नियंत्रण के लिए जुताई किया का प्रभाव यह दर्शाता है कि परीक्षण के दोनों वर्ष में कॉलर रोट की घटना शून्य जुताई में अधिक दर्ज की गई थी। अधिकतम औसत बीमारी 37.12 प्रतिशत दर्ज की गई जिसके बाद परम्परागत जुताई में (31 80) देखी गई । न्यूनतम पद गलन रोग की घटना न्यूनतम जुताई में 16.73 प्रतिशत दर्ज की गई जो कि दोनों वर्षों में देखी गई। रोग नियंत्रण के लिए सीड प्रिमिग का प्रभाव यह दर्शाता है कि परीक्षण के दोनों वर्ष में बिना किसी प्राइमिंग के पद गलने की घटना अधिक दर्ज की गई। मसूर की फसल में अधिकतम औसत रोग की उग्रता में 14.40 प्रतिशत की वृद्धि दर्ज की गई। दोनों वर्षों में बीज प्राइमिंग से न्यूनतम पद गलन बीमारी 12.68 प्रतिशत दर्ज की गई।

जैव नियंत्रण कारको, *ट्राइकोडर्मा* स्पी. (40 पृथक्कृत), *फ्लूरोसेंट स्युडोमोनास* (30 पृथक्कृत), तथा *बेसिलस* स्पी. (22 पृथक्कृत), को छ.ग. मे पद गलन से संकमित मसूर क्षेत्रो से पृथक्क किया गया तथा इनका *इसक्लोरोशियम रोल्फसाई* के विरूद्ध *इन विट्रो* द्विसंवर्धन विधि एवं *इन* विवो पात्र परीक्षण विधि द्वारा मुल्यांकन किया गया। *ट्राइकोडर्मा* स्पी. के 40 पृथक्कृतों में से पृथक्कृत T32, T7 तथा T3 *इसक्लोरोशियम रोल्फसाई* के कवकजाल वृद्धि को नियंत्रित की तुलना में अधिक कमशः 91.69 प्रतिशत, 91.16 प्रतिशत एवं 90.88 अवरोधन किया जबकि सबसे कम अवरोधन पृथक्कृत T16 (50.26 प्रतिशत) के द्वारा किया गया। *इन विवो* पात्र परीक्षण के सर्दभ में सबसे न्युतम पद गलन मृत्यु दर कमशः T23 (28.96 प्रतिशत), T40 (29.16 प्रतिशत) तथा T17 (29.16 प्रतिशत) में देखा गया। *फ्लूरसेंट स्यूडोमोनास* के 30 पृथक्कृतो में से P26 *इसोक्लोरेशियम रोल्फसाई* के कवकजाल के वृद्धि को नियंत्रित की तुलना में 79.01 प्रतिशत सार्थक रूप से प्रभावशाली ढ़ंग से कम किया इसके पश्चात P7 (78.42 प्रतिशत), तथा P13 (77.78 प्रतिशत) पाया गया। जबकि न्युनम अवरोधन पृथक्कृत P28 (17.07 प्रतिशत) द्वारा देखा गया। अन्य कारको से संबंलधित *फ्लूरसेंट स्यूडोमोनास* पृथक्कृत *इसोक्लोरेशियम रोल्फसाई* के इसक्लेरोशिया बनने की प्रकिया को भी अवरोध करता है। *इन विवो* पात्र परीक्षण स्थिति में सभी उपचार पद गलन आपतन को कम करने तथा मसूर के वृद्धि को बढाने में नियंत्रण के तुलना में सार्थक रूप से बेहतर थे। पृथक्कृत P15, P26 तथा P22 न्युनतम मृत्यु दर कमशः 15.24 प्रतिशत, 15.57 प्रतिशत तथा 15.73 प्रतिशत किये एवं अधिकतम मृत्यु दर P8 (60.79 प्रतिशत) मे देखा गया। इन विट्रो द्वि संवर्धन में *बेसिलस* स्पी. *इसक्लेरोशिया रोल्फसाई* में कवकजाल वृद्धि को प्रतिरोधी किया द्वारा अवरोधीत किया। अधिकतम अवरोधन, पृथक्कृत B21 (72.33 प्रतिशत) तथा B9 (70.39 प्रतिशत) में अभीलेखित किया गया। जबकि न्युनतम अवरोधन B14 (18.73 प्रतिशत) द्वारा किया गया। इन विवो पात्र परीक्षण में पृथक्कृत B21 (17.35 प्रतिशत), B19 (22.64 प्रतिशत)तथा B22 (27.24 प्रतिशत) न्युनतम मृत्यु दर संख्या प्रदर्शित किये एवं पौधे कि ओज सूंचकांक को बढ़ा दिये। जबकि अधिकतम मृत्यु दर पृथक्कृकत B9 (81.95 प्रतिशत) में पाया गया। जैवनियंत्रक कारको के निजी पृथक्कृतों *ट्राइकोडर्मा* स्पी. (*Tricho*-32), *फ्लूरसेंट स्यूडोमोनास* (*Pf*-26) तथा *बेसिलस* स्पी. (*Bs*-21) से चूर्ण (टाल्क) अधारित नियंत्रण तैयार किये गये तथा मसूर में पद गलन के प्रति बीजोपचार एवं मृदा अनुप्रयोग हेतु उपयोग किये गये। चना के पद गलन रोग के नियंत्रण हेतु प्रभावकारी मात्रा पता लगाने के लिए जैवनियंत्रण कारको के विभिन्न मात्राओं (5, 10, 15, 20, 25 तथा 30 ग्राम प्रति किलो बीज) के साथ बीजोपचार किया गया तथा यह निष्कर्ष निकाला गया की जब जैविक कारको का अनुशासित से अधिक मात्र में उपयोग प्रभावशाली ढ़ंग से रोग नियंत्रण कर सकता है तथा पौध वृद्धि को बढ़ा सकता है। ग्रीन हाउस स्थिति में जैवनियंत्रण कारको का दोनो बीजोपचार तथा मृदा अनुप्रयोग रूप में उपयोग नियंत्रित तथा बीजोपचार के अकेले उपयोग की उग्रता में प्रभावशाली ढ़ंग से पद गलन आपतन को कम किया तथा पौध ओज में वृद्धि किया।

वानस्पतिकों का मूल्यांकन किया गया एगवे ने परीक्षण किए गए सभी सांद्रता में 100 प्रतिशत की अधिकतम मायसेलियल अवरोधन दर्ज की जिसके बाद हेना ने 34.4, 71.3 और 90 प्रतिशत के साथ क्रमशः 5, 10 और 15 प्रतिशत एकाग्रता के साथ क्रमशः 65.25 दर्ज किया।

विशैलां भोजन तकनीक द्वारा PDA माध्यम में फफूदनाशीयों (7 सर्वांगीण तथा 4 गैर सर्वांगीण) के क्षमता का विभिन्न सान्द्रता (20, 50, 100, 200 एवं 500) पी.पी.एम स्तर पर *इसक्लेरोशियम रोल्फसाई* के प्रति प्ररीक्षण किया गया। यह निष्कर्ष निकाला गया कि सर्वागीण फफूदनाशी जैसे हेक्जाकोनाजोल 5 प्रतिशत ई.सी., प्रोपीकोनाजोल 25 प्रतिशत ई.सी. तथा सयुक्त उत्पाद टेबुकोनाजोल 50 प्रतिशत + ट्राइफ्लॉक्सीस्ट्रोबीन 25 प्रतिशत डब्ल्यू.जी., केप्टान 70 प्रतिशत + हेक्जाकोनाजोल 5 प्रतिशत डब्ल्यू.पी., प्रोपीकोनाजोल 13 प्रतिशत + डाइफेनकोनाजोल तथा कार्बोडाजीम 37.5 प्रतिशत + थीरम 37.5 प्रतिशत सभी सांद्रता स्तर पर रोगजनक का सपूर्ण अवरोधन किये। वही संपर्क फफुंदनाशी मेन्कोजेब 75 प्रतिशत डब्ल्यू.पी., थीरम 7 प्रतिशत डब्ल्यू.एस. तथा प्रोपिनेब, 70 प्रतिशत डब्ल्यू.पी केवल अधिक सांद्रता (100 पी.पी.एम) स्तर पर ही *इन विट्रो* स्थिति में *इसक्लेरोशियम रोल्फसाई* के प्रति अवरोधी पाये गये। पात्र परीक्षण में फफूंदनाशीयों से बीजोपचार करने पर भी इसी प्रकार परिणाम मिले। *इन विट्रो* द्वि संवर्धन तथा *इन विवो* पात्र परीक्षण में प्रोपिनेब 70 प्रतिशत डब्ल्यू.पी, *इसक्लेरोशियम रोल्फसाई* के प्रति अत्यधिक प्रभावशाली पाया गया तथा जैविक नियंत्रण कारको *ट्राइकोडर्मा* (*Tricho*-32) *फ्लूसेन्ट स्यूडोमोनास* (*Pf*-26) एवं

बेसिलस स्पी (*Bs-21*) के साथ अनुकूलता प्रदर्शित किया। इसलिए प्रोपिनेब 70 प्रतिशत कवकनाशी का उपयोग मसूर के पद गलन के रोग का एकीकृत प्रबंधन हेतु जैव नियंत्रण कारकों के साथ किया जा सकता है। बीज ड्रेसिंग विश्लेषण *ट्राइकोडर्मा स्पी.* (*Trich-13*) *स्यूडोमोनास फ्लूरोसेन्स* (*Pf-26*) एवं *बेसिलस स्पी.* (*Bs-21*) एवं प्रोपिनेब 70 प्रतिशत डब्ल्यू.पी. का संयुग्मन आने वाले पौध को अधिकतम रक्षा प्रदान किया। पात्र परीक्षण में *Pf-26+ Bs-21* + प्रोपिनेब 70 प्रतिशत डब्ल्यू.पी. से उपचारित बीज का अंकुरण प्रतिशत पूर्ण ढंग से बढ़ा दिया तथा मसूर के पद गलन आपतन को कम किया।

ट्राइकोडर्मा के अधिकांश जाति, कवकनाशी के संयोजन के साथ पौधों की वृद्धि को बढ़ावा देने वाली गतिविधि के लिए सकारात्मक प्रतिक्रिया दिखाई दी। *ट्राइकोडर्मा* आइसोलेट्स के साथ उपचारित बीज, पौधे की ऊँचाई में उल्लेखनीय वृद्धि (टी 8 में 53.62, टी 7 में 58.28 सेंटीमीटर तथा नियंत्रण उपचार में 53.57 सेंटीमीटर) रहा। *ट्राइकोडर्मा* तथा कवकनाशी के सभी उपचारों में *ट्राइकोडर्मा हर्जियानम*+प्रोपीनेब और *ट्राइकोडर्मा म्यूटेंट* के संयोजन के उपचार ने मसूर के पौधे की मृत्यु दर को काफी कम कर दिया और अन्य *ट्राइकोडर्मा* आधारित संयोजन से बेहतर था। *ट्राइकोडर्मा हार्जियानम*+प्रोपीनेब संयोजन और *ट्राइकोडर्मा म्यूटेंट* के कुछ अन्य *ट्राइकोडर्मा* उपभेदों, कवकनाशी और संयोजन उपचार के साथ, दो साल तक क्षेत्र की स्थिति में तुलना किया गया। *ट्राइकोडर्मा हर्जियानम*+प्रोपीनेब और *ट्राइकोडर्मा म्यूटेंट* से बीजों का उपचार करने से बीजों की वृद्धि में काफी सुधार देखा गया, रोग की उग्रता में कमी आई है और उपज में सुधार दोनों वर्षों में हुआ ।

पद गलन रोग के विरुद्ध कुल 271 मसूर प्रविष्टियों की जांच की गई, जिसमें से 2018–2019 के दौरान 139 प्रविष्टियों की जांच की गई, जबकि 2019–2020 में 132 प्रविष्टियों की जांच की गई। पहली रबी सीजन 2019–2020 के दौरान, सभी दाल प्रविष्टियों को पद गलन के लिए अतिसंवेदनशील होने की संभावना थी। जबकि, दूसरी रबी सीजन 2018–19 के दौरान, केवल 3 जर्मप्लाज्म, डीपीएल –62, वीएल –1 और वीएल –4, इस बीमारी के लिए अत्यधिक प्रतिरोधी पाए गए, जबकि, 10 जर्मप्लाज्म डीपीएल –15, आशा, एनडीएल –1, पीएल– 5, रंजन, पीएल –406, पीएल –234, वीएल –103 किस्में फोकन और देहाती मसूर को प्रतिरोधी के रूप में पहचाना गया, बाकि सभी अन्य प्रविष्टियां बीमारी के लिए संवेदनशील से अतिसंवेदनशील तक पाया गया।

CHAPTER-1
INTRODUCTION

Pulses are considered to be as a wondrous gift produced in the nature to both humankind and earth. They nourish the mankind with their proteinaceous and nutritive nature as well the soil with its ability to improve soil fertility. Pulses traditionally have been considered as an important component of cropping systems in the Indo-Gangetic plans and became an important constituent in the Indian daily vegetarian diet. Its production occupies about one fifth of the total area under food grain production and shares about one twelfth of the national food grain basket. Indian Council of Medical Research (ICMR) has recommended an intake of 50 g of pulses/ capita /day in daily human consumption.

Among pulses, Lentil (*Lens culinaris M.*) is an important *Rabi* crop and accounts up to 7% of the total pulses production in the country. Lentils are earliest known crop to be cultivated and archaeological evidences dating back to 7000 years have been found in the Middle East and from Iberian Peninsula (Zapata et al., 2004). The origin of lentil was considered to be as Central Asia. It is one of the first foods to have been cultivated and have been an important food since prehistoric times (Sarker and Erskine 2006). Lentils have been used as a staple food during lent in many Catholic countries. It had become a very important part of the diet in many parts of the world, especially South Asia which has a large vegetarian population (Singh, 1999). They were introduced into India before the 1st century AD and still lavishes as a high regard traditional cuisine made of spiced lentil known as dhal.

The Lentil (*Lens culinaris M.*) belonging to the family Leguminosae is a cool season legume crop and a bushy annual grown for its seeds. The name came from its characteristic lens shaped seeds. It also known by many common names viz., Malka masoor (bold seeded), Masuri (small seeded), Massour, Mangu/Margu, Masura, Renuka, Mangalaya etc. (Kay, 1979). It is a bushy annual self-pollinated diploid (2x=2n=14) (Muehlbauer, 1995) and self-pollinating with a haploid genome size of an estimated 4063 Mbp (Arumuganathan and Earle, 1991). The plant grows up to the height of 40 cms and the seeds are developed in pods, each

pod containing two seeds within in it. Protein amount in Lentil ranges from 22 – 30% (Wang and Daun, 2006) and is the vegetable with highest level of protein after soybean (Bhattacharya *et al*., 2005). 100 g of dried seeds contain 340 – 346 calories, 12% moisture, 20 .2g protein, 0.6 g fat, 65.0 g total carbohydrate, about 4 g fibre, 2.1 g ash, 68 g Ca, 325 mg P, 7.0 mg Fe, 29 mg Na, 780 mg K, 0.46 mg thiamine, 0.33 mg riboflavin, 1.3 mg niacin (Adsule *et al*., 1985; Muehlbauer *et al*., 1985). Lentil is the richest in the amino acids than that of other vegetarian diets that are imposed by poverty on different people throughout the world (Iqbal *et al*., 2006).

Currently the leading producers of lentil in the world are India, Canada, Turkey, China and Syria. India stands in 2nd position next to Canada with annual production of 1.61 MT (INDIASTAT, 2018). The major lentil producing states are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and Bengal (Kumar *et al*., 2013). Among rabi pulses, lentil next to chickpea, being grown on an area of 1.55 m ha with a productivity of 1034kg/ha in India. The consumption of lentil is more in India than any other country in the world and produces more than 50 varieties in different states. Chhattisgarh contributes around 0.34% of total lentil production with an annual production of 0.05 M tonnes and being grown in around 0.16 m ha of area annually. The varieties K – 75, JG – 14, Vishal, IPL -81, Lens – 4076 etc., were mostly grown in Chhattisgarh.

The yield of lentil remains low (1.1 tonnes ha^{-1}) in India (INDIASTAT 2018) and still relatively low compared to its yielding potential (3.6 tonnes ha^{-1}) under well managed production due to biotic and abiotic stresses (Kumar *et al.,* 2017). This lentil low production was attributed to various insect pests, diseases, poor agronomic practices and lack of improved cultivars and crop production technologies (Ghazanfar *et al*., 2010). The biotic constraints that suffers the crop includes plant diseases caused by different fungi, bacteria, viruses, nematodes, plant parasitic weeds and insect pest thereby resulting in huge economic losses. The major diseases hampering the production of lentil are Wilts, Rusts, *Botrytis* Grey mold, *Ascochyta* blight and *Stemphylium* blight. Lentil wilt / root rot complex was caused by various plant pathogenic fungi such as *Sclerotium rolfsii*, *Fusarium* spp, *Rhizoctonia solani* and *R. bataticola.*

Out of this, Collar rot disease caused by *Sclerotium rolfsii* Sacc. is very important and a polyphagus pathogenic fungus causing substantial losses in quality and productivity of yield. *Sclerotium rolfsii* Sacc. is very fast spreading and nonspecialized soil borne fungal pathogen having worldwide importance and has a host range of over 500 species, includes ornamentals, vegetables, fruits and field crops (Punja and Grogan, 1988). It has wide geographic diversity and commonly found in tropics, sub tropics and other temperate regions. It causes many diseases like leaf spot, leaf blight, southern blight, collar rot, stem rot, root rot etc., on various hosts. This ability to survive on various hosts, prolific growth and production of sclerotia contributes to the great economic losses associated with this pathogen. Its profuse growth rate makes it well suited facultative parasite and a most successful pathogen throughout the world.

Being a soil borne pathogen, its influence on foliar parts of the plants is less common and hence foliage diseases are not frequently found on plants. It produces white mycelium with fan shaped pattern on plant part during infection. Clamp connection may be present in hyphae. It produces a large number of mustard grains sized light to dark brown structures known as sclerotia, measuring 0.5 to 1.5 mm in diameter. They can overwinters and persist for many years in the soil which makes the pathogen difficult to kill. The pathogen is capable of infecting the plant at any time, i.e., from seedling to flowering stage. The destruction of the pathogen is observed more during seedling stage than at flowering stage. The affected plant indicates root rot symptoms marked by dark brown discoloration at the collar region, covered by a white cottony mycelial growth. Infected young seedlings show damping-off symptoms. In earlier stages of infection, rapeseed like sclerotia can be observed attached to mycelium around the collar at advanced stages; the plants gradually turn pale, droop and dry (Njambere and Chen, 2011). Germinated seedlings were killed within 7 days after emergence. Symptoms were evident as yellowing collapse (Nene *et al.* 1978). From infected plants, the mycelium spreads out to the nearest plants by the soil surface. Like this, the disease spreads out entire field causing a huge loss in yield and productivity of the lentil. The losses in yield are appreciably very high leading to consistent decrease in area under which the

crop is grown. Hence, controlling the disease is very essential to increase both yield and productivity of the crop.

Despite of many achievements in modern agriculture, management of diseases is not being successful still now. Being a soil borne pathogenic fungus, the most effective way to control *Sclerotium rolfsii* Sacc. is seed treatment with chemicals, botanicals and biocontrol agents. Moreover, seed treatment requires very low quantities of seed treating materials compared to foliar application Hence, it reduces the risk of environmental pollution, health hazards and economically not much costlier to farmers. Benomyle, Carbendazim and Vitavax had shown occurrences against" against seed borne fungi *viz., Sclerotium rolfsii* Sacc., *Aspergillusspp, Penicillium spp, Alternaria alternate, Rhizopus* etc.

Application of fungicide to control diseases causes several adverse effects i.e. development of resistance in the pathogen, residual toxicity, pollution to the environment etc. It was reported that, despite advances in antifungal therapies, many problems remained to be solved for most antifungal drugs available (Grasela *et al.* 1990). Therefore, it is essential to adopt eco-friendly approaches for enhancing crop yield and better crop health. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Jun-Dong *et al.,* 2006). Flavanoids, triterpenoids, steroids and other phenolic compounds in plants have also been reported to have antimicrobial activity (Rojas *et al.,* 1992; Hostetmann *et al.,* 1995). In nature also, many beneficial micro-organisms are found which can be able to supresses the growth of pathogens. Botanicals such as Neem, Garlic, Mehandi, Tulasi, Kanuga etc, and biocontrol agents like *Trichoderma, Pseudomonas,* are reported to be effective against soil borne pathogenic fungi (Singh *et al.,* 2014).

Biological control of plant diseases is gaining attention due to increased pollution concerns caused by the excessive use of pesticides for crop protection and development of pathogen resistance. The use of environmental friendly microorganisms has proved to be useful in plant growth promotion due to their role in nutrient cycling (Bhattacharyya and Jha, 2012) and disease control. Plant growth promoting rhizobacteria (PGPR) inoculation has proven to be a promising agricultural approach that plays an important role in crop protection, growth

promotion or biological disease control. The mechanism of antifungal effects lies on the production of a variety of antimicrobial compounds that act in different ways. The antagonistic effects are caused by cytolysis, leakage of potassium ions, disruption of the structural integrity of membranes, inhibition of mycelial growth and the protein biosynthesis (Quan *et al*., 2010). One of the most popular bacteria studied and exploited as biocontrol agent is the *Pseudomonas* species (Laslo, 2012).

The genus *Trichoderma* is cosmopolitan in nature and survives on decaying wood and vegetative matter. Species of *Trichoderma* are frequently dominant components of the soil microflora in widely varying habitats. This may be attributable to the diverse metabolic capability of *Trichoderma* species and their aggressively competitive nature. Different species of *Trichoderma* are found in nature like *T. asperellum*, *T. atroviride*, *T. harzianum, T. hamatum, T. koningii*, *T. virens* and *T. viride* etc. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. *Trichoderma*, a filamentous soil inhabiting mycoparasite, is used in commercial preparation for biological control of many fungal plant pathogens (Jash, 2006). The mechanisms like antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solublization and sequestration of inorganic nutrients and inactivation of pathogen enzymes (Harman, 2000).

The potential of using *Trichoderma* as a biocontrol agent was suggested by Weindling (1932), who was the first to demonstrate the parasitic activity of the members of this fungal genus to pathogens such as soil borne plant pathogenic fungi e.g. *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* sp. etc. Dennis and Webster (1971) described the antagonistic properties of *Trichoderma* in terms of antibiotic production and hyphal interactions in the control of *Sclerotium rolfsii*. However, with the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, several *Trichoderma* species were formulated in a commercial production for protection and growth enhancement of a number of crops in several countries (McSpadden and Fravel, 2002).

The combination of bio-agents along with lower dose of fungicides has been successfully used for the control of several diseases (Kumar and Dubey 2001; Moradi *et al*. 2012). The benefits of this approach include improving plant growth and quality, reducing the amount of chemical application, reducing the possibility of developing resistance in the pathogens, potential environmental hazards and cost of disease management.

Fluorescent *Pseudomonas* spp. are aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller *et. al*. 1988). In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soilborne pathogens (Weller *et. al*. 2002).

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In the light of this importance of the crop and disease management, the present study was conducted with the following objectives:

Objectives of investigation:

Survey and Collection of *Sclerotium rolfsii* Sacc. of lentil from lentil growing areas of Chhattisgarh.

1. Isolation, purification and pathogenicity test of different isolates of *Sclerotium rolfsii* Sacc. of lentil
2. Study of morpho-cultural variations among the different isolates of *Sclerotium rolfsii* Sacc. of lentil
3. Study of soil factors affecting development of collar rot disease in lentil.
4. Screening of lentil varieties/wild relatives for resistance to collar rot of lentil.
5. Management of collar rot of lentil. (Evaluation combination of bio agents and fungicides against isolates of collar rot of lentil)

CHAPTER-II
REVIEW OF LITERATURE

The available literature pertaining to research investigation is reviewed critically under the following major heads:

2.1.: About lentil

Lentil (*Lens culinaris* M.) is an edible pulse and belongs to the family *Leguminosae* (*Fabaceae*). It is a bushy annual plant of the legume family, grown for its lens-shaped seeds. It is about 40 cm (16 in) tall and the seeds grow in pods, usually with two seeds in each. Lentils have been part of the human diet since the ceramic (before pottery) Neolithic times, being one of the first crops domesticated in the Near East. Archaeological evidence shows they were eaten 9,500 to 13,000 years ago. Lentil colours range from yellow to red-orange to green, brown and black. Lentils also vary in size, and are sold in many forms, with or without the skins, whole or split.

2.1.1. Lentil in culture

The lens (double-convex shaped) is so called because the shape of a lens is basically the same shape as lentils. Lens is the Latin name for lentil. Lentils were a chief part of the diet of ancient Iranians, who consumed lentils daily in the form of a stew poured over rice. Lentils are also commonly used in Ethiopia in a stew-like dish called *kik*, or *kik wot*, one of the dishes people eat with Ethiopia's national food, injera flat bread. Yellow lentils are used to make a non-spicy stew which is one of the first solid foods. Ethiopian women feed to their babies. In Pakistan, lentils are often consumed with *Roti*/bread or rice.

In India, lentils soaked in water and sprouted lentils are offered to gods in many temples. It is also a practice in South India to give and receive sprouted peas by women who perform *Varalakshmi Vratam*. It is considered to be one of the best foods because the internal chemical structures are not altered by cooking.

2.1.2. "Nutritional status of lentil"

With about 30% of their calories from protein, lentils have the third-highest level of protein, by weight, of any legume or nut, after soybeans and hemp. Proteins include the essential amino acids isoleucine and lysine, and lentils are an

essential source of inexpensive protein in many parts of the world, especially in West Asia and the Indian subcontinent, which have large vegetarian populations. Lentils are deficient in two essential amino acids, methionine and cysteine. However, sprouted lentils contain sufficient levels of all essential amino acids, including methionine and cysteine.

Lentils also contain dietary fiber, folate, vitamin B1, and minerals. Red (or pink) lentils contain a lower concentration of fiber than green lentils (11% rather than 31%). Health magazine has selected lentils as one of the five healthiest foods. The low levels of Readily Digestible Starch (RDS) 5%, and high levels of Slowly Digested Starch (SDS) 30%, make lentils of great interest to people with diabetes. The remaining 65% of the starch is a resistant starch that is classified RS1, being a high quality resistant starch, which is 32% amylose. Lentils also have some anti-nutritional factors, such as trypsin inhibitors and relatively high phytate content. Trypsin is an enzyme involved in digestion, and phytates reduce the bio-availability of dietary minerals. The phytates can be reduced by soaking the lentils in warm water overnight. Lentils are a good source of iron, having over half of a person's daily iron allowance in a one cup serving.

2.1.3. "Nutritional value of lentil per 100 g dry weight"

Energy -	353Kcal
Carbohydrates-	60
Sugars -	2g
Dietary fibre-	31g
Fat -	1g
Protein-	26g

Vitamins"

Thiamine (B1)-	(76%)	0.87 mg
Riboflavin (B2)-	(18%)	0.211 mg
Niacin (B3)-	(17%)	2.605 mg
Pantothenic acid (B5) –	(42%)	2.120 mg
Vitamin B6 –	(42%)	0.54 mg
Folate (B9)-	(120%)	479 µg
Vitamin C -	(5%)	4.4 mg

Trace metals

Calcium-	(6%)	56 mg
Iron -	(58%)	7.54 mg
Magnesium-	(34%)	122 mg

Phosphorus-	(64%)	451 mg
Potassium-	(20%)	955 mg
Sodium -	(0%)	6 mg
Zinc-	(50%)	4.78 mg

Other constituents

Water -10.4 g

Units µg = micrograms, mg= milligrams, IU,

International Units Source: USDA Nutrient Database

2.1.4. Area, production and productivity

Among all the *Rabi* pulses, it is next to chickpea in area and production. It is one of the ancient crop cultivated as early as 8000 BC as evident from archaeological excavations. Due to its domestication and cultivation since long, it has become an important pulse crop in many countries. It has become an important dietary component in the developing countries like Afghanistan, Bangladesh, Egypt, India, Turkey, Syria, Pakistan, Iraq, Nepal, Spain, China, Morocco, Ethiopia, Tunisia, Sudan, Iran, etc. Many of these countries are major producer too. Globally it is cultivated in India, Canada, Australia, Turkey, Syria, Pakistan, Bangladesh, Spain, China, Morocco, Ethiopia, Chile, Argentina, USA, Oceania etc.

In India total coverage area under this crop is about 1.27 million hectare and production 0.97 million tonnes and average productivity 765 kg/hectare (Anonymous, 2016-17). Major lentil growing states of India are UP, MP, South eastern Rajasthan, Bihar and West Bengal which contribute more than 80% of lentil area and production, whereas, Assam, Chhattisgarh, Haryana, J & K, Punjab, Maharashtra, Jharkhand, Tripura and Uttarakhand also contribute up to some extent.

The cultivation of lentil is well adapted to the conditions prevailing in Rajasthan and is cultivated in about 75677 hectares with annual production of 75877tonnes and productivity 1003 kg/hectare (Anonymous, 2017-18). The major lentil growing districts in Rajasthan are Bharatpur, Dholpur, Kota, Bundi, Bhilwara, Jhalawar, Sawai-madhopur, Pratapgardh, Chittorgarth and Baran districts.

2.1.5. Distribution of Lentil

Lentil (*Lens culinaris* Medikus) is one of the world's oldest domesticated leguminous crops, (Zohary and Hopf, 2000). Cultivated lentil is thought to have been originated and first domesticated in western Asia and then introduced into the Indo-Gengetic Plain around 2000 B.C. (Cubero, 1981). Lentil has also been rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later to the new world, including Latin America, Mexico Chile, Argentina, Colombia and more recently Canada, and cultivated in most sub-tropical and warm temperate regions (Abraham, 2015). Lentil remains stable food crop in the Middle Eastern and Indian diets, and one popular in the cuisines throughout the world (Anonymous, 2013).

Lentil is a short and slender annual cool-season food legume, which was domesticated early in the Fertile Crescent of the Middle East (Sarker *et al*., 2010). The botanical features of lentil (*L. culinaris*) can be described as annual bushy herb with slender almost erect or sub-erect, much-branched, softly hairy stems that are slender angular, 15-75 cm in height and show hypogeal types of germination (Muehlbauer *et al.,* 1997). It is a brushy annual plant of the legume family, grown for its lens-shaped seeds, which produces many small purse-shaped pods containing one to two seeds each. Lentil is classified into two groups by seed size, namely the Chilean and Persian types. The large seeded Chilean type has 1000 seed weight of 50 g or more. The small seeded Persian type has 40 g or less of an average weight per 1000 seeds.

Lentil has been considered to be the poor man's meat due to an affordable other sources of protein. About one-third of the calories in lentil come from protein, which is the third-highest level of protein by weight of any legume. In many parts of the world, lentil is the cheapest protein food and contains dietary fiber, vitamin B and minerals, iron, among the cool season legume crops, lentils are the richest in their important amino acids (lysine, arginine, and leucine) contents; however, there is shortage of certain lentil amino acids, including methionine and cystine (Muehlbauer *et al*., 2002). Lentil is important, especially for women of child-bearing age, children and vegetarians. The crop has great

significance in cereal-based cropping systems because it fixes nitrogen and the straw provides animal feed (IBC, 2007; Muehlbauer, 2011).

The Lentil Improvement Program of the Ethiopian Institute of Agricultural Research (EIAR) in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) has released some lentil varieties; previously these varieties were resistant to Fusarium wilt and had yield potentials of up to 2.6 t ha^{-1} (Mulugeta, 2009). According to FAO (2009) production database, currently Ethiopia contributes to 2% of the world total production, and it was the first producer of lentil in Africa accounting to 84% of the total regional production (96,524 tons), followed by Morocco (8.8%), Malawi (1.9%), Egypt (1.6%) and Tunisia (1.2%). Currently, lentil is considered as a cash crop that fetches higher price than most of the cereals and pulses grown in India (Abraham, 2015).

2.1.6. Production and Consumption of Lentil

Lentil provides an important source of food and nutritional security for the rural poor, especially those who cannot produce or cannot afford costly livestock products as source of essential proteins. Lentil is recognized as one of the most nutritious pulse crops ranking next to chickpea amongst pulses. It contains 24-26% protein, 3.2% fiber and 57% carbohydrate. It is a rich source of minerals containing 68 mg calcium, 300 mg phosphorus and 7 mg iron per 100 g lentil seed. It is also rich in vitamin C and riboflavin (Ali and Mishra, 2000). Furthermore, because of its high lysine and tryptophan contents, its consumption with wheat or rice provides a balanced diet. The chemical composition and nutritive values of lentil haulm vary with variety, soil, climatic conditions, sowing date, stage of harvest and storage conditions (Demirel *et al.*, 2012).

The major lentil-growing countries of the world include Australia, Canada, China, Ethiopia, India, Nepal, Syria, Turkey and USA (Ahlawat, 2012). The total lentil cultivated area in the world is estimated at around 4.34 million hectares with annual production and productivity of 4.95 million tons and 1260 kg ha^{-1} respectively (FAO, 2014). Most of the production, which reaches around 56%, is consumed locally and only 44% of the production is supplied to the global markets

(Kumar *et al.,* 2013). Canada is the leading lentil exporting nation worldwide (Bedard *et al.,* 2013).

Among grain legumes, lentil is one of the principal crops widely grown in diverse agro-ecological zones of Ethiopia (Muehlbauer and Tullu, 1997; Schneider and Anderson, 2010). Food legumes are grown throughout Ethiopia and account for 13% of the cropped land that is concentrated in the Amhara and Oromia regions (Rashid *et al.,* 2010). The country's potential lentil producing zones are six zones of Amhara region: North Gondar, North Shewa, North Wollo, South Gondar, South Wollo and Waghemra; three zones in Oromia region are East Shewa, North Shewa and Southwest Shewa, while South Tigray is the major lentil-producing zone in the Tigray Region (Senait *et al*., 2006). The average seed yield of lentil in farmers' fields in India generally ranges from 0.6 to 0.8 t ha^{-1} (Senait *et al*., 2006). Not surprisingly, lentil yield that was restricted to about 2.0 t ha^{-1} has been reported from experiments performed under controlled field experimental conditions in India (Teklu *et al*., 2006).

2.1.7. Lentil Improvement in India

Crop Improvement Genetic improvement programmes of Lentil were initiated through collection of genetic resources, evaluation, and their utilization through hybridization programme. In early phase of varietal development, selection from available variability was common method to identified high yielding lines. Later hybridization and induced mutation method were also used for developing the high yielding and disease resistant lines. Concerted efforts were made to emanate large seeded varieties and consequently a large number of such varieties suitable for India were developed and released i.e., IPL-406, DPL-62, L-4076, JL-3, JL-1, IPL-81, IPL-316 and RVL-31. The JL-1 and JL-3 varieties were developed by Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur and RVL-31 and RVL 11-6 were developed in the year of 2014 and 2016 respectively by Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior. These high yielding varieties have also revealed additional tolerance to wilt which are important for the region. Traditionally grown cultivars produced good harvest under normal rainfall, but fail to maintain their productivity in deficit rains and sudden rise in temperature and moisture stress at terminal stage. Therefore, efforts

have been made to develop early maturing and high yielding cultivars in Lentil. A good progress has been made in development of short duration and high yielding varieties of Lentil like IPL-316, IPL-81, JL-3, and JL-1. The popular varieties of Lentil of this decade are JL-3, JL-1, IPL-81, DPL-62 and PL-5 in India.

2.1.8. Major Biotic Constraints to Lentil Production

Currently, the consumption of lentil is decreasing mainly because of the high level of limiting factors on its production and productivity in Ethiopia. The yield-limiting factors include cultivation of local varieties for long period, inappropriate seedbed type, poor field management, use of unhealthy seed, lack of lodging resistance, lack of seedling vigor, high rate of flower drop, low pod setting, poor dry matter, low harvest index, low or no response to inputs, and subject to various biotic and abiotic stresses. In India, there are about ten important lentil diseases, among which Fusarium wilt, root rots and rust are the major ones (Abraham, 2015). Fusarium wilt is the most important biological constraint to lentil productivity worldwide, causing severe damage to leaves, stems, roots and pods as well as seed quality reduction (Garkoti *et al.*, 2013). Singh (2015) reported that lentil is attacked by a number of seed- and soil-borne pathogens, such as vascular wilt (*Fol*), root rot (*Rhizoctonia solani*), dry root rot (*R. bataticola*), downy (*Peronospora lentis*) and powdery (*Erysiphe pisi*) mildews, rust (*Uromyces vicia-fabae*), and collar rot (*Sclerotium rolfsii*).

2.1.9. Disease importance

A major limiting factor in profitable cultivation of this crop in Rajasthan is the attack of several diseases mainly caused by fungi, which takes heavy loss of the crop at all the stages of growth right from sowing to harvest and storage. Like all other crops, lentil suffers from various diseases caused by fungi and other microorganisms. Important diseases incited by fungi are wilt (*Fusarium oxysporum* f. sp. *lentis)*, Rust [*Uromyces fabae* (Pers.) de Bery], Grey mould (*Botrytis cinerea)*, Powdery mildew (*Erysiphe polygoni* f. sp. *lentis),* Root rot (*Rhizoctonia solani)*, Collar rot (*Sclerotium rolfsii* (Sacc.) Curzi) and Ascochyta blight (*Ascochyta lentis)*. The diseases, which affect the foliage cause extensive

damage to tissues, interfere to the photosynthetic process and cause severe losses in yield (Anonymous, 2005-06).

Amongst these diseases, wilt of lentil caused by *Fusarium oxysporium* f. sp. *lentis* is one of important seed and soil borne diseases and importan biological constraint to productivity of lentil worldwide (Bhalla *et al*., 1992) and plays a major role in reducing lentil yeild (Hamdi and Hassanein, 1996). It is an important disease in the major lentil growing areas of the states. Wilt incidence as high as 50-78% has been reported in some fields of Madhya Pradesh (Khare *et al.,* 1979). In West Asian countries like Syria, the yield losses range from 5-72% (Bayaa *et al.,* 1986). The most of the lentil cultivars are susceptible to this disease. It is emerging as a major and wide spread problem in Rajasthan.

The disease appears in either early stage of crop growth (seedling) or during the reproductive stage (adult stage) (Khare, 1981; Stoilova and Chavdarov, 2006). The wilt pathogen survives in the soil as chlamydospores that can remain viable for several years (Erskine *et al.*, 1990) and is capable of colonizing residues and roots of most crops grown in rotation with lentil. The incidence of the wilt disease is increasing, causing substantial lentil yield losses.

2.2 "*Sclerotium rolfsii*"

2.2.1 History, taxonomy and occurrence of *Sclerotium rolfsii*

Rolfs (1892) for the first time recorded a tomato blight disease caused by *Sclerotium rolfsii* Sacc. in florida and recognized the small, round sclerotia as the outstanding morphological characteristics of the organism. The name *Sclerotium rolfsii* was given by Saccardo (1911), who characterized the fungus as an imperfect form i.e. without sexual spores.

Curzi in 1931 proposed the generic name *Corticium* for the fungus, based on studies of the perfect stage in pure culture. West (1947) suggested that the generic name may be changed to *Pellicularia*, since it was characterized by aerolate hymenia, short celled, stout hyphae and right angled branching of mycelia. However, according to Talbot (1973) the basidial stage of *Sclerotium rolfsii* is a species of *Athelia* in corticiaceae. Aycock (1966) reported that hyphae have clamps in the form of forks and hooks or H-like connections.

Subramanian (1971) stated typical character of *S. rolfsii* Sacc. Mycelium very floccose, not ropy, producing numerous sclerotia. Sclerotia pinkish buff to olive brown to clove brown colour, globose, 0.8-2.5 mm in dimeter. *P. rolfsii* formed hymenium aerolate, putty coloured, 30-40µ thick. Basidia obovoid, 7-9 x 4-5µ each bearing 2 or 4 paralled or divergent sterigmata, 2.5 x 4-6µ long. Taebot (1973) reported that according to the basidial stage of *Sclerotium rolfsii*. It is a species of *Athelia* in corticiaceae.

2.2.2 Distribution and economic importance

S. rolfsii is prominently distributed in tropical and subtropical countries. It is common where high temperature exist during the rainy season. Reported that the fungus survived in the soil for years together by producing sclerotial bodies and causing the disease on various hosts. At present, 5000 host species belonging to 100 plant families are susceptible for this pathogen (Aycock, 1966).

Collar rot of chickpea is caused by *S. rolfsii*. This disease is widespread in most tropics and warm countries, Bangladesh, Colombia, Egypt, Ethiopia, Kenya, Mexico, Nepal, Pakistan, Philippines, Sudan, Syria, Uganda, and Zambia (Nene *et al*., 1996). It is also prevalent in India.

Collar rot is a serious threat, which under conducive conditions causes 55-95% mortality of the crop at seedling stage (Gurha and Dubey, 1982). *S. rolfsii* is a devastating soil-borne plant pathogenic fungus with a wide host range (Aycock, 1966; Punja, 1988), has prolific growth and ability to produce persistent sclerotia contributing to high degree of economic losses (Mahen *et al*., 1995).

2.2.3. Pathogen

Oduro and Tetteh (1978) reported Sclerotium rot (*S. rolfsii*) for the first time on soybean varieties raised at the University of Science and Technology, Kumasi, Ghana. Collar and pod rots were observed.

Khare et al. (1979) reported that various diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew, downy mildew, which are caused by *Fusarium oxysporum* f.sp. lentis, *Sclerotium rolfsii, Rhizoctonia solani, Uromyces fabae, Erysiphe polygoni* and *Peronospora lentis,* respectively are known to infect lentil.

Punja (1985) reported that the most common hosts are the legumes, crucifers and cucurbits. The diseases caused by the fungus are more serious in tropical and sub-tropical regions. The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance through out the world.

2.2.4. Survey

Rafique *et al.* (2015) conducted the survey of lentil wilt in seven districts of Pakistan during (2011-12 and 2012-13). This revealed, 100% mean disease prevalence and 25.7% mean incidence.

Altaf *et al.* (2014) surveyed nine districts of Pakistan with 28 locations during the crop season of year 2012-13 and out of which 21 showed 100% disease prevalence of lentil wilt.

Naimuddin and Chaudhary (2009) surveyed Bundelkhand region of U.P. and observed that per cent plant mortality in lentil due to different soil borne fungal pathogens ranged between 19.95-33.30, The mortality was more in year 2006-07 (24.68-33.30%) than in 2005-06 (19.95-25.69%). *F. oxysporum* f. sp. *lentis* was most important and widely prevalent in all surveyed districts. Its share in causing plant mortality ranged between 49.76 and 59.17%.

Incidence ranged from 5 to 18% in 2005-06, and from 7 to 23% in 2006-7Where maximum wilt incidence ranging between 13-18% was recorded in Mahsi districts in 2005-06 and that of 9-23% was recorded in 2006-07 (Srivastava *et al*., 2008).

In India, lentil wilt was first reported from undivided Bengal in 1934 (Anonymous, 1934). The pathogen causes serious disease and is widespread in India. Aerial inspections would also be useful as *Fusarium* wilts often occur in patches or along rows in fields and these patches are usually visible from the air (Kochman *et al.*, 2002).

Ferreira and Boley (1992) reported that *Sclerotium rolfsii*, a soil-borne fungal pathogen causes disease in a wide range of agricultural and horticultural crops. *S. rolfsii* has at least 500 species hosts in 100 families.

2.2.5. Symptomatology

Wilson (1953) described the symptoms of collar rot as, mycelium covering the plant stem near the soil surface. The production of abundant white mycelium, and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease. Later, affected plants/branches turned yellow or drooped while retaining their green colour, followed by drying and turning straw colored. White mycelial strands appeared at the collar region and above, covering the base of the branches, whitish, brownish, irregular shaped sclerotia mingled with mycelial strands on branches.

Nene *et al.* (1978) reported that *S. rolfsii* initiated the rotting of seeds and seed surfaces were covered with white mycelial mat in soil. Germinated seedlings were killed within 7 days after emergence. Symptoms were evident as yellowing of leaves followed seedling collapse. The region and down ward with the whitish mycelium in earlier stages of infection, rapeseed like sclerotia can be observed attached to mycelium around the collar.

Kumar and Dubey (2000) described the symptoms of collar rot on pea as the plants showed dark brown lesions near the collar region which increased in size and covered half or more of the root and collar region of the stem. Affected portions rotted after some time, with discoloration of vascular tissues in the region. In cases where seedling survival was prolonged because of late infection, plants showed leaf yellowing and dropping, brown lesions at the nodes, and, ultimately, death of the plant.

Dantas *et al*. (2002) reported that collar rot of common bean (*Phaseolus vulgaris*), caused by *Sclerotium rolfsii*, can induce high losses. The isolates were obtained from the stem of infected plants and the inoculation was done by deposition of ten sclerotia on the collar of previously wounded plants. Some cultivars and lines showed susceptibility to the isolates and some cultivars and lines showed resistance to the isolate.

Rao *et al.* (2002) conducted an experiment on stem rot or collar rot of flora bean (*Dolichos lablab*) caused by *S. rolfsii*. The symptoms of the disease included appearance of initially small, oval, straw to brown lesions at the collar region followed by wilting of the lower leaves and gradual drying of the whole plant.

White mycelial growth developed on the rotted portion, small and brown, round to oval sclerotia were produced. The pathogen was identified based on morphological observations and pathogenicity test.

Kator *et al*. (2015) studied the disease causing potential of *Sclerotium rolfsii* on some tomato cultivars and bioassay was conducted. The cultivars showed disease symptoms such as chlorosis, wilting, damping off, blighting and necrosis.

2.2.6. Purification, Isolation and Identification

Sulladmath *et al.* (1975) reported that oat (*Avena sativa*) plants grown in experimental plots, were severely affected by *S. rolfsii* at the flag leaf stage. The affected plants indicated root-rot symptoms marked by dark brown discolouration at the collar region, covered by a white cottony mycelial growth. On uprooting such plants, the stem breaks easily at ground level. In advanced conditions, numerous sclerotia were seen on the collar region. Isolation from the infected portions yielded *Sclerotium rolfsii* Sacc.

Siddaramaiah *et al*. (1978) reported that about one per cent of the Niger plants wilted in 1978 at Botanical Garden of College of Agriculture, Dharwad, Minute examination of the wilted plants showed that collar portion of the plants were sunk and covered with white mycelial mat and large number of sclerotial bodies around the collar portion. Heavily infected plant died within a week. Repeated isolation of the infected portion of the plant yielded the fungus *Corticium rolfsii.*

Padole et al. (2009) studied incidence of collar rot in 15 to 45 days old chickpea crop which ranged from 5-30% in 51 locations surveyed nearby Jabalpur. Investigations on variations in 51 isolates of *Sclerotium rolfsii* showed considerable variations with regard to cultural and morphological characters on PDA and grouped them into three pathotypes. The pathogenicity test showed the isolates to vary in number of days taken to initiate plant mortality and 100% mortality.

2.2.7. Pathogenicity

Kilpatrick and Merkle (1967) reported the effect of different levels of *S. rolfsii* inoculum on foot rot of wheat and found that, 0.5 and 1.0% inoculums was superior to 3, 5 and 10%. However, considerable amount of infection was

recorded in two per cent inoculum and 100% disease in 6% and above inoculum level.

Sengupta and Das (1970) studied the cross inoculation of isolates of *S. rolfsii* from groundnut, wheat, potato, guava and Bengal gram. They concluded that Bengal gram was the most susceptible host against *S. rolfsii*. Although isolates were most virulent to their appropriate hosts.

Datar and Bindu (1974) proved the pathogenicity of *S. rolfsii* on sunflower by soil inoculation method under glasshouse condition. The inoculum was prepared by growing the fungus on sterilized maize bran medium and mixed with the sterilized soil one week before sowing. Typical symptoms were produced within a week of inoculation in the field.

2.2.8. Mass multiplication of the pathogen

The pathogen *Sclerotium rolfsii* was mass multiplied on sterilized sorghum grains presoaked overnight in 2 percent sucrose solution (Upadhyaya and Mukhopadhyay, 1986). Different substrate has been used by different workers for mass multiplication of *S. rolfsii in vitro* such as sterilized sorghum grains (Uma Maheswari *et al.*, 2002; Patibanda *et al.*2002), wet wheat bran: vermiculate (1:1 w/w) (Prasad *et al.*, 1999) and sand maize meal medium (Rajani *et al.* 2006;Anahosur 2001; Dutta and Das, 2002; Rao *et al.* 2004)

Hussain *et al*. (2006) reportated that using wheat grains fully covered with mycelium of the pathogen is simple, less time consuming, highly effective and much less inoculum requiring compared with other techniques. It gives consistent and reproducible results.

Amule *et al*. (2014) conducted an experiment to find out the most effective screening techniques for identifying host plant resistance against chickpea collar rot caused by *Sclerotim rolfsii* in pot house. Out of four techniques employed, chickpea grain inoculation techniques was found best

2.2.9. Morphological and cultural characters of *Sclerotium rolfsii*.

Subramanian (1971) stated typical character of *S. rolfsii* Sacc. Mycelium very floccose, not ropy, producing numerous sclerotia. Sclerotia pinkish buff to olive brown to clove brown, globose, 0.8 - 2.5 mm in diameter. *P. rolfsii* formed

hymenium aerolate, putty coloured, 30 – 40 µ thick. Basidia obovoid, 7-9 x 4-5 µ each bearing 2 or 4 paralled or divergent sterigmata, 2.5 x 4-6 µ long. Basidiospores elliptical to obovate, hyaline, smooth, rounded or apiculate at base, 6-7 x 3.5-5 µ.

According to Mordue (1974) the mycelium is very floccose, snow white, thick, cottony and grown rapidly all over the blotting paper during blotter tests while sclerotia are the resting infective propagules of the fungus and can be seen on the mycelial strands. They are globose to ellipsoid, pinkish, buff to olive brown to clove brown. Young sclerotia appear white, producing characteristic exudate droplets around them, which were slowly become dark as age and resemble mustard seeds, 1-2 mm in diameter when mature.

The classification of *S. rolfsii* were given on the basis of sclerotia formation types, their fine structure, mycelial compatibility grouping and divided into A, R and middle type on the basis of colony morphology. Of the total 50 isolates, 16 were A types, 7 were R type and 27 isolates were middle type (Chen *et al.,* 1998). They further stated that mature sclerotia are composed of an outer melanized rind 2 to 4 cell layers thick, an underlying cortical layer and an innermost medullary region comprising loosely interwoven hyphae. Hernandez and Ysla (1997) evaluated cultural and morphological characteristics in eight isolates of *S. rolfsii* and found variability in their mycelial growth rate, number and diameter of the sclerotia, mycelial density, the presence of rhizomorphs and duration of sclerotial formation. Variability of *S. rolfsii* in their growth rate, mycelial colour and sclerotial production was also classified by Radwan *et al.* (1987).

Ansari and Agnihotri (2000) were grouped 44 isolates of *S. rolfsii* obtained from different locations in India and identified on the basis of morphological characters of the sclerotia and their arrangement on semi-synthetic medium. They found that there was positive correlation between the oxalic acid production and the virulence of the isolate. Sarma *et al.* (2002) studied variability in 26 Indian isolates of *Sclerotium rolfsii* and reported that, the isolates varied in colony morphology, mycelial growth rate, sclerotial formation, teleomorph production and

sclerotial size and colour. They also saw the mycelial incompatibility among the isolates.

Akram et al. (2008) observed variability among the 12 isolates of *S. rolfsii* was determined on the basis of their sensitivity to different fungicides. Mycelial incompatibility among the isolates was also studied and out of 66 combinations, only 26 combinations (39%) showed compatible reactions. Based on mycelial compatibility, 39% vegetative compatibility groups (VCG) were identified among the isolates.

Bankar *et al.* (2017) studied on morphological and cultural variability of *S. rolfsii.* Significant variability with reference to mycelia and sclerotial characters was observed on different media. This investigation revealed that the maximum mycelial growth was observed in Potato Dextrose Agar (9 cm) and more test weight (262 mg) of sclerotial bodies was recorded in Sabourauds dextrose agar. Cultural studies showed the maximum dry mycelial weight of fungus in potato dextrose broth (750 mg) followed by oat meal broth (663 mg).

Mahato and Biswas (2017) reported that ten isolates of *S. rolfsii* expressed significant differences with respect to cultural, morphological, and pathogenic characters. In pathogenicity test, all the isolates showed their pathogenic temperament to tomato plant where as in host range study, all test crop species showed susceptible except wheat (*Triticum vulgare*) showed some extent of resistance. Three isolates were found to be very fast growing (diam. > 9 cm), three were fast growing (8-8.9 cm), three moderately fast growing (5-6 cm) and one was observed slow growing (< 3 cm). Four isolates were white, one each of extra white and cottony white, two isolates were light white and another two were dull white. The colour of sclerotia ranged from brown to dark brown, shape ranged from spherical to oval and also irregular, sclerotial weight ranged from 3.7 to 8.6 mg. Six isolates were observed scattered, two peripheral and two were reported be central in Sclerotia arrangement. Sclerotia took a range of 9 to 15 days after inoculation for maturity. A range of 154.0 to 395.0 sclerotia production per plate was reported from the tested isolates.

2.3. Soil factors affecting development of collar rot disease in lentil”

"Nene (1979) observed factor affecting dry root rot in chickpea and founded that vertisols seem to favour the disease more than alfisols". The "alfisol extract medium supports less sclerotia production than vertisol extract medium. The dry root rot is observed more in vertisol at ICRISAT center in both pigeonpea and chickpea.

Sugha *et al.*, 1994 investigated that the soil characteristic soil pH in relation to fusarium wilt in chickpea, found that the maximum wilt occurred at pH 5.2, with slight decline toward neutrality and significant reduction of disease with increasing soil alkinity."

Krishna and Krishnappa (1996) reported *Fusarium oxysporum* f.sp. *ciceri* spore was high in vertisol (1300 CFU/g soil) when compared to alfisol (1153 CFU/g soil). The fungal population was high in vertisol which was significantly influenced by soil type with a higher severity in vertisol when compared to alfisol. Total mortality was observed on 20-24 days after sowing in vertisol while the wilt incidence was only 73.4% in alfisol."

"Mathur and Sinha (1968) studied the disease development in gaur and gram attacked with *Sclerotium rolfsii*, observed that the infection in guar was maximum at pH 6.6 (54.2%) and in gram at 5.7 (89.6%). Alkaline condition reduced the disease in both the crops."

Chattopadhyay and Mustafee (1977) reported that *Sclerotium. Rolfsii* from jute, showed considerable increase in population at all pH levels tested, however, growth was good at pH 5 and 6."

Kulkarni V.R. (2007) tested 9 soil pH levels for saprophytic activity of *Sclerotium rolfsii* Sacc. and reported that, the "fungus showed moderate to good growth over a pH range of 5.5 to 9.5. However, maximum fungal colonization of sorghum seeds was recorded at pH 6, where the saprophytic activity of the fungus was 76.67 per cent, which was on par with 6.5, 5.5, 8.0 and 9.0 pH (70, 60, 60 and 56.67%, respectively). Lower saprophytic activity of 46.67 per cent was observed at pH of 9.5".

Banyal *et al* .(2008) explain the "role of different soil parameters *viz.*, texture, pH, moisture, nutrients (available nitrogen, phosphorous, potassium and

organic carbon) and population dynamics of S. *roltsii* in the development of collar rot of tomato. Lighter soils were more favourable to the disease than the heavy textured soils. The maximum disease incidence was obtained at a pH 6.5. Low moisture favoured the disease development as compared to high moisture. The phosphorous and potassium had negative correlation, whereas nitrogen, soil pH, per cent organic carbon and pathogen population showed positive correlation. Nitrogen enhanced the disease whereas phosphorous and potassium decreased it."

Sundar *et al.* (2009) tested effect of different soil type on fungicidal control of *Rhizoctonia solani* causing seedling mortality in mungbean. Three soil of different textural group viz. sandy, loam and silt clay were used. The result revealed that disease incidence was more in silt clay soil and loam soil than sandy soil".

Sahu *et al* (2015) studied the effect of different soil type on collar rot incidence. Four different soil type found in Chhattisgarh state viz. clay loam, sandy loam, gravely and clay on collar rot incidence were taken for study. Significant difference in mortality percent was observed among treatments of soil type. Clay loam was found to be most favorable in disease development with highest mortality (77.50 %), followed by sandy loam soil with 70 per cent mortality. However least mortality was observed in gravely soil with 47.50 %.

Billah *et al*.(2017) studied on the pathogenic effect of *Sclerotium rolfsii* on different crops and revealed that "*Sclerotium rolfsii* can attack any parts of a plant that touch the soil, but it most commonly attacks a plant at or just below the soil line. The fungus produces white fungal strands (mycelia or hyphae) around infected plant parts and can be observed on the soil surrounding the plant. The pathogens of sclerotial diseases cause damping-off of seedlings, stem canker, crown blight, root, crown, bulb, tuber and fruit rots. The mycelia of S. rolfsii spread rapidly and can remain active in soil for long period as sclerotia. Losses due to affect of this pathogen can vary considerably depending on theenvironmental conditions, crop type and soil conditions."

Mahato *et al.* (2017) "revealed that the available quantity of different soil edaphic components *i.e.,* Nitrogen (N), Phosphorous (P), Potassium (K), Organic carbon (OC), soil pH and No. of Sclerotia / gm of soil were ranged from 185.0 -

488.0 kg/ha, 17.0-63 kg/ ha,122.0-446.0 kg/ ha, 0.33-0.98%, 5.1-6.6 and 0.2-1.2 respectively and disease incidence was ranged from 7.36% to 21.06% in red and lateritic zone of West Bengal. Disease incidence showed significantly positive correlation with available soil Nitrogen, Organic carbon, Soil pH and Sclerotia population of soil but negatively correlated with available soil Potassium (K) and Phosphorous (P). Sandy clay loam soil, 6.5-7.0 pH level, and 15% moisture level of soil found highly favorable for collar rot disease.

2.4. Collection and characterization of native isolates of *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp.

2.4.1. Isolation and Identification of *Trichoderma spp*.

Kim *et al.* (1992) worked on isolation and identification of species of *Trichoderma* antagonistic behavior to soil dwelling pathogens and its activities in the rhizosphere.

Singh and Majumdar (1995) categorized 27 isolates of soil fungus into 10 groups on the basis of colony characters on PDA, including presence of aerial mycelium, sporulation, ring formation and pigmentation. The isolates were also grouped as either medium growth, fast or very fast on their growth.

Choudhary and Sen (1999) studied five isolates of *Trichoderma* (T_2, T_5, T_8 and T_{10}) with their morphological features using light microscopy, cultural characteristics in four semi-solid media and scanning electron microscopy (SEM). The results obtained had sufficient diagnostic features to infer that all the isolates selected were biotypes of *Trichoderma*.

Kucuk and Kvanc (2003) isolated *Trichoderma harzianum* T_9, T_{10}, T_{15} and T_{19} isolates from 31 different soil samples and determined the biological control and antifungal effects of these isolates against various plant pathogenic fungi. Among these isolates, *T. harzianum* T_{19} showed a wide range of inhibitory effects on plant pathogens.

Karkare *et al.* (2007) isolated *Trichoderma* from rhizosphere of sesame plant and on the basis of its morphological characters, isolated species of *Trichoderma* to be *Trichoderma harzianum*.

Vishwanath *et al.* (2008) isolated 17 *Trichoderma* spp. from 64 soil samples collected from rhizoplane of infected acid lime trees. Among the 17

isolates of *Trichoderma* spp. isolate TCT4 effectively inhibited the test pathogen *Fusarium solani* both in dual culture and volatile compounds method".

2.4.2. Biochemical characterization of fluorescent *Pseudomonas and bacillus* isolates "collected from different locations of Chhattisgarh"

King *et al.* (1954) developed Pseudomonas Agar P (aka King A media) for enhancing pyocyanin and pyorubin production and Pseudomonas Agar F (aka King B media) for enhancing fluorescein production for easiness of identification of pyocyanin, "the blue-green water- and chloroform-soluble pigment produced by typical strains", "*P. aeruginosa* is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odor *in vitro*. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of pyocyanin and fluorescein, as well as its ability to grow at 42°C" where as *P fluorescens* produce only fluorescein pigments.

Collins (1955) tested rapid nitrate to be "useful in distinguishing *P. aeruginosa* (positive after addition of Zn powder) from *P. fluorescens* (positive before addition of Zn powder) and *P. putida* (both negative). A shortened gelatin test differentiated *P. fluorescens* (positive) from *P. putida* (negative). *P. fluorescens* and *P. putida* were very sensitive to low levels of kanamycin and resistant to carbenicillin, a pattern just the opposite of that obtained with *P. aeruginosa*."

"Lelliott *et al.* (1966) described methods for testing each fluorescent Pseudomonad for characterization through eleven tests: production of phenazine, gelatinase, catalase and arginine dihydrolase, use of D-trehalose, L-tryptophan and citrate, reduction of nitrate, egg yolk reaction and growth at 41°C and 4°C. The use of D-trehalose and L-tryptophan was tested by the method described by (Lemanceau *et al*., 1995) and the use of citrate, by the method described by"Simmons (1926)".

Donna *et, al. (*1972) tested that a rapid nitrate test "was found to be useful in distinguishing *P. aeruginosa* (positive) from *P. fluorescens* and *P. putida* (both negative). A shortened gelatin test differentiated *P. fluorescens* (positive) from *P. putida* (negative").

Blazevic *et al*. (1973) reported of diagnostic tests for differentiation of *P. fluorescens* and *P. putida* isolates. *P. aeruginosa* only fluorescent Pseudomonads that can grow at 42○C whereas *P. fluorescens* grow at 4°C. In the present investigation not any isolates showed growth at 4°C and 42°C and further suggested that the shortened test for nitrate reduction, together with the marked sensitivity to kanamycin and resistance to carbenicillin would provide a rapid means of accurately identifying *P. fluorescens* and *P. putida* and separating them from P. aeruginosa. However, rare nitrate-negative *P. aeruginosa* or rare nitrate-positive *P. fluorescens* or *P. putida* should not be misidentified using both of these characteristics. Growth at 41°C and 4°C was tested according to (Stanier *et al.*, 1966).

Alberto Pichardo Reyes *et al* (1981) studied *Pseudomonas aeruginosa* is the only gram-negative bacteria capable of producing the very distinctive water-soluble pigment pyocyanin. A retrospective and prospective analysis was performed with a total of 835 strains of *P. aeruginosa*; 818 (98%) produced pigment within 48 h of incubation, and 96% of those which produced pigment were positive after overnight incubation. Seventeen strains (2.0%) failed to produce pigment; 15 were mucoid strains from patients with cystic fibrosis. They evaluated the reliability of this characteristic as a unique test for the identification of this organism by using Tech agar (BBL Microbiology Systems, Cockeysville, Md.) medium. "Tech agar is an effective, simple, and inexpensive medium for *P. aeruginosa* identification and may be used as a unique test for all potential *P. aeruginosa* isolates (beta hemolytic on blood agar; lactosenegative, oxidase-positive colonies). Nonpigmented mucoid strains, as well as other nonpigmented organisms, will require additional testing to ensure proper identification".

Sheath *et al.* (1981) reported that most of the *Pseudomonas* spp. belongs to *P. fluorescens* and *P. putida* complex. There was no clear distinction between *P. fluorescens* and *P. putida*.

Krieg and Holt (1984) observed that most of the tests conducted for identification of fluorescent Pseudomonads have been based on physiological and nutritional tests.

Paleroni (1984) reported that the pure culture of fluorescent *Pseudomonas* species on king's B medium can be identified using ultraviolet light illumination by its fluorescence. and its rapid identification of potentially and economically viable bioagents is possible through various methods of biochemical characterization (Weller *et al.*, 2002; Ongena *et al.*, 1999; Zehnder *et al.* 2000; Singh *et al.*, 2000), coupled with determination of mole percent G+C of the DNA (Murmur and Doty., 1962).

Gilardi (1991) observed that *P. fluorescens* produced pyoverdines and *Pseudomonas aeruginosa* produced pyocyanin. Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, non fluorescent, phenazine pigment in addition to their colonial morphology and the characteristic grapelike odor of aminoacetophenone. Pyocyaninis are toxic to a wide range of fungi and bacteria (Hassan and Fridovich 1980).

Hildebrand *et al.* (1992) reported no clear distinction between *P. fluorescens* and *P. putida* on the other hand; these two species are identified based on trehalose utilization and gelatine liquefaction. In this, *P. fluorescens* showed a positive reaction for both the tests whereas, *P. putida* showed negative reaction. No references

Koomen and Jeffries (1993) identified as *Pseudomonas* spp. based on Gram negative reaction and fluorescent pigment production. All the fluorescent bacterial antagonists were Gram negative, rod shaped and all produced blue green pigment on King's B medium (Noori and Saud, 2012).

Bossis *et al.* (2000) observed that fluorescent Pseudomonads include all *Pseudomonas* species with the ability to produce fluorescent pyoverdine siderophore (s), noticeably *P. aeruginosa, P. syringae, P. putida* and *P. fluorescens*. *Pseudomonas fluorescens* is one of the fluorescent Pseudomonads that secrete pyoverdins (Meyer, 2000) for its essential requirement for iron. Pyoverdine is a yellow-greenish fluorescent siderophore involved in high affinity transport of iron into the cell (Budzikiewicz, 1992).

Kieboom and Bont (2001) were observed that several strains within the family Pseudomonadaceae such as *P. putida* S12 show significant intrinsic resistance to multiple antibiotics.

Angayarkanni *et al.* (2005) reported that *P. fluorescens* can dissolve solid gelatin into a liquid form in room temperature with the presence of an enzyme known as PPT1a, PPT1d, PPT2a and PPT3b, were found to be positive for gelatin hydrolysis.

Jagadish (2006) reported that *Pseudomonas* Sp. B-25 strain produced nonpigmented and non fluorescent colonies on nutrient agar and King's B agar medium and also suggested other medium for *Pseudomonas spp. viz.,* SIM agar, Urea broth, Tributyrin agar medium.

Li *et al.* (2009) reported that *fluorescent Pseudomonas* strains were gram negative, obligate aerobes, arginine dihydrolase positive, and oxidase positive. Also, the fluorescent strains were positive for the production of levan from sucrose.

Li *et al*. (2009) tested of YB128 strain as *Bacillus licheniformis* indicated Simmons Citrate-positive, V.P-positive, contact enzyme-positive, M.R positive, starch and gelatin hydrolysis.

Belkar and Gade (2012) reported fifteen isolates of *Pseudomonas fluorescens* were isolated from rhizospheric soil of different field crop collected. All the cultural and biochemical studies confirmed them to be *P. fluorescens.* The isolates showed positive response for siderophore production and phosphate solubilization, while negative for IAA and HCN production.

Pankaj Kumar *et al*. (2012) identified seven isolates of *Bacillus* spp. based on morphological, physiological and biochemical characteristics. All seven isolates showed Gram-positive, rod shaped, endospore forming with white, dry and fold, opaque and irregular edged colonies on Nutrient Agar Medium plates and found positive for catalase, oxidase, glucose and almost all were negative for H2S production, methyl red, glucosamine and sorbose.

Agrawal *et al.* (2014) reported that out of the 24 *P. putida*, 9 isolates (37.5 %) showed proteolytic activity (casein hydrolysis) by inducing clear zones around the cells on skim milk agar medium, 7 isolates (29.17 %) showed lipolytic activity, 14 isolates (58.33 %) were negative for nitrate test and 10 isolates (41.67) gave positive result for nitrate test (of which 6 isolates P23, P43, P59, P80, P144 and P174 were positive before addition of zinc and 4 isolates P187, P191, P192 and

P207 showed positive response after addition of zinc). Only three isolates P56, P130 and P191 were positive for phenylalanine test as indicated by appearance of green color after addition of few drops of 10 % aq. ferric chloride to the cultures grown in phenyl alanine amended medium. Lecithinase production was observed as opaque precipitate around colonies of four *P. putida* isolates viz. P59, P80, P123 and P166 resulting in lecithin positive result in egg yolk medium. None of the isolates showed growth at 4°C and 42°C. the isolates P2, P3, P45, P144, P192 and P207 liquefied gelatin. All the isolates were resistant to antibiotic carbenicillin and sensitive to kanamycin.

2.4.3. **Identification of bio agent**

Microorganisms that can grow in the rhizosphere are ideal to use as biocontrol against fungal pathogen since the rhizosphere provides the front line defense for roots against the pathogen. Pathogens encounter the antagonism from rhizosphere micro-organism before and during primary infection and also during secondary spread on the root. The review on the evaluation of biocontrol agents viz., *Trichoderma harzianum, Trichoderma viride, and Pseudomonas fluorescens* quoted below.

T. harzianum and T. viride were found effective in reducing the mycelial growth of *R. bataticola* (Deshmukh and Raut, 1992; Anju and Verma, 2007; Mandhare and Suryawansi, 2008). *T. harzianum* found most effective in inhibiting the mycelial growth of *R. bataticola* (Haque *et al.*, 1990; Pant and Mukhopadhyay, 2001; Ammajamma *et al*., 2009). Dinakaran *et al*. (1995) observed the effect of three biocontrol agents against root rot of sesame caused by *M. phaseolina*. Seed treatment with *T. viride, T. harzianum* and *Bacillus subtilis* reduced levels of root rot incidence. Sankar and Jeyarajan (1995) reported that *T. harzianum* and *T. viride* significantly reduced *M. phaseolina* root rot incidence in sesame to 10.1 and 12.8 per cent, respectively, compared with 60 per cent incidence in the control plots. Carbendazim treatment did not increase the rhizosphere and soil populations of the antagonists. The soil population of *Trichoderma* spp. was maximum in plots which were applied with *Trichoderma* spp. Seed treatment with *T. harzianum* significantly increased root length, shoot length, yield and oil content.

Raguchander *et al*. (1998) reported that *T. viride* was effective in inhibiting the mycelial growth of *R. bataticola* by greatly reducing the sclerotial number.

Jayashree *et al*. (2000) noticed that *P. fluorescens* strain pf1 effectively inhibited the mycelial growth of *M. phaseolina*, the pathogen causing dry root rot in black gram cv. Co 5 and sesame cv. Tmv 6. Application of pf1 as seed treatment (10 g/kg seed) followed by soil application (2.5 kg/ha) against root rot effectively supported higher plant growth, better native rhizobium nodulation and grain yield. Sclerotial number and root rot incidence were also greatly reduced.

Kumar *et al.* (2002) reported that plant growth-promoting rhizobacterial strains belonging to fluorescent *Pseudomonads* were isolated from the rhizosphere of rice and sugarcane. Among fourty strains that were confirmed as *Pseudomonas fluorescens*, eighteen exhibited strong antifungal activity against *R.bataticola*, mainly through the production of antifungal metabolites.

Sindhan *et al*. (2002) found that *T. harzianum, T. viride* and *P. fluorescens* inhibited the mycelial growth and sclerotial production in *R.bataticola*. Kaswate *et al.* (2003) evaluated the antagonistic effect of *Trichoderma viride* and *Pseudomonas fluorescens* against isolates of *R. bataticola* from pigeon pea, chickpea, green gram, cluster bean, field pea, cotton, okra and safflower. Plates inoculated with *R. bataticola* and each of the biological control agents were work out for the inhibition of mycelial growth. *T. viride* was the most effective in the inhibition of the various isolates of *R. bataticola* (100% inhibition) and *P. fluorescens* (73.98-78.94%).

Rajpurohit (2004) reported that seed treatment with *T. viride* at the rate 4 g/kg was highly effective against *M. phaseolina* and reduced the disease from 28.7 to 12.5 per cent and increased seed yield from 399 to 490 kg/ha.

Karthikeyan *et al*. (2006) evaluated three isolates of *T. viride*, and one isolate in each of T. *harzianum* and *P. fluorescensin vitro*, to assess their antagonistic potential against *M. phaseolina* causing root rot of groundnut. *T. viride* and *T. harzianum* were the most effective in reducing the mycelial growth and sclerotial formation of *M. phaseolina* culture filtrates of *T. viride* inhibited the growth of the pathogen as well as sclerotial germination to a greater extent.

Nair *et al*. (2006) conducted a pot experiment to determine the effects of biological control agents (*T. harzianum, T. viride* and *B. subtilis*) on *R. bataticola* (*M. phaseolina*) infecting sesame cv. var-1 and found that seeds treated with spore suspension of *T. harzianum* were superior over all the other treatments. The germination percentage was maximum (92%) in case of *T. harzianum* followed by *T. viride* (89.33%).

Durai (2004) made efforts to manage charcoal rot of sesame caused by *M. phaseolina*. The most effective treatment was the combination of mancozeb (100 ppm) + soil application of bioagents *viz*., native isolate of *T. viride* (10 g/kg of soil) and *P. fluorescens* (20 ml/kg of soil). The treatment significantly reduced disease incidence (10.67%) and enhanced plant growth components.

Singh *et al.* (2005) evaluated fungal antagonists against *M. phaseolina* causing root rot of mungbean. Out of four bioagents (*Aspergillus sp,Gliocladium virens, T. harzianum and T. viride*) tested against the root rot pathogen. *T. harzianum* proved most effective, which inhibited maximum mycelial growth (71.85%). Highest disease control 66.67% was recorded by *T. harzianum* followed by *G. virens* 50.00% incidence under green house conditions.

Lokesha and Benagi (2007) evaluated the efficacy of biocontrol agents *in vitro* and *in vivo* on *M. phaseolina* which causes dry root rot of pigeonpea. The results revealed that the both bioagents; *Trichoderma virens* and *Pseudomonas fluorescens* significantly inhibited the mycelial growth of *M. phaseolina* by 78.22 per cent and 76.66 per cent respectively. Talc based formulations of these bioagents were applied, seed treatment (4g/kg seed) along with soil application (2 g talc powder mixed with farm yard manure/pot) supported the maximum plant stand and less root rot incidence compared to other treatment and found significantly superior to chemical seed treatment (2g/kg seed). Seed treatment + soil application with *T. virens* recorded less root rot incidence (2.89%) among the four bioagents used.

Aly (2007)an *in vivo* experiment was conductedto determine the efficacy of *Trichoderma* spp. against *M. phaseolina*. Greenhouse evolution of the interaction between *M. phaseolina* isolates and *Trichoderma* spp. isolates revealed a very significantly effective result. *Trichoderma* play a role as source of variation in

survival, plant height, and dry weight of plant, while *M. phaseolina as source* of variation in pre-emergence damping-off and post-emergence damping-off of plant.

Ramezani (2008) evaluated the efficacy of four fungal bioagents viz., *T. hamatum, T. harzianum, T. polysporum* and *T. viride* under *in vitro* condition against the eggplant root - rot caused by *M. phaseolina*. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum*. Soil application of talc - based formulation of *T. harzianum, T. polysporum* and *T. viride* effectively controlled the root- rot of egg-plant under field condition.

Usha *et al*. (2009) noticed that among the antagonists assay that, *T. viride, T. harzianum* and *B. subtilis* were found inhibitory to *M. phaseolina*. Further, the root rot incidence was low (14.1 per cent) with *T. viride* (seed treatment + soil application) followed by *T. viride* and *T. harzianum* (seed treatment).

Manjunatha and Naik (2010) evaluated several isolates of two bio-control agents, *T. viride* and *P. fluorescens.* Bio-control agents were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions and subsequently used for field studies. The most effective isolate of each bio-control agent and the common chemical for seed treatments were too used as experimental trail. It was found that trial combining with soil application through bioagent enriched farm-yard manure, along with seed treated with the bio-control agent showed maximum germination, least root rot incidence and highest yield, as compared to the other biological or chemical seed treatments included.

Govindappa *et al.* (2010) isolated three biocontrol agents such as *T. harizianum, P. fluorescens* and *Bacillus subtilis* from safflower rhizosphere soil and tested individually for their effectiveness in controlling root-rot of safflower. Talc based formulations were prepared and treated the seeds at different concentrations, for assessing their ability to induce plant growth and in turn control root rot disease. Among bioagents, *P. fluorescens* and *T. harzianum* (10 g/kg) proved to be effective in controlling disease under laboratory, greenhouse and field conditions. The efficacy of these biocontrol agents are equivalent to the standard fungicide Bavistin (carbendazim). Seed treatment with these biocontrol agents enhanced the seed germination and growth parameters against root-rot disease.

Jyothirmai *et al*. (2011) evaluated the plant growth promoting microorganisms viz., *Trichoderma* spp. and fluorescent pseudomonads which was isolated from the rhizospshere of groundnut, exhibited the suppression of *R. bataticola*. *Trichoderma* spp. and fluorescens *Pseudomonas* isolate 1 effectively restricted the radial growth of *R. bataticola*by 69.92 % and 67.31 % respectively. In green house studies, talc based formulations of potential *Trichoderma* spp. and fluorescens *Pseudomonas* isolate 1 effectively suppressed the seedling disease and improved the plant growth, shoot and root dry weight and pod yield.

Patel *et al*. (2011) tested *in vitro* antagonistic acitivity of *P. fluorescens* via dual culture which exhibited significance reduction in the radial growth of *R. bataticola* over control (90 mm colony diameter) and in the field trial study seed treatment with four strains significantly reduced the disease incidence of dry root rot in all treatments in comparison to uninoculated control, however *P. fluorescence* strains BHUPf4 was found more effective in mycelial growth reduction as well as in disease reduction followed by strains BHUP5, BHUP6 and BHUPsb.

Govindappa *et al*. (2011) screened thirty eight isolates of *Pseudomonas fluorescens* for biological control of *M.phaseolina* root-rot of safflower. These results indicated that the seedling assay is more important for selection of promising biocontrol agents besides they also increased the plant growth by controlling the disease as evidenced by induction of defense enzymes.

2.5. Management of collar rot of lentil.

The management of lentil collar rot can be done through cultural practices, use of resistant varieties, biological control and chemical protection. In the absence of resistant/tolerant varieties, it would be too difficult to manage the disease caused by soil-borne pathogens because of complex soil physico-chemical properties, environmental conditions and biological origin. The following sub-sections deal with possible management options of lentil collar rot.

2.5.1. Management throught cultural Practices

Alternation in date of sowing has great impact on the occurrence of the plant diseases. Thakur and Khare (1990) reported that *C. dematium* and *C. lindemuthianum* late sowing (13^{th} July) were higher in early sowing (23^{rd} June to

3rd July) than. They further stated that cv. J-45 showed less incidence than Pusa Baishakhi and K-851.

Subramanyam (1979) observed that the sowing date of the crop has to be so adjusted that the best temperature and moisture conditions may prevail for the rapid growth of the host to escape the critical period of disease incidence. The disease incidence was high when the sowing of the crop was further delayed by August 27th but the decline in percentage of disease intensity was gradual.

Verma *et al.* (2009) evaluated the effect of 4 different dates of sowing using 4 different varieties on the incidence of pod blight of soybean and reported least disease incidence in crop sown on 30th June, in variety JS 80-21

The cultural practices include deep plowing and leaving the soil fallow. These practices helped in reducing the pathogen population in the soil but do not eliminate it completely (Agrios, 2005). Also, management practices to reduce the effects of waterlogged soil include genotype choice and the proper design of field drainage systems to discharge excess water (Silvia *et al*., 2016). Selecting cultivars that mature early and adjusting the planting date, if possible, can reduce disease incidence by escaping a portion of lentil growth period from weather conditions favorable to the disease. Use of clean seed for sowing and/or the use of fungicidal seed treatments can eliminate or reduce contaminating inoculum sources. Lentil crops grown on raised beds produced significantly superior agronomic characteristic; yield attributes trials, seed and straw yield as compared to the flat bed sown crop (Rathore *et al.,* 2010). Merkuz and Getachew (2012a) reported that growing resistant and moderately resistant varieties on raised seedbed that drain excess water with recommended seeding rate could reduce plant mortality caused by chickpea wilt.

Changes of micro-environment are complex and often interrelated because they affect both host and root pathogens. Some factors may affect the lentil plant negatively and the fungus positively, leading to an apparent increase in lentil wilt. Under the traditional management systems, lentil yield from Vertisols is far below the potential yield (Berhanu, 1985). Feng *et al.* (2010) also demonstrated significant effects of the ridge-furrow system on Siberian wild rye (*Elymus sibiricus*). Field observation showed that *E. sibiricus* plants in the ridge-furrow

system grew more rapidly and robustly than that in flat bed. Salih and Ageeb (1987) showed that sowing date and plant population significantly affected the incidence of wilt and root rot complex.

2.5.2. . *Trichoderma spp.* as a biocontrol

2.5.2.1. *Trichoderma*: an Introduction

More than two hundred years ago, when it was first described by Persoon in 1794**,** mycologists mistook *Trichoderma* Pers. Fr. for a gasteromycetes. The true nature of this fungus was realized only half a century later. Although, *Trichoderma* has not yet given us any "Wonder drugs" like penicillin, but the ability of some species to produce enzymes and/or attack or inhibit other fungi causing diseases in plants has attracted major research efforts in several areas specially biological control of plant disease. At present *Trichoderma* is a readily exploitable source and how this source can be fully utilized for economic gains depends on clear understanding of the systematics and mechanisms of action of this fungus (Samuels, 1996).

Genus *Trichoderma* has traditionally been classified as fungi imperfecti. They produce only asexual spores i.e., conidia (Singh *et al*., 2006). With the recent advances in molecular taxonomy, many of the species have been reclassified as belonging to the genus Hypocrea of the class ascomycetes (Gams and Bisset, 1998; Druzhinina *et al*., 2005). *Trichoderma* spp. are characterized by rapid growth rate on agar (solid medium), generally producing green conidia in abundance, conidiophores (structures that bear conidia) erect or straggling, highly ramified, more or less conical, weakly or strongly verticillate, bearing divergent flask-shaped phialides singly or in clusters, from which subglobose to ellipsoidal, slimy, non-septate phialospores (conidia) are borne, often gathering in balls at the openings of the phialides. Chlamydospores thick-walled, transformed hyphal cells, considered to be the survival structures) are commonly formed. They are generally intercalary or rarely terminal, globose to ellipsoidal, hyaline and smooth walled (Cook and Baker, 1983). *Trichoderma* spp. are very common saprophytic soil-fungi, capable of rapidly decomposing soil organic matter. Many species have the ability to parasitize other fungi and have the ability to colonize plant roots and rhizosphere (Singh *et al.,* 2006). *Trichoderma virens* (earlier classified as *Gliocladium virens*)

is a well-studied biocontrol agent with at least one commercial formulation (SOILGARD) available in the market (Howell, 2003). In addition to biocontrol properties, *Trichoderma* spp. also produce several hydrolyzing enzymes of industrial significance like cellulases, hemi-cellulases, xylanases and hence are important in the industry (Mach and Zeilinger, 2003). Some species of *Trichoderma* like *T. longibrachiatum* and *T. citrinoviride* are opportunistic human pathogens on immunocompromised patients and hence they are medically important (Kuhls *et al.*, 1999). In brief, *Trichoderma* spp. represent a group of fungi of immense economic importance in agriculture, industry and medicine.

2.5.2.1.1. *Trichoderma*: systematic

About 35 species of *Trichoderma* are currently recognized on the basis of morphological and molecular data. *Trichoderma* is a genus of filamentous deuteromycetes with only one known teleomorph, hypocrea Fr. The basic *Trichoderma* morphology has been described as: Colonies having rapid growth, abundant powdery green conidia and well defined conidiophores. The characteristics of colonies growing on agar media are subtle and it produces submered mycelium. Reverse of the colonies may be uncoloured or in some species buff, yellow, amber, reddish or yellow- green. Conidiation is effuse and chlamydospores are usually present. Conidiophores in most species are repeatedly branched in a regular dendritic manner. However, in spite of the tremendous economic importance of this fungus, the taxonomy of *Trichoderma* is still problematic with no well-defined concept of species.

Rifai (1969) made the first attempt at classification of *Trichoderma* species. Based on his studies, he released a comprehensive taxonomic monograph for this genus. The classification of *Trichoderma* given by Rifai relies primarily on morphological characters. For Rifai, the key to the species of *Trichoderma* lay in the link between *Trichoderma* and the sexually producing genus *Hypocrea* Fr. and many of his aggregate species were based on cultures from ascospores. He classified the different species of *Trichoderma* in 9 species aggregates, which he defined as "aggregations of morphologically very similar and often hardly separable species".

The 9 species aggregates defined by Rifai are:

1) *Trichoderma piluliferum*
2) *Trichoderma polysporum*
3) *Trichoderma hamatum.*
4) *Trichoderma koningii*
5) *Trichoderma aureoviride*
6) *Trichoderma harzianum*
7) *Trichoderma longibrachiatum*
8) *Trichoderma pseudokoningii*
9) *Trichoderma viride*

2.5.2.1.2.. Mechanism of biological control

A biocontrol agent is a microorganism that adversely affects another (the host target pathogen) growing in association with it. These biocontrol agents interfere with the life process of plant pathogens involving antibiosis, competition, mycoparasitism, predation or induced systemic resistance (Howell, 2003).

Even though several commercial formulations based on *Trichoderma* are available in the world market for use as biofungicides, their efficacy, in most cases, are not comparable with chemical pesticides. This is normal, as *Trichoderma* spp. are living entities, the activity and survival of which are dependent on biotic and abiotic environmental factors. This limitation has been overcome, to some extent, by combining *Trichoderma* spp. with chemical fungicides in the form of an integrated plant disease management (Mukhopadhyay *et al*., 1992). However, the growing demand for a ban on many chemical fungicides is likely to make the issue complicated, unless we have strains of *Trichoderma* with improved biocontrol potential, as well as survival under adverse environmental conditions. One of the most interesting aspects of the science of biocontrol is the study of the various mechanisms employed by the biocontrol agents to effect disease control (Howell, 2003). Identification of biocontrol activity was studied under dual culturing of 17 biological control agents (BCAs) in the presence of the phytopathogenic fungi *Phoma betae* (*Pleospora betae)*, *Rosellinia necatrix*, *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *dianthi* in three different media by Hermosa et al. (2000). Classically, three principal mechanisms of action of *Trichoderma* spp. have been recognized- mycoparasitism (parasitism of one fungus by another fungus),

antibiosis (production of antimicrobial metabolites and thus inhibiting other fungi) and the universal phenomenon of competition for food, space or oxygen (Chet, 1987).

2.5.2.1.2(a) Mycoparasitism

Mycoparasitism is a complex process involving tropic growth of the biocontrol agent towards the target organism, coiling and finally dissolution of the target organism's cell wall/cell membrane by the activity of enzymes (Weindling, 1932; Chet, 1987; Sharon *et al*., 2001). One of the most prominent quality of the genus *Trichoderma* is its ability to parasitize other fungi. Weindling in 1932 for the first time ascribed the biocontrol of *Rhizoctonia*. *solani* (causing citrus seedling disease) by *T. lignorum* to mycoparasitism. Weindling described in detail the mycoparasitism of *R. solani* hyphae by the hyphae of the biocontrol agent, including coiling around pathogen hyphae, penetration, and subsequent dissolution of the host cytoplasm. This phenomenon occurred regardless of the supply of external nutrients to the host or mycoparasite. Although he considered the possibility that under certain circumstances *T. lignorum* might act as a competitor for nutrients with *R. solani*, he much favored mycoparasitism as the principal mechanism for biocontrol. In a typical mycoparasitic interaction, the parasite (e.g., *Trichoderma*) receives the chemical stimulus released by the host (e.g., *Rhizoctoniasolani*) and gets chemotropically attracted towards the host. This is followed by coiling of the host hyphae, running adpressed to the host, production of appressoria-like structures, penetration of the host and derivation of nutrients, and finally, lysis of the host (Chet, 1987; Chet *et al*., 1998). The role of recognition in *Trichoderma*- fungal interactions was demonstrated by an experiment where nylon fibres were coated with lectins, and the coated lectins were coiled around by *T.harzianum*, but not the fibres that had not been coated (Inbar and Chet, 1992). This experiment underlined the possible role of signal interplay in this novel host-parasite interaction. The lysis of the host associated with the mycoparasitism is accomplished by the production of several hydrolytic enzymes mainly chitinases, glucanases and proteases (Chet *et al*., 1998).

Studies on the molecular and cellular aspects of the process of mycoparasitism indicate that it is an extremely complex process involving several

steps and numerous separate genes and gene products. *Trichoderma* can detect its host from a distance and on detection it starts branching in an atypical way towards the fungus. This process is probably induced by nutrient gradients arising from the host (Chet., 1987). Dennis and Webster, 1971a&b conducted experiments using plastic threads similar in diameter to *Pythium ultimum* hyphae and concluded that the coiling of *Trichoderma* is not merely a contact stimulus. *Trichoderma* hyphae were never observed to coil around plastic threads. Later studies done by Elad et al., 1983 indicated the role of lectins in the process of host recognition by *Trichoderma*. Elad *et al*., 1983, isolated a lectin from *R.solani* hyphae and culture filtrate which, they concluded, binds to the galactose residues in cell walls of *Trichoderma*. Extracts from *S. rolfsii* also exhibited agglutinin activity but its properties were different from that of *R. solani*. Inbar and Chet (1992) provided direct evidence for the role of lectins in mycoparasitism. They observed that *T.harzianum* coiled around nylon fibers, which had been treated with concanavalin A, a lectin, purified from *S. rolfsii*. Attachment of *Trichoderma* to host hyphae is followed by a series of degenerative events and the host cell wall structure is disrupted which promotes osmotic imbalances triggering cell disruption and killing the pathogen.

2.5.2.1.2(b) Antibiosis

This is the second major mechanism implicated in the biocontrol of pathogens by *Trichoderma*. Two years after reporting the involvement of mycoparasitism in *Trichoderma*- host fungus interactions, Weindling in 1934 reported that a strain of *T.lignorum* produced a "lethal principle" that was excreted into the surrounding medium. He characterized it and demonstrated that it was toxic to both *R.solani* and *Sclerotinia americana* and named it "gliotoxin". Later in 1983, Howell and Stipanovic isolated and described a new antibiotic "gliovirin", from *Gliocladium virens* that was strongly inhibitory to *Pythium ultimum* and *Phytophthora* but was ineffective against *R.solani, Theilaviopsis basicola, Rhizopus* arrhizus, *Bacillus thuringensis* and *Pseudomonas fluorescens*. Lifshitz et al., (1986) attributed the control of *Pythium* species on peas by *T.harzianum* to the production of an antibiotic. Similarly, suppressive activity of *T.virens* to damping off of Zinnias was correlated to production of antibiotic gliotoxin by the bioagent

(Lumsden *et al.,* 1992). Mutation studies with *Trichoderma* strains have revealed that mutants deficient for antibiotic production often lack the ability to control *Pythium* damping off disease (Wilhite et al., 1994).Wilhite *et al.,* (2001) cloned a 5-kb partial cDNA encoding a putative peptide synthase (Psy1). The disruption of psy1 indicated a role in siderophore production in *T. virens*. However, the disrupted strains exhibited normal biocontrol properties against *R. solani* and *P. ultimum*, indicating that the iron competition may not play important role in biocontrol in this system. Wiest *et al*., (2002) identified a 62.8 kb continuous open reading frame encoding a peptaibol synthetase from *T. virens*; the mutation of the gene eliminated the production of all the peptaibol isoforms, thus confirming that this gene is responsible for the synthesis of the peptaibols. A putative peptide synthetase gene has recently been identified in *T. harzianum* (Vizcaino *et al.,* 2005). At present *Trichoderma* species are reported to produce a number of antibiotics. These include gliotoxin, glioviridin, viridian and viridiol (Howell *et al.,* 1993).

2.5.2.1.2(c). Competition

If mycoparasitism and antibiosis are not the principal mechanisms in the biocontrol process, then what exactly it is? Competition is considered as a 'classical' mechanism of biological control and has gained adherents in recent years. This is through rhizosphere competence i.e., ability to colonize and survive on the root surface and adjoining soil zone under the influence of roots (Howell, 2003). It involves competition between antagonist and plant pathogen for space and nutrients (Chet, 1987). It is assumed that the mechanism of competition is involved in biocontrol, if no evidence for mycoparasitism or antibiosis is found in a particular *Trichoderma*-host fungus interaction (Alexander, 1982; Cook and Baker, 1983). Howell (2002) used ultraviolet light irradiation to produce mutants of *T. virens*, deficient for both mycoparsitism and antibiotic production. However, the mutants still retained biocontrol efficacy equal to that of the parent strain against both *P. ultimum* and *R. solani* causing cotton seedling disease. This indicated that neither mycoparsitism nor antibiosis is the principal mechanisms involved in the biocontrol of seedling disease in cotton.

Rhizosphere competence is important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. *Trichoderma* spp., either added to soil or applied as seed treatment, grow readily along with the developing root system of the treated plant (Zhang *et al*., 1996; Harman, 2000; Howell *et al.,* 2000). Although competition through rhizosphere competence may not be among the principal mechanisms that drive biocontrol, the replacement of the endogenous fungi might play important role in reducing the inoculum levels (Harman, 2001). Similarly, the rapid colonization of the dead/necrotic tissues by *Trichoderma*, and thus preventing further spread could help reducing build-up of further inoculum and spread of the pathogen as has been demonstrated by Mukherjee *et al*., (1995) in case of foliar application of *T. viride* against *Botrytis cinerea* in chickpea. The omnipresence of *Trichoderma* in agricultural and natural soils throughout the world proves that it must be an excellent competitor. In studies conducted by Elad and Kapad,(1999), he presented information regarding biocontrol of *B. cineria* by *T. harzianum* strain T-39. *B. cineria* conidia require external nutrients for germination and infection. When conidia of T-39 were applied to leaves, germination of conidia of the pathogen was slowed, an effect attributed in part to competition.

2.5.2.1.3. Evaluation of potential Trichoderma spp. isolates against S. rolfsii

Dennis and Webster (1971b) described isolation technique of resident *Trichoderma* isolates from the rhizosphere of healthy plants in the fields having high incidence of various diseases. Production of volatile metabolites by six resident *Trichoderma* isolates was evaluated by Inverted plate technique.

Henis and Chet (1975) reported that antagonists may act against pathogens in one or more of the following mechanisms, competition, antibiosis, parasitism, predation or induce resistance in plant; hydrolytic enzymes excreted by antagonists is a well known feature of mycoparasitism.

Agrawal et al. (1977) reported that *Trichoderma harzianum* was highly antagonistic to *Sclerotium rolfsii* causing collar rot of lentil. Filtrate of this organism also checked the growth of *Sclerotium rolfsii* on potato dextrose agar. *Trichoderma harzianum* could check the mortality of lentil caused by *Sclerotium*

rolfsii under pot condition. The culture of *Trichoderma* spp. was more effective when used with the seeds as compared to that used in soil.

Mathur and Sarbhoy (1978) tested five fungi *viz., Trichoderma viride, Trichoderma harzianum, Aspergillus flavus, Fusarium oxysporum* and *R. bataticola* known to have some antagonistic properties. *Trichoderma harzianum* and *Trichoderma viride* were showing antagonism against *Sclerotium rolfsii* under laboratory conditions.

Elad et al. (1980) reported that *T. harzianum* was found to be an effective biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* under both greenhouse and field conditions.

Mukhopadhyay (1987) reported that *Trichoderma* spp. has long been known as effective antagonist against plant pathogenic fungi. *Trichoderma* spp. are known to inhibit the growth of *Sclerotium rolfsii* on lentil.

Claydon et al. (1987) reported antifungal properties of volatile compounds (*Alkyl pyrones*) produced by *T. harzianum*. Species of *Trichoderma* have been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics.

Gaikwad and Kapgate (1990) reported that the spore suspension of *T.harzianum* and *P. pinophilum* prevented the germination of sclerotia of *S.rolfsii* under *in-vitro* conditions.

Mukhopadhyay *et al.* (1992) reported that combined application of biological agents and fungicides as seed treatment, first with *T. virens* and then with 0.1% carboxin was effective in controlling *Sclerotium rolfsii* in chickpea, lentil and groundnut.

Rathore *et al.* (1992) reported volatile activity of *T. viride* against *F. solani* which vacuolated most hyphae of the pathogen and that the hyphae of the pathogen were comparatively thin as compared to control.

Sugha *et al.* (1993) studied the conidial coating of the antagonists *Trichoderma viride, T. harzianum* on seeds and found the significant reduction in seedling mortality (47-65%) of chickpea as compared with the untreated control under *in-vitro* conditions.

Michrina *et al.* (1995) and Pandey and Uapadhyay (1997) reported the effectiveness of diffusible volatile compounds by *T. viride* and *T. harzianum in vitro*.

Virupaksha (1997) tested the antagonistic organisms against *Sclerotium rolfsii*. Among them, *Trichoderma harzianum* and *Trichoderma viride* were found to be effective in inhibiting the mycelium growth and reducing production of sclerotial bodies irrespective of inoculation periods. He also observed inhibition zone and reduction in size of sclerotial bodies in presence of antagonists.

Prasad *et al.* (1999) reported that isolates of *Trichoderma* and *Gliocladium* spp. inhibited mycelial growth (54.9 to 61.4%) and suppressed the sclerotial production (31.8 to 97.8%) of *Sclerotium rolfsii* under *in-vitro* conditions.

Arora (1999) found that, *T. harzianum* significantly inhibited the growth of *S. rolfsii*, the causal organism of root disease of lentil (*Lens esculenta*) on PDA medium.

Desai and Schlosser (1999) reported that *Trichoderma* species has ability to infect, macerate and kill the sclerotia of *S. rolfsii*.

Mondal (1999) tested 55 isolates of *T. harzianum*, isolate TF-24 showed 93% inhibition of mycelia growth of *S. rolfsii* on PDA.

Biswas and Sen (2000) studied the dual culture of 11 isolates of *T. harzianum*. Isolate *viz.*, T8, T10 and T12 were effective against *S. rolfsii* as they over grew the pathogen up to 92%, 85% and 79%, respectively *in vitro* conditions.

Das *et al.* (2000) evaluated *Trichoderma harzianum, T. viride* and *T. koningii in vitro* against *Sclerotium rolfsii,* causing collar rot of tomato. *Trichoderma harzianum* was the most effective in inhibiting the mycelial growth in dual culture.

Patel and Anahosur (2001) reported that *Trichoderma harzianum* showed mycoparasitic property by overgrowing, or ceasing the mycelial growth and reducing the sclerotial production of *S. rolfsii* under laboratory conditions.

Dutta and Das (2002) observed 61.5, 59.1 and 57.2 per cent inhibition in mycelial growth of *S. rolfsii* by *T. harzianum, T. viride* and *T. koningii,* respectively. They also found reduction in sclerotia production by all the antagonists.

Faruk *et al.* (2002) tested six isolates *of Trichoderma harzianum in vitro* and also *in vivo* against *Sclerotium rolfsii* causing cabbage seedling disease. They found the isolates of *T. harzianum* significantly reduced the radial colony growth of *S. rolfsii*.

Pranab *et al*. (2002) studied the efficacy of *Trichoderma harzianum, T. viride,* and *T. koningii* for the management of collar rot of tomato caused by *Sclerotium rolfsii* under *in vitro* condition. *T. harzianum* was the most inhibitory to *S. rolfsii,* showed 61.5% inhibition in mycelial growth of the pathogen, *T. harzianum* inhibited more than 90% sclerotial production.

Yogendra and Singh (2002) studied the effect of *Trichoderma* based biocontrol agents, *viz., T. viride* and *T. harzianum* on the growth of *Sclerotium rolfsii in vitro.* Growth inhibition increased with the increase *in* culture filtrate concentration. The maximum (75%) growth inhibition was observed at 50% concentration of culture filtrate of *T. viride*, whereas in case of *T. harzianum,* maximum growth inhibition was 64.44% after 96 hrs of incubation.

Faruk *et al.* (2002) tested the isolates of *Trichoderma* spp. as biocontrol agent against *Sclerotium rolfsii.* Four isolates of the antagonist significantly reduced the radial growth of *S. rolfsii* in dual culture on PDA. The *Trichoderma* reduced the post emergence mortality due to S. *rolfsii.*

Revathy and Muthusamy (2003) studied the antagonistic effect of *Trichoderma harzianum, T. hamatum and T. viride* on *Sclerotium rolfsii*. *T. viride* was the most effective in inhibiting the growth of *S. rolfsii* (55.8% inhibition over the control).

Prasad *et al. (*2003) tested the efficacy of isolates of *Trichoderma spp* in suppressing the growth *of Sclerotium rolfsii,* the cauliflower collar rot pathogen by dual culture method. They found that *T. harzianum* (44.1%) isolate was superior to *T. viride* (39.1%) isolate in reducing the colony diameter of *S. rolfsii.*

Kashem (2005) conducted experiments to determine the efficacy of *Trichoderma* in controlling foot and root rot and collar rot of lentil. He found that *Trichoderma harzianum* and *Trichoderma viride* as seed treatment, soil treatment, seed + soil treatment were effective in controlling collar rot of lentil.

Hannan (2005) studied integrated management of foot rot of lentil, chickpea and grasspea. He found that post-emergence death of lentil plants, chickpea and grasspea due to foot rot *(Fusarium oxysporum* and *Sclerotium rolfsii)* was reduced by treating seeds with BAU Biofungicide and BINA-fertilizer either alone or in combination.

Rudresh *et al.* (2005) conducted experiment with inhibitory effect of *Trichoderma* culture filtrate and non-volatiles on the growth of *S. rolfsii*.

Amin *et al.* (2010) tested six isolates of *Trichoderma viride*. for their ability to produce volatile metabolites against seven fungal plant pathogens *viz.*, *Fusarium oxysporum* (causing chilli wilt), *Rhizoctonia solani* (causing sheath blight of rice), *Sclerotium rolfsii* (causing collar rot of tomato), *Sclerotinia sclerotiorum* (causing web blight of beans), *Colletotrichum capsici* (causing anthracnose of chilli fruit), *Helminthosporium oryzae* (causing brown spot of rice) and *Alternaria brassicicola* (causing Alternaria blight of cabbage). Studies indicated that *T. viride* (*Tv*-1) was most effective in reducing the mycelial growth and sclerotia production.

Kashem *et al.* (2011) conducted experiment with 14 isolates of *Trichoderma* spp. (*Trichoderma harzianum* and *T. viride*) for control of foot and root rot of lentil (*Lens culinaris* Medik). The isolate TG-2 of *T. harzianum* showed the highest inhibition of the pathogen in field condition.

Rawat *et al.* (2012) sceened ten *Trichoderma* isolates for their antagonistic potential against two major soil borne plant pathogens *viz.*, *Sclerotium rolfsii* and *Fusarium oxysporum* causing root rot and wilt in lentil and chickpea, respectively. Under laboratory conditions, high antagonistic activity against both the test pathogens by all the *Trichoderma* isolates was observed in lentil and chickpea.

Bhuiyan *et al.* (2012) reported that total of 20 *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops were screened against *S. rolfsii* following dual plate culture technique. The screened isolates of *Trichoderma* significantly reduced the radial growth of *S. rolfsii*. The isolate TH-18 of *T. harzianum* showed the highest inhibition of radial growth of *S. rolfsii*.

Darvin *et al.* (2013) evaluated the effect of *Trichoderma* spp. on radial growth of *Sclerotium rolfsii*. The results from this experiment revealed that *T.*

viride (TvL), *T. harzianum* 4 (Th4) and *T. harzianum* 14 (Th14) isolates were found effective and showed lowest radial growth of 3.50 cm and highest per cent inhibition (56.25%) of *S. rolfsii.*.

2.5.2.1.4. Plant growth promoting activities of *Trichoderma* spp.

Zaidi and Singh (2004) found that *Trichoderma* are most widely used biocontrol agents since they are reported to have antifungal, antinematodes, plant growth promoting and plant defense inducing activities. However, the major limitation of these biocontrol agents is not only their relatively short shelf life but also inconsistent field performance. Zaidi and Singh (2004) reported multiplication of *Trichoderma* on cow dung .Therefore, the approach was to use easily available and relatively inexpensive substrates especially those used as organic compost like cow dung or FYM, Vermicompost and poultry manure for the multiplication and delivery of *T. harzianum.*

Khan *et al*. (2005) reported that *Trichoderma harzianum* was examined for its effects on emergence and vigour of rice seedlings through seed or soil treatments. All doses of *T. harzianum* in both the experiments significantly increased seedling emergence, root and shoot length, fresh and dry weight of root of rice seedlings, as compared to check. Maximum increase in seedling emergence (44.67%) was observed when bioagent was applied as soil treatment @ 8 gm/kg soil. There was similar trend of increase in root and shoot length, root and shoot weight from soil and seed treatments. Higher doses of the antagonist exhibited maximum increase in seed germination and seedling vigour.

Ha, (2010) conducted experiments on several crops such as: peanut, tomato, cucumber and durian concluded that selected *Trichoderma* strains could reduce significant diseases caused by fungal pathogens including: *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* and *Pythium* spp. The efficacy of *Trichoderma* species on soil borne fungal disease is higher than fungicides and maintain longer. The value obtained through development, exploitation and use of *Trichoderma* products are not only plant disease control but also gave the local people opportunities to reduce health risks, costs and environmental damage due to over fungicide usages. Moreover, crop treated with

Trichoderma grew better and had higher yields as compared to the one without application.

John *et al.* (2010) reported that *Trichoderma* showed growth promoting action on soybean. *Trichoderma* enhanced growth of shoot and root systems and fruit yield after 12 weeks of growth. *Pythium* and *Fusarium* infected plants treated with *Trichoderma* had ~194% and 141% more height than pathogens alone. The fruit yield treated with *Trichoderma* was ~66 per plant whereas the yield was only 41 for a control plant. The plants infected with *Pythium* and *Fusarium* treated with *Trichoderma* had fruit yields of 43 and 53 respectively and those were 5 and 1.6 times higher than plants infected with pathogens.

2.5.2.2. Fluorescent *Pseudomonas* spp. as a biocontrol

Pseudomonas is an important component of the rhizosphere, and certain isolates can enhance plant health (Schippers *et al.,* 1987; Weller, 1988; Cook *et al.,* 1995). Plant growth-promoting *Pseudomonas* species (collectively known as plant growth-promoting rhizobacteria, PGPRs) exert their beneficial effect via several different mechanisms but principally by active exclusion of pathogens from the rhizosphere. Traits with a confirmed role in this process include allelopathic factors, such as toxins, antibiotics and siderophores. Recent reports also indicate that some PGPRs can protect plants against pathogen infection by eliciting induction of systemic resistance (ISR) (Pieterse *et al.,* 1996).

Rangeshwaran and Prasad (2000) reported that *Pseudomonas putida* completely inhibited *S. rolfsii* in dual culture. Petri plate assay revealed that the *Pseudomonas* sp. exhibited antifungal activity against the plant pathogens, *Pythium ultimum, Rhizoctonia solani, Phytophthora capsici, Botrytis cinerea* and *Fusarium oxysporum* (Kim *et al*., 2000).

Pal *et al*. (2001) were reported *Pseudomonas fluorescens* to be antagonistic to *Aspergillus flavus, Sclerotium rolfsii* and *Aspergillus niger in vitro.*

Tripathi and Johri (2002) studied *in vitro* biocontrol potential of fluorescent Pseudomonads recovered from rhizoplane and rhizosphere of pea and wheat against *Colletotrichum dematium, Rhizoctonia solani* and *Sclerotium rolfsii* and found them to restrict the growth of all the three pathogens but were most effective against *Rhizoctonia solani*.

Anith *et al*. (2002) studied that fluorescent pseudomonad isolates tested against *Phytophthora capsici* on potato dextrose agar (PDA) and carrot agar (CA) by dual culture technique was found to inhibit the growth of pathogen in 5-7 days.

Reddy *et al.* (2009) isolated and characterized Eight *Pseudomonas fluorescens* strains tested for their in-vitro antagonistic activity against *R. solani*. They found that all the strains tested, exhibited antagonistic activity against *R. solani* and one isolate, Pf 003 gave 78% inhibition compared to control.

Deepti and Johri (2003) observed that Pseudomonads suppress soil-borne fungal pathogens by producing antifungal metabolites such as pyoluteorin, pyrrolnitrin, phenazines, and 2, 4-di-acetyl phloroglucinol.

Saikia *et al.* (2004) reported *Pseudomonas aeruginosa* RS B29 is inhibit the growth of *Fusarium oxysporum* f.sp. *ciceri, Fusarium udam, Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* under *in vitro* conditions by producing an antifungal metabolite.

Rini *and* Sulochana (2007) reported that the inhibitory potential of pseudomonad isolates also differed significantly. Against *R. solani*, *Pseudomonas fluorescens* isolates P28 and P51 exerted the maximum inhibitory effect as evidenced by the widest inhibition zone (14.25 and 14 mm respectively for P28 and P51 in KMB and 7.5 mm for both in PDA).

Rakh *et al.* (2011) isolated 11 *Pseudomonas spp.* from rhizospheric soil, were evaluated for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas* cf. *monteilii* 9, showed highest antagonistic activity against *Sclerotium rolfsii*. In dual cultures, the *Pseudomonas* cf. *monteilii* 9 inhibited the *Sclerotium rolfsii* up to 94 % in terms of dry weight. *Pseudomonas* cf. *monteilii* 9 produced diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophore which affect *Sclerotium rolfsii* growth in vitro. This strain also produced a clear halo region on skim milk agar plates, indicating that it excretes protease which played vital role in inhibition of *S. rolfsii*.

Sinha and Simon (2013) results of lab and field experiment conducted on bio control agent strains of *Pseudomonas fluorescens*. The bacterial isolates were characterized to study the antagonist behavior in which the isolate of *Pseudomonas fluorescens* had given positive HCN production and IAA production. All thirteen

strains were evaluated for their bio-efficacy in dual culture against important plant pathogen *Alternaria porri*. It was observed in screening test of strain that of PsL-4, PsL-6 and PsL-8 were found superior.

Saravanan *et al.* (2013) focused on the antagonistic potential of fluorescent Pseudomonas in vitro and its inoculation effect on growth performance of *Lycopersicon esculentum* in *Rhizoctonia solani* infested soil. Isolates Pf5 and Pf6 were antagonistic against 14 bacterial species, and two pathogenic fungi (*F. oxysporum* and *R. solani*).

Prashant *et al.* (2014) was screened different media for growth and colony formation of test bacterium *Pseudomonas fluorescens* and to check its antagonistic activity on fungal plant pathogens by in vitro dual culture techniques. In dual culture method, *P. fluorescens* on co-inoculation with fungal pathogens decreased their growth rate. Maximum inhibition was observed in Sclerotium rolfsii (63.15%) followed by *Fusarium oxysporum* (61.85%) *R. bataticola* (55.56%) and *R. solani* (53.15 %).

Solanki *et al*. (2014) were screened 220 bacteria isolated from tomato rhizosphere for *in vitro* antagonistic activity against *Rhizoctonia solani* AG-4. Five potent antagonistic strains viz., *Pseudomonas spp*. (M10A and MB65), *P. aeruginosa* (MPF14 and MB123) and *P. fluorescens* (MPF47) were identified on the basis of physiological characters and 16S r DNA sequencing. These strains were able to produce hydrolytic enzymes, hydrogen cyanide, indole acetic acid, although, only few strains were able to solubilize phosphate. Two strains (MB123 and MPF47) showed significant disease reduction in glasshouse conditions were further evaluated under field conditions using three different application methods.

Agrawal *et al.* (2014) reported that there is variability among 24 *P. Putida* isolates for PHB production and release of inorganic phosphate as potential isolate for industrial, biocontrol and plant growth promoting agents. All the isolates (except P56) were amplified with polyhydroxyalkanoate gene-specific primer PhaJ but the variability in PHB production may be because of the fact that synthesis of PHB is pathway-dependent and environment dependent. One of the potential *P. putida* isolate P132 can contribute as a candidate agent for both biocontrol and PGPR applications. Identified as one of the most efficient PHB producer and

phosphate solubilizer, in vitro detection of P132 showed the presence of genes for polyhydroxyalkanoate.

In conclusion, siderophore producing bacteria MPF47 have strong biocontrol abilities and its application as soil mix + seedling root treatments provided strong shield to plant roots against *S. rolfsii* and could be used for effective bio management of pathogen.

2.5.2.2.1. Isolation, purification and biochemical characterization Fluorescent *Pseudomonas* collected from different geographical locations of Chhattisgarh

The genus *Pseudomonas* belongs to the gamma-subclass of the Proteobacteria and includes mostly fluorescent Pseudomonads as well as a few non-fluorescent species. Fluorescent *Pseudomonads* belongs to the family Pseudomonadaceae are aerobic, gram-negative plant growth promoting rhizobacteria and possess many traits that make them well suited as biocontrol agent, the free living rhizosphere bacteria are usually referred as plant growth promoting rhizobacteria., these rhizobacteria that play a major role in the plant growth promotion, induced systemic resistence and biological control of pathogens etc. (Bergey"s Manual of Determinative Bacteriology, 1974).

Fluorescent pseudomonads are ubiquitous bacteria that arecommon inhabitants of the rhizosphere and are the most studied group within the genus Pseudomonas (Ahmadzadeh and Tehrani, 2009). Plant growth promoting rhizobacteria are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Ahemad and Kibret, 2014).One of the most popular bacteria studied and exploited as biocontrol agent is the Pseudomonas species (Ahmad *et al*., 2008). Most of the identified Pseudomonas biocontrol strains produce antifungal metabolites such phenazines, pyrrolnitrin, pyoluteorin and cyclic lipopeptides like viscosinamide. It was demonstrated that

viscosinamide prevents the infection of sugarbeet by Pythium ultimum (Bloemberg and Lugtenberg, 2001). These bacterial strains beside the antagonistic effect also influence the defense system of plants (Laslo *et al.*, 2012).

P. aeruginosa secretes a variety of pigments, including pyocyanin (bluegreen), fluorescein (yellow-green and fluorescent, now also known as pyoverdin), and pyorubin (red-brown). *P. aeruginosa* is capable of growing in diesel and jet fuel, where it is known as a hydrocarbon-utilizing microorganism (or "HUM bug"), causing microbial corrosion.

2.5.2.2.2. The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms

Singh (2013) studied that Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that enhances plant growth and yield via various plant growth promoting substances as well as biofertilizers. The use of beneficial soil microorganisms such as PGPR for sustainable and safe agriculture has increased globally during the last couple of decades. PGPR as biofertilizers are well recognized as efficient soil microbes for sustainable agriculture and hold great promise in the improvement of agriculture yields.

Telangre *et al*. (2013) reported that the plant height, fresh weight and dry weight were recorded at 120 DAS, in which seed treatment with *P. fluorescens* @10 g/kg seed significantly increase the plant height, fresh weight and dry weight of pigeonpea. Hence the results concluded that *P. fluorescens* alone or in combination with chemicals not only reduce the wilt incidence but also enhance the growth of lentil, which ultimately helps in increasing the crop yield.

A) Direct mechanism

2.5.2.2.2.1. Production of Auxin - Indole 3- acetic acid

Geeta (2001) studied 28 P-solubilizing bacterial strains for the production of PGPS. The production of IAA among the strains varied from 3.61-35.45 µg/25 ml of broth.

Patten and Glick (2002) demonstrated that bacterial IAA from *P. putida* played a major role in the development of host plant root system. Similarly, IAA

production in *P. fluorescens* HP 72 correlated with suppressing of creeping bent grass brown patch (Suzuki *et al.*, 2003).

Vessay (2003) studied that microbial Indole acetic acid could be involved in the growth stimulation observed in greenhouse assay. Production of plant growth regulators by the microorganisms is another important mechanism often associated with growth stimulation. Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). This review focused on the known, the putative, and the speculative modes-of-action of PGPR. These modes of action include fixing N2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant–microbe symbioses.

Rashmi (2004) studied P-solubilizing involving *Serratia* isolates showed production of IAA in the range of 4.10 to 28.08 µg/25 ml broth and that of GA in the range of 1.35 to 8.60 µg/25 ml.

Suneesh (2004) reported that all the 48 fluorescent Pseudomonads isolated from the moist deciduous forest Western Ghats produced IAA and GA in the range of 1.63 to 17.00 µg/25 ml of broth and 0.72 to 5.27 µg/25 ml of broth respectively. Several fluorescent Pseudomonads have been used as seed or root inoculants for higher growth and yield of crops like wheat (Brown, 1974), potato (Burr *et al.*, 1978) and sugarbeet (Suslow and Schroth, 1982).

Tanimoto (2005) reported that at relatively high concentrations, natural auxins, such as IAA, stimulate shoot elongation and root induction while reducing root elongation.

Gravel *et al.* (2007) investigated the production or degradation of indole acetic acid (IAA) by the two microorganisms as possible mechanisms for plant growth stimulation. Both *P. putida* and *T. atroviride* were shown to produce IAA. The production of IAA by the two microorganisms was stimulated in vitro by the addition of L-tryptophan, tryptamine and tryptophol (200 mgml ¯1) in the culture medium. *P. putida* and *T. atroviride* also increased the fresh weight of both the

shoot and the roots of tomato seedlings grown in the presence of increasing concentrations of L-tryptophan (up to 0.75 mM).

Yadav and Verma (2014) observed that the highest significant number and dry weight of nodules root and shoot dry weight plant ¯1, yield of grain and straw and uptakes of N and P by chickpea were recorded due to combined inoculation of *R. leguminosarum* and *Pseudomonas aeruginosa* over uninoculated soil and treatment with *R. leguminosarum* alone. This co-inoculation involved direct mechanism of more nodulation and N2 - fixation caused by *R. leguminosarum* where as improved acquisition of P and Fe caused by *P. aeruginosa*. Also, indirectly by production of phytohormone (IAA) by both of these microorganisms which stimulated the growth of host plant. *P. aeruginosa*, and suppressed plant diseases like wilt and root rot caused by *Fusarium oxysporum* f. sp. *Cicer* and *Rhizoctonia solani*, respectively.

B) Indirect mechanism

2.5.2.2.2.2 Siderophore production

The term siderophore (Griron carrier) was introduced by Lankford in 1973. Neilands, 1993 to designated relatively low molecular weight virtually ferric specific ligands of microbial origin, the biosynthesis of which is enhanced at low molecular concentration. The molecular masses of siderophores fall in the range 500-1000 Da, a certain amount of commercial scaffolding being required to effort stability and hence, specific for Fe (III) that is the hallmark of this ligands.

For the first time Kloepper *et al.* (1980) reported the significance of siderophores produced by certain genera of PGPR in plant growth promotion. Siderophores are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore producing PGPR in rhizosphere increases the rate of Fe3+ supply to plants therefore enhance the plant growth and productivity of crop. Further, this compound after chelating Fe3+ makes the soil Fe3+ deficient for other soil microbes and consequently inhibits the activity of competitive microbes (Schippers *et al*., 1987).

Scher and Baker (1982) reported the excreted pseudobactin chelates iron because it has a higher affinity for iron than do the siderophores from most microorganisms that are deleterious to plant growth.

Demange *et al. (*1987) characterized fluorescent *Pseudomonas* for the production of yellow-green pigments termed pyoverdines which fluoresce under UV light and function as siderophores.

Hofte *et al.* (1991) reported pyoverdin production by *Pseudomonas aeruginosa* 7 NSK 2 that was able to increase the yield of barley, wheat, maize, cucumber and spinach.

Budzikiewicz (1993) observed that siderophores are iron-specific compounds which are secreted under low iron stress and we found that production of siderophores in the medium employed was inversely proportional to the iron concentration in the medium. In soil, plant roots normally coexist with bacteria and fungi which may produce siderophores capable of sequestering the available soluble iron and hence interfere with plant growth and function. Siderophores are low molecular weight, non-ribosomal peptides, secreted under low iron stress conditions and capture iron from the environment. Siderophores are also thought to facilitate biocontrol by sequestering iron from pathogens, thus limiting their growth and for the solubilization of extracellular ferric iron by most bacteria and fungi.

Hofte (1993) reported the production of siderophores is wide spread in the microbial world; most aerobic and facultative anaerobic species at least one of such compounds. That are secreted by microorganisms to take up iron from the environment and their mode of action in suppression of disease were thought to be solely based on competition for iron with the pathogen (Bakker *et al.,* 1993; Duijff *et al*., 1997).

Manwar *et al.* (2004) examined that *Pseudomonas* culture and purified siderophores showed good antifungal activity against the plant deleterious fungi, *viz. Aspergillus niger. A. flavus, A. oryzae, F. oxysporum* and *Sclerotium rolfsii.*

2.5.2.2.2.3 HCN production

HCN production ability has been found to be associated with effective disease suppression ability in different pathosystems. Prior studies have suggested that HCN may be a distinctive marker for deleterious soil microorganisms (Schippers *et al*., 1993), and, in fact, HCN producing bacteria have been exploited for weed biocontrol (Owen & Zdor, 2001).

Defago *et al. (*1990) reported HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens.

Ramette *et al.* (2003) reported that hydrogen cyanide (HCN) a broad spectrum antimicrobial compound involved in biological control of root diseases, is produced by many plant associated fluorescent Pseudomonads and HCN synthase is encoded by three biosynthetic genes (*hcn* A, *hcn* B and *hcn* C). However, little is known about the diversity of these genes in fluorescent *Pseudomonas* sp. and other bacteria. It has been demonstrated that HCN-producing ability was a positive attribute for plant-associated biocontrol *Pseudomonas* strains, when linked to the ability to also produce Phl.

2.5.2.2.2.4 Production of Antibiotics

Production of antibiotics by several strains of fluorescent pseudomonads has been recognized as a major factor in suppression of root pathogens.

Weinberg (1970) reported that *Pseudomonas fluorescens* produced PCA (Phenazine-1-carboxylic acid), whereas *Pseudomonas aureofaciens* 30-84 not only produced PCA but also lesser amounts of 2-hydroxyphenazines. The major phenazine derived by *Pseudomonas aeruginosa* was pyocyanin (1- OH-5-methyl phenazine). Phenazines (Ph3) are N-containing heterocyclic pigments synthesized by *Pseudomonas* (Leisinger and Margraff, 1979).

Smirnov and Kiprianova (1990) showed that bacterization of wheat seeds by *Pseudomonas fluorescens* strains 30-84 and 2-79 provided primary protection against *Gauemannomyces graminis tritici* on account of release of phenazine. Almost all phenazines exhibited broad spectrum activity against bacteria and fungi.

Hill *et al. (*1994) in one study, it was reported that mutants of the biocontrol PGPB *Pseudomonas fluorescens* BL915 that no longer produced the antibiotic pyrrolnitrin lost the ability to prevent *Rhizoctonia solani* induced damping-off of cotton plants.

Schnider *et al. (*1994) observed when a DNA fragment, isolated from the wild-type bacterium that restored pyrrolnitrin synthesis to these mutants was transferred to two strains of *P. fluorescens* that did not normally synthesize this antibiotic, the transformed strains gained both the ability to synthesize pyrrolnitrin

and inhibit *Rhizoctonia solani*-induced damping-off of cotton plants. When an antibiotic-producing (wild-type) strain of *Pseudomonas fluorescens* was genetically manipulated to overproduce the antibiotics pyoluteorin and 2, 4-diacetylphloroglucinol, the resultant strain protected cucumber plants against disease caused by *Pythium ultimum* to a greater extent than did the wild-type strain that caused take all disease in wheat.

Picard *et al.* (2000) observed that DAPG was also a major determinant in the biological control activity of *Pseudomonas fluorescens* against black root rot of tobacco caused by *Thielaviopsis bassicola* and that of *Pseudomonas fluorescens* F113 against damping off of sugarbeet caused by *Pythium ultimum*.

2.5.2.2.2.5. Solubilization of phosphates by fluorescent pseudomonads

Phosphorus (P) is one of the most essential plant nutrients which profoundly affect the overall growth of plants (Wang *et al*. 2007) by influencing various key metabolic processes such as cell division and development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis and respiration of plants.

Goldstein (1986) studied that the biological process of conversion of unavailable /fixed form of inorganic phosphorous into primary orthophosphate (H2PO4) and secondary orthophosphate (H2PO4) has been termed as mineral phosphate solubilization.

Raghothama (1999) studied that phosphorus frequently is the least accessible macronutrient in many ecosystems and its low availability is often limiting to plant growth. Several *Pseudomonas* species have been reported among the most efficient phosphate-solublizing bacteria and as important bio-inoculants due to their multiple biofertilizing activities of improving soil nutrient status, secretion of plant growth regulators and suppression of soil-borne pathogens (Rodriguez and Fraga, 1999; Gulati *et al.*, 2008; Vyas *et al.*, 2009).

Meur *et al.* (2012) reported that sequential feeding of relatively cheap carbohydrates such as xylose is a practical way to achieve more cost effective medium-chain-length (mcl) PHA production. Reduction in cost can be achieved using two kinds of carbon sources, one for biomass production and the other for synthesis of PHA. Metabolite utilization diversity is also important because

changes in their composition may affect the patterns and activities of rhizobacterial populations which are dependent upon rhizospheric nutrients for growth[].

2.5.2.3.. *Bacillus* spp. as biocontrol agent

Sing and Diwivedi (1987) reported that *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Streptomyces diastiaticus* reduced the mortality caused by *Sclerotium rolfsii*, a causal agent of foot rot of barley. In pot tests S. rolfsii killed 83.3% of barley plants. But the addition of suspension of antagonists reduced the mortality to 13.3, 20.0, 33.3, 40.3, 53.3 and 46.6%, respectively[].

Iqbal *et al*. (1995) tested the microorganisms for antagonism to *Sclerotium rolfsii*. All the organisms viz., *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma viride*, *Gliocladium virens*, *Aspergillus candidus*, *Paecilomyces lilacinus* and *Bacillus spp*. significantly inhibited the mycelial growth of *S. rolfsii*. *Trichoderma harzianum*, *T. koningii* and *T. viride* overlapped the pathogen and suppressed the growth by 63.6%, 54.9% and 51.89%, respectively[].

Ahmadzadeh *et al*. (2003) found that the antagonistic rhizobacteria, more specifically *fluorescent pseudomonads* and certain *Bacillus* species possessed the ability to control fungal and bacterial root diseases of agronomic crops. Seed treatment with *Pseudomonas aeruginosa* RS B29 controlled fusarium wilt and charcoal rot diseases of chickpea, in both green house and field conditions[].

Abeysinghe (2009a) reported effective mode of application of *Bacillus subtilis* CA32r on control of *Sclerotium rolfsii* on *Capsicum annuum*. Best protection was achieved by combination of root bacterization prior to transplant and soil application of CA32r. Root bacterization resulted in maintaining higher numbers of bacteria at the collar region of chilli plants and may have shielded the most vulnerable area from the pathogen, resulting enhanced protection. Since the application of CA32r resulted in a significant reduction of the number of viable *S. rolfsii* propagules in the soil indicates that *B. subtilis* CA32r possesses not only protective but also eradicative potential.[]

Vishwanath *et al.* (2012) conducted experiment to screen potential biocontrol agents, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas* sp.-I,

Bacillus sp.-I and *Bacillus* sp.-II for the management of *Sclerotium rofisii* the causal agent of collar rot of sunflower in dual culture test and they overgrew the pathogen upto 76.2, 88.8, 80.01, 35.9 and 77.8 per cent *in vitro,* respectively.

Gomashe *et al*., (2014) *Bacillus subtilis* has the potential to produce bioactive compound antagonistic against plant pathogenic fungi *Sclerotium rolfsii*".

Shifa *et al.* (2014) observed that *B. subtilis* G-1, *B. amyloliquefaciens* B2 and *B. subtilis* EPCO 8 were found effective biocontrol agents in inhibiting the mycelial growth of *S. rolfsii in vitro* in dual culture assay. Gholami *et al.* (2014) reported that *B. subtilis* sub sp. *subtilis* and *B. subtilis* subsp. *spizizenii*, which demonstrated the largest inhibition growth against *S. rolfsii* under *in vitro* dual culture".

Darma *et al*. (2016) reported a strong antifungal-producing bacteria from bamboo powder for biocontrol of *Sclerotium rolfsii* causing Sclerotium rot in melon. *Bacillus subtilis* BMB26 produced an extracellular metabolite that effective against a wide range of pathogenic fungi, including *S. rolfsii*, and resistant to heat and protease. Furthermore, to evaluate the application of cell-free filtrates BMB26 on melon plants, a cotyledon test was done under greenhouse condition. A significant decrease was observed in disease incidence (up to 77%) occurred in BMB26-treated melon leaves inoculated with *S. rolfsii* after 4 days post-inoculation. This result showed that Bacillus subtilis BMB26 has a potential application as a bio-control agent against phytopathogenic fungi".

Suneeta *et al.* (2016) screened 26 isolates of *Bacillus* spp. against collar rot pathogen *in vitro.* Among them, five strains of *Bacillus* spp. showed highest antagonistic activity against *S. rolfsii*".

2.5.3. Management throught Botanicals

Dubey and Kishore (1990) reported that volatile fraction of two medicinal plants; *Azadirachta indica* and *Eucalyptus globules* were more effective in suppressing the sclerotial germination of *Macrophomina phaseolina* than non-volatile fractions.

Jalal and Ghaffar (1992) studied antifungal characteristics of *Ocimum sanctuml* L. and found that its leaf extract completely inhibited the growth of *S. rolfsii* and other fungi. Ethanol extracts of *Aframomum melegueta* and *Ocimum gratissimum* at 3 to 5% concentration showed total inhibition (100%) of the mycelia growth of *S. rolfsii.*

Mahfuzul (1997) tested plant extract like garlic (*Allium sativum*), ginger (*Zingiber officinale*), nisinda (*Vitex negundo*), dolkalmi (*Ipomoea fistulosa*) and marigold (*Tagetes erecta*) against major seed borne fungal pathogens of chilli. Among these plant extracts, garlic was found to be most effective.

Kurucheve and Padmavathi (1997) reported that, *Allium sativum* (garlic) clove recorded the minimum mycelial growth of (176 mg). Under the *in vitro* condition.

Prithiviraj et al. (1998) reported that antifungal properties of *Allium sativum* are well known against plant pathogens. It contains different antimicrobial components like allicin, E-and Z-ajoene, iso-E-10-devinylajoene, and so forth, which are effective against bacteria, yeasts and phytopathogenic fungi.

Yoshida et al. (1999) studied antifungal properties of *Allium sativum* against plant pathogens. It contains different antimicrobial components like allicin, E-and Z-ajoene, iso-E-10-devinylajoene, and so forth, which are effective against bacteria, yeasts and phytopathogenic fungi.

Hanthegowda and Adiver (2001) tested different plant extracts against *S. rolfsii.* Among different plant extracts, 1:20 dilution of *Parthenium hysterophorus, Polyalthia longifollia* and *Azadirachta indica* significantly inhibited the mycelial growth of *S. rolfsii*.

Okereke and Wokocha (2006) reported that the inhibition of damping-off disease of tomato incited by *S. rolfsii* was highest with soil drenching of neem seed (62.4%).

Suleiman and Emua (2009) reported that 55% growth inhibition of *Pythium aphanidermatum* with neem leaf extract and followed by ginger rhizome extract (70%).

Aslam *et al*. (2010) observed 44.73% mycelial growth inhibition of important damping-off pathogen, *Rhizoctonia solani in vitro* when neem leaf extract was supplemented in potato dextrose agar medium.

Farooq *et al*. (2010) reported the maximum inhibition of mycelial growth of *Sclerotium* (*Athelia*) *rolfsii* causing southern *Sclerotium* rot in sugar beet, by *Azadirachta indica* (73.8%).

Yeni (2011) reported that 80% concentration aqueous extract of *Z. officinale* inhibited *Fusarium oxysporum* to 66.70%, 80% aqueous extract of *O. gratissimum* inhibited *Botrydioploidia theobromae* to 60.00% also 73.33% inhibition of *Aspergillus flavus* was recorded using 30% ethanol extract of *Z. officinale*, the same concentration of *Ocimum gratissimum* inhibited *A. niger* to 70.00%.

Islam and Faruq (2012) studied effect of seed treatment with neem leaf, garlic clove, allamonda leaf, ginger rhizome, kalijira seed, bel leaf, turmeric rhizome, katamehedi leaf and onion bulb against damping-off disease of some winter vegetable in the net house. All the treatments were significantly reduced percent damping-off of cabbage, tomato and egg plant over untreated control. The most effective treatment was neem leaf extract followed by garlic clove and allamonda leaf extracts in terms of suppressing damping-off disease incidence with increasing plant growth characters.

Darvin (2013) evaluated extracts of eight plant species *viz.*, Ashoka (*Polyalthia longifolia*), Garlic (*Allium sativum*), Ginger (*Zingiber officinalis*), Neem (*Azadirachta indica*), Seetha Phal (*Annonas squamosa*), Tulasi (*Ocimum sanctum*), Milk weed (*Calotropis gigantean*) and Peri Winkle (*Vinca rosea*) at 10% concentration on development of *Sclerotium rolfsii* causing stem rot in groundnut. Among these plant extracts, clove extract of garlic was most effective and recorded lowest mycelial growth (0.0 cm) and highest per cent inhibition (PI) (100%) under *in vitro* condition.

Mahato *et al*. (2014) studied the effect of different plant oils and plant extracts on radial growth of *Sclerotium rolfsii* Sacc. Among the plant oils and plant extracts, Karanja oil (88.49%) and *Murraya exotica* leaf extract (86.15%) were found effective in reducing the growth of *S. rolfsii*.

Sab *et al.* (2014) studied the bio efficacy of ten botanicals through poison food technique and dual culture technique against *S. rolfsii* causing collar rot of chickpea. Among the ten botanicals tested, cent per cent mycelial inhibition was observed in aqueous extract of Agave at different concentrations.

Gupta *et al.* (2015) studied the antifungal activity of crude extracts of some plants against *Fusarium udum* in pigeonpea. On screening, the crude extracts of 20 plants for their antifungal activity, the crude extract of leaf of *Phyllanthus nursi* Linn, and *Vitex negundo* exhibited maximum toxicity against the test fungus.

2.5.4 Management throught fungicide chemicals

2.5.4.1. Evaluat ion of fungicides against *S. rolfsii*

2.5.4.2 *In vitro* evaluation of fungicides

The first report that southern blight of peanuts caused by *Sclerotium rolfsii* controlled with PCNB was done by Cooper, (1956). The poison food technique was followed for *in vitro* studies on the effect of certain fungicides on *Sclerotium rolfsii*. The best inhibition was shown by Calixin, Vitavax, Duter, Ferbam, Cereson wet, Sandoz seed dressing 6334 and Brassicol which allowed no growth of the fungus (Chauhan, 1978).

Johnson and Subramanyam (2000) found carbendazim as least effective on *S. rolfsii*. Das *et al*. (2002) studied in vitro efficacy of Thiram and Mancozeb at 0.1% concentration against tomato isolate of *S. rolfsii* and reported that Thiram had inhibited 70.3% of mycelial growth and 96.5% of sclerotial production of *S. rolfsiii*.

Gupta and Sharma (2004) reported that penconazole, hexaconazole, propineb and mancozeb inhibited mycelial growth. Propineb was found to be the most effective in reducing disease incidence on crown and pods. Among the biocontrol agents, *Gliocladium virens* and *Trichoderma viride* were found to be the most effective against the *Sclerotium rolfsii*.

Mathur and Sarbhoy (2004) reported that all the fourteen fungicides tested were not effective at and below 500 ppm. Four fungicides viz., Cereson wet (2000 ppm), Rhizoctol (2000 ppm), Formaldehyde (1000 ppm) and Vapam (1000 ppm) were found effective against *Sclerotium rolfsii* when tested in vitro.

Sheoraj *et al*. (2005) studied the efficacy of Mancozeb, Thiram, Carboxin, Dithane M-45, sulfur dust, Carbendazim, Ziram, Streptocycline, Thiophanate methyl and Blue copper at 2500 ppm in controlling *S. rolfsii* causing collar rot in lentil *in vitro*. Mancozeb, Thiram and Carboxin recorded 100% per cent control against the pathogen.

Tripathi and Khare (2006) found that complete inhibition of growth of *S. rolfsii* by Carbamate, iprobenfos, chlorothanonil, propineb and ediphenphos at 500 and 1000 ppm. Carbendazim completely inhibited the growth of *S. rolfsii* only at 1000 ppm. Benzimidazole was found least effective fungicide *in vitro*.

Yaqub and Saleem (2006) tested six fungicides viz., Benomyl, Sancozeb, Thiovit, Dithane M-45, Carbendazim and Topsin-M against *Sclerotium rolfsii* by food poison method. At low concentration, no fungicide inhibited the growth of *S. rolfsii*, however, at high concentration Dithane M-45 and Sencozeb significantly reduced the growth of *sclerotium rolfsii.*

Toorray *et al*. (2007) evaluated seven fungicides (each at 1000, 1500 and 2000 ppm) against *Sclerotium rolfsii* under *in-vitro* condition. Complete inhibition of growth of *S. rolfsii* was recorded by Captan, Thiram, Mancozeb, Hinosan (edifenphos) and antracol where as Chlorothalonil showed partial inhibition at low concentration. Bavistin (carbendazim) did not show much inhibition at all concentrations.

Johnson and Reddy (2008) evaluated in vitro efficacy of fungicides (Hexaconazole, Propiconazole, Mancozeb, Chlorpyriphos and Quinalphos) against *S. rolfsii*. Among the five pesticides tested for their efficacy, Hexaconazole at a concentration of 1000, 1500 and 2000 ppm and Propiconazole at a concentration of 500, 750 and 1000 ppm completely inhibited the growth of *S. rolfsii*.

Arunasri *et al*. (2011); who reported that the Triazoles (Hexaconazole, Propiconazole, Difenconazole) were highly inhibitive to the growth of *S. rolfsii*.

And the combi products containing triazoles *viz.*, Avatar, Merger and Nativo were highly inhibitive to the growth of *Sclerotium rolfsii*.

Bhuiyan *et al*. (2012) screened six fungicides namely Provax-200, Bavistin, Ridomil, Dithane M-45, Rovral 50 WP and Tilt at 100, 200 and 400 ppm concentration for their efficacy against the radial colony growth of *S. rolfsii*. The complete inhibition was obtained with Provax-200 at all the selected concentrations.

Singh *et al*. (2012) evaluated different fungicides against *Sclerotium rolfsii* Sacc. causing collar rot under *in vitro* conditions. Out of all ten tested fungicides at 2500 ppm concentration, four showed 100 per cent suppression of the pathogen over the control while in rest others significant reduction in radial growth and size of sclerotia were observed.

Nawar (2013) studied the inhibitory effect of fungicides against the growth of *Sclerotium rolfsii* under *in vitro* condition. Rhizolex fungicide was found highly effective and gave 100% reduction in growth when used at lower concentration 12.5ppm.

Sangeetha and Sharmrao (2013) the root rot/wilt causing pathogens are *Sclerotium rolfsii Rhizoctonia bataicola* and *Fusarium* sp. In vitro screening of fungicides was undertaken to identify an effective compound against these pathogens. Mancozeb, Carbendazim, Thiophanate methyl, Hexaconazole, Propiconazole, Carbendazim + Mancozeb and Carboxin + Mancozeb were found to be effective in inhibiting mycelia growth of all the three pathogens.

Begum *et al.* (2014) tested eight fungicides *in vitro* against *S. rolfsii*. The result revealed that maximum (100%) inhibition was observed in Carboxin, Propiconazole, Hexaconazole, Difenconazole and Carbendazim at all three concentrations viz., 500, 1000 and 1500 ppm followed by captan (79.30, 82.76 and 85.23%) and Triadimenfon (49.13, 60.23 and 65.33%) over control.

Chaurasia *et al*. (2014) tested nine fungicides, viz., Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Manzate, Parasan and Sulfex against *Sclerotium rolfsii in vitro* by food poison method. All the fungicides have showed adverse effect on the growth of *Sclerotium rolfsii*. Manzate has been found to be the best as it gave 100% inhibition of growth at 0.1% concentration.

Das *et al*. (2014) evaluated the potential of six systemic fungicides (i.e. Propiconazole, Hexaconazole, Mycobutanil, Thiophanate Methyl, Tebuconazole & Carbendazim); three non-systemic fungicides (i.e. Captaf, Mancozeb & Copper oxychloride) and three combo fungicides (i.e. Metalaxyl 8% +Mancozeb 64% , Carbendazim 12% + Mancozeb 63% & Carboxin 37.5% + Thiram 37.5%) against *Sclerotium rolfsii* using poisoned food technique, in vitro. The result showed that the effect of Hexaconazole (systemic) has been highly effective in suppressing radial expansion of hyphae.

Mahato *et al.* (2014) studied the effect of different fungicides, on radial colony growth of *Sclerotium rolfsii* Sacc. One systemic (Carbendazim 50%), three contact (Mancozeb, Copper oxychloride, Chlorothalonil) and three combinations of systemic and contact fungicides (Carboxin 37.5% + Thiram 37.5% WP, Metalaxyl 8% +Mancozeb 64%, Cymoxanil 8% + Mancozeb 64% WP) were evaluated against *S. rolfsii* in laboratory. Carboxin+Thiram was the best combination of fungicides to restrict the fungal growth effectively.

Khan and Javaid (2015) carried out *in vitro* bioassays using four fungicides namely Tegula (Tebuconazole), Thiophanate Methyl, Ridomil Gold (metalaxyl + mancozeb) and Mancozeb at 50, 100, 250 ppm concentrations. All the concentrations of these fungicides significantly decreased radial growth of *S. rolfsii* over control.

Shahiduzzaman (2015) tested fungicides Provax 200 (Carboxin + Thiram) and Bavistin 50 WP (Carbendazim), against *Sclerotium rolfsii* the maximum and significant growth reduction was achieved with only Provax 200 compared to control.

Khalequzzaman (2016) studied the effect of chemical, against foot and root rot of lentil. The lowest foot and root rot (21.67%) was obtained when seed treatment was done with Provax 200 (2.5 g/kg seed).

2.5.4.3. *In-vivo* evaluation of fungicides

Junaid *et al*. (1991) reported that in pot experiment for the control of *Sclerotium rolfsii*, seed treatment with Bavistin gave the best results followed by

PCNB (quintozene) and Thiram. Seed and soil treatments with quintozene slightly reduced seedling vigour.

Sharma *et al*. (1994) found that in pot tests with 10 fungicidal seed treatments against *Sclerotium rolfsii* in highly infested soil, the best results were obtained with triadimenol at 5 g/kg seed, followed by carboxin.

Tiwari (1997) reported that the percolation of four systemic fungicides (Carbendazim, tridemorph, edifenphos and thiophanate-methyl) was effective against *Sclerotium rolfsii* on chickpea in two different soil types tested in polyethyle columns. The results indicated that the percolative ability of all the fungicides was higher in sandy clay loam than in clay loam and the efficacy of each fungicide against *Sclerotium rolfsii* decreased with soil depth.

Charde *et al.* (2002) found that seed treatment with propiconazole and hexaconazole weresuperior in checking stem rot of groundnut caused by *S. rolfsii* and increasing the shoot and root.

Dutta and Das, (2002) reported that Seedling root dip in mancozeb (0.1%) and thiram (0.1%) effectively reduced collar rot of tomato caused by *S. rolfsii*

Tajane *et al.*, (2002).Seed treatment of soybean with hexaconazole and propiconazole inhibited *S. rolfsii*. These fungicides were found to be absorbed by roots and translocated to shoot and leaf length.

Singh *et al*. (2004) reported the effect of different concentrations of six fungicides on the incidence of collar rot disease of betelvine caused by *Sclerotium rolfsii*. The results revealed that 3-thioallophanate and mancozeb effectively controlled the collar rot disease when applied as soil drench.

Hoque *et al*. (2014) studied the efficacy of four fungicides in controlling foot and root rot of lentil under field condition. The test fungicides were Rovral (0.2%), Secure (0.2%), Bavistin (0.2%), Captan (0.2%). Tested fungicides significantly decreased incidence of foot and root rot of lentil and increased yield. Best performance was found with Secure (0.2%) in controlling the incidence of foot and root rot.

2.5.5 Soil Application of Biocontrol Agents

Soil Application of Biocontrol Agents While seed treatment gives the advantage of antagonist being the first colonizer of the rhizosphere, soil application

has the advantage of inhibiting the pathogen even away from the rhizosphere. Further, as the quantity of inoculum required for soil application is high, in general, better management is obtained with soil application strategy. Soil application of P. Fluorescence was effective in controlling collar rot of groundnut incited by S. rolfsii (Patil *et al*., 1998). Soil application of T. harzianum @ 60 g kg-1 of natural soil reduced stem rot of groundnut caused by *S*. *Roofs* up to 83 per cent (Biswas and Sen 2000). Biswas et al. (2000) observed that application of T. harzianum inoculum to soil and seed dressing at the time of sowing in the pots exhibited percent disease reduction through seed dressing from 33 to 50%, and through direct soil application it was 72 to 83%. Sclerotium wilt of groundnut caused by *S. rolfsii* was effectively reduced to 92.58 per cent, when *T. harzianum* was applied @ 10 g, kg -1 soil (Patibanda *et al*., 2002). Soil application of T. harzianum (H) inoculum was superior in reducing the percentage disease incidence and increased shoot length (24 g), root length (17.0), and yield 1509 kg ha-1 against root rot of groundnut caused by *Sclerotium rolfsii*, Saralamma and Vithal Reddy (2003)

2.5.6. Integrated disease management

Ramayallareddy (2002) found that integrated use of *T. viride, P. fluorescens,* neem cake and thiram for seed treatment of groundnut improved seed yield and controlled soil microflora *viz., A. niger, Alternaria spp, Curvularria sp, Fusarium spp, Penicillium sp, R. stolonifer, R. solani, S. rolfsii and Verticillium sp.*

Arunasri (2003) reported that seedling root dip in thiram @ 0.1 per cent + seedling root dip in *Trichoderma* suspension (T1) + seedling root dip in *Pseudomonas* spp. (B1) reduced the *S. rolfsii* incidence in Crossandra to about 6.66 per cent compared to control (73.66%).

Tewari and Mukhopadhyay (2003) conducted an experiment on integrated seed treatment with vitavax (carboxin @ 1gm/kg seed) and *Gliocladium virens* (Gv). Application of carboxymethyl cellulose (CMC) with *G.virens* powder (10^9spores) in combination with vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with Gv powder + CMC+vitavax significantly increased seedling

emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment Gv powder+vitavax and Gv suspension + vitavax in a sick plot.

Manjula *et al*. (2004) reported that under controlled environment conditions, *P. fluorescens* GB 10, GB 27, *T. viride* pq 1 and the systemic fungicide Thiram reduced the mortality of *S. rolfsii* inoculated to groundnut seedlings by 58.0%, 55.9%, 70.0% and 25.9%, respectively. The use of either *P. fluorescens* ot *Trichoderma virens* or Thiram alone or in combination with each other significantly reduced the stem rot caused by *S. rolfsii* in groundnut plant compared to control under greenhouse conditions.

Kumar *et al.* (2008) Seed treated with *T. harzialllm* + carboxin provided maximum protection to the crop against collar rot of chickpea and giving maximum seedling emergence, final plant stand and grain yield.

Abeysinghe (2009) suggested that a combination of *Bacillus subtilis* with *Pseudomonas* strains can lead to greater plant protection against *R. solani* and *S. rolfsii* than the biocontrol exhibited by these strains when they were used individually.

Kumar and Dubey (2012) conducted an experiment to study the effect of integrated application of biocontrol agent and fungicides for management of collar rot of chickpea. Two fungicides (carboxin and thiram) and two bio-control agents (*Pseudomonas fluorescens* and *Trichoderma harzianum*) were evaluated as seed treatment in different combinations against *S. rolfsii*, the causal organism of collar rot of chickpea. Seed treated with *T. harzianum* (4g/kg seed) + carboxin (O.5g/kg seed) provided maximum protection to the crop by giving maximum seedling emergence (495.0120 m'), final plant stand (480A/20m') and grain yield (l8.2q/ha). Other treatment combinations significantly increased seedling emergence, final plant stand and grain yield compared to control.

Singh *et al.* (2013) selected *Trichoderma* spp. and *Pseudomonas* spp. for seed and seedling treatment in tomato, to assess the synergistic effect of compatible isolates for plant growth promotion and management of *S. rolfsii*. He concluded that the application of a consortium of compatible bioagents enhanced

the plant growth and biological control of the pathogen in contrast to treatment with single bioagent.

Dwivedi and Prasad (2016) conducted an experiment to identify integrated management of *Sclerotium rolfsii* causing different types of diseases like collar-rot, sclerotium wilt, stem-rot, charcoal rot etc in more than 500 plants species. *S. rolfsii* may be controlled through biological agents, chemical agents, plant extracts and soil solarization. But in present study among all these control measures, biological, soil solarization and medicinal plant extract were the more significant than chemical control.

Rajendraprasad *et al.* (2017) observed that the combination of potential *Trichoderma harzianum* -1 and *Pseudomonas fluorescence* bacterial biocontrol agents proved effective in increasing germination and to reduce pre and post emergence collar rot and increasing the shoot and root weight and fresh and dry weight of tomato plants in the pots when inoculated with *Sclerotium rolfsii*.

2.5.7. Evaluation of different antagonists (seed dressers) against lentil disease under field condition:

Seed treatment of the biological control agent helps the antagonist to grow along with the root, and occupy the rhizosphere with the advantage of being the primary colonizer. Several reports were published on the use of seed treatment method in biological control. Harman *et al*. (1998) suggested the application of *Trichoderma* or Gliocladium to seed as an alternative approach to introducing them into the soil. Podile and Dube (1988) reported that seed coating with *P. fluoroscens* (PN-3) controlled stem rot pathogens of peanut (*S. rolfsii* and *R. solani*) in pot experiments. Muthamilan and Jeyarajan (1992) found that seed pelleting with Trichoderma harzianum (5x 109 conidia ml-1) as the best treatment in controlling root rot caused by *S. rolfsii*. Bhatia *et al*. (2005) reported that fluorescent Pseudomonas PS-I and PS–II coated seed sown in *S. rolfsii* infected soil significantly increased seed germination by 13.1 and 8.5 per cent respectively as compared to control. Singh *et al*. (2013) selected Trichoderma spp. and *Pseudomonas* spp. for seed and seedling treatment in tomato, to assess the synergistic effect of compatible isolates for plant growth promotion and management of *S. rolfsii*. He concluded that the application of a consortium of

compatible bioagents enhanced the plant growth and biological control of the pathogen in contrast to treatment with single bioagent. Belkar *et al.* (2013) reported that the seed treatment with Pseudomonas fluorescens @ 10g/kg of seed+ Bradyrhizobium japonicum @ 20g/kg of seed + Pseudomonas striata @ 20g/kg of seed with minimum stem rot incidence, i.e. 8.86%, 13.33%, 20.00% at 20 DAS and 17.73%, 33.33% and 40.00% of flowering, respectively.

Mukherjee, *et al.*, evaluated that "Using gamma-ray-induced mutagenesis, we have developed a mutant (named G2) of *Trichoderma virens* that produced two- to three-fold excesses of secondary metabolites, including viridin, viridiol, and some yet-to-be identified compounds. Consequently, this mutant had improved antibiosis against the oomycete test pathogen *Pythium aphanidermatum*. A transcriptome analysis of the mutant vis-à-vis the wild-type strain showed upregulation of several secondary-metabolism-related genes. In addition, many genes predicted to be involved in mycoparasitism and plant interactions were also upregulated. We used tamarind seeds as a mass multiplication medium in solid- state fermentation and, using talcum powder as a carrier, developed a novel seed dressing formulation. A comparative evaluation of the wild type and the mutant in greenhouse under high disease pressure (using the test pathogen *Sclerotium rolfsii*) revealed superiority of the mutant over wild type in protecting chickpea (*Cicer arietinum*) seeds and seedlings from infection. We then undertook extensive field evaluation (replicated micro-plot trials, on-farm demonstration trials, and large-scale trials in farmers' fields) of our mutant-based formulation (named TrichoBARC) for management of collar rot (*S. rolfsii*) in chickpea and lentil (*Lens culinaris*) over multiple locations in India. In certain experiments, other available formulations were included for comparison. This formulation consistently, over multiple locations and years, improved seed germination, reduced seedling mortality, and improved plant growth and yield. We also noticed growth promotion, improved pod bearing, and early flowering (7–10 days) in TrichoBARC-treated chickpea and lentil plants under field conditions. In toxicological studies in animal models, this formulation exhibited no toxicity to mammals, birds, or fish".

Subhash chandra (2020) reported that the highest per cent disease control was found in T8 Metalaxyl 8% + Mancozeb 64% + Neem leaf extract + P.

fluorescens (87.75%) followed by T6 Metalaxyl 8% + Mancozeb 64% + P. fluorescens (81.54%), T7 Metalaxyl 8% + Mancozeb 64% T. harzianum (79.62%) T5 Metalaxyl 8% + Mancozeb 64% Neem leaf extract 10% (73.88%), T1 Metalaxyl 8% + Mancozeb 64% (71.50%), T3 P. fluorescens (68.83%), T4 T. harzianum (66.88%) and T2 Neem leaf extract 10% (64.68%). as compared to T9 control (0.0).

2.5.8. Screening of lentil varieties for resistance to collar rot of lentil.

Akram *et al*. (2008) evaluated ninety-eight chickpea germplasm accessions under greenhouse conditions to identify sources of genetic resistance against collar rot disease incited by the fungus *Sclerotium rolfsii*. Out of 98 germplasm accessions only 5 genotypes *viz.,* FLIP 97- 132C, FLIP 97-85C, FLIP 98-53C, ILC -5263 and NCS 9905 exhibited highly resistant response to disease.

Amule *et al*. (2014) conducted the experiments during 2009 and 2010 to find out the most effective screening techniques for identifying host plant resistance against chickpea collar rot caused by *Sclerotim rolfsii* in pot house. Out of four techniques employed, chickpea 'grain inoculation technique' was found best. The minimum post emergence mortality (6.7%) occurred at 4.0 percent concentration of Pyraclostrobin which is significantly relatively less in comparison to control (26.8%) during the two consecutive year of testing. Among 88 chickpea desi genotype GNG 1958 was found resistant to disease, in Kabuli types, two entries i.e GNG 1969, BG 2086 were resistant.

Bayaa, *et al.,(*1994) Conducted the experiment the inheritance of resistance to lentil (*Lens culinaris* Medik.) vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis* was investigated in a cross between resistant (ILL5588) and susceptible (L692–16-l(s)) lines. $F_{2:4}$ progenies and $F_{6:8}$, $F_{6:9}$ recombinant inbred line (RIL) populations were assessed for their wilt reaction for three seasons in a well-established wilt-sick plot. Resistance to wilt was conditioned by a single dominant gene in the populations studied. The map location of the *Fw* locus was identified for the first time through linkage to a random amplified polymorphic DNA (RAPD) marker ($OPK\text{-}15_{900}$) at 10.8 cM. Two other RAPD markers ($OP\text{-}BH_{800}$ and $OP\text{-}DI5_{500}$) identified by bulked segregant analysis were associated in the coupling phase with the resistance trait, and another marker ($OP\text{-}C04_{650}$)

was associated with repulsion. The DNA markers reported here will provide a starting point in marker-assisted selection for vascular wilt resistance in lentil.

Bayaa, *et al.* (1997) evaluated that host-plant resistance is the best means to control the key disease of lentil-vascular wilt, caused by Fusarium oxysporum Schlecht. emend. Snyder & Hansen f.sp. lentis Vasudeva and Srinivasan. Systematic screening for resistance to lentil wilt was initiated in the field in 1993, in a wilt-sick plot in North Syria, with a core collection of 577 germplasm accessions from 33 countries. A subset (88 accessions) of mostly resistant accessions was re-screened in 1994. The most resistant accessions came from Chile, Egypt, India, Iran and Romania. Variation among accessions in the temporal pattern of wilting was analyzed. The limited wilting in resistant accessions followed a linear model through time, whereas the pattern for susceptible accessions was better described with an exponential model. This temporal variation emphasizes the need for repeated scoring during screening for resistance to lentil vascular wilt to identify 'late-wilters'. To overcome spatial variation in inoculum density, the efficacy of using wilt scores from a systematically-repeated susceptible control in covariate analysis was tested. Covariance analysis significantly improved overall screening by 3% in 1993, but the improvement was non-significant in 1994. The results emphasize the relative uniformity of disease pressure in the wilt-sick plot and suggest that covariance analysis of a systematically arranged control will be of greater benefit in land which is less uniformly infected.

Bayaa *et al.,* (1995) Reported that vascular wilt caused by*Fusarium oxysporum* f. sp.*lentis* Vasud. & Srin. is the major disease of the cultivated lentil (*Lens culinaris* Medikus). Host plant resistance is the most practical method of disease management. Wild lentils represent an unexplored potential source for disease resistance and other characters. Screening 219 accessions of wild*Lens* Miller and 2 accessions of*Vicia montbretii* Fisch. & Mey. (syn. *Lens montbretii* (Fisch et Mey) Davis et Plitm.) for resistance to a Syrian isolate of this fungus at the seedling stage was conducted under artificial inoculation in a plastic house. Resistance at the reproductive growth stage was confirmed in pots in a plastic house and in a wilt-sick plot. Three accessions each of *Lens*

culinaris ssp.*orientalis* (Boiss.) Ponert and*L. nigricans* M.B. Godr. ssp.*nigricans* Godr. and 2 of*L. nigricans* ssp.*ervoides* (Brign.) Lad. maintained their resistance at the reproductive growth stage in the plastic house. All accessions of*L. culinaris* ssp.*odemensis* Lad. and*V. montbretii* were susceptible. However, in the sick-plot only three accessions (ILWL 79 & ILWL 113 of *L. culinaris* ssp.*orientalis* and ILWL 138 of*L. nigricans* ssp.*ervoides*) maintained a good level of resistance. Resistance at the seedling stage was often found in accessions collected from northern and western sites of the distribution of the genus at low elevations. The most resistant accessions in the field at the reproductive growth stage were from Syria and Turkey.

Konde *et al.* (2017) screening out fifteen varieties of soybean against two diseases root rot caused by *Macrophomina phaseolina* and collar rot caused by *Sclerotium rolfsii* under epiphytotic conditions. Study of varietal resistance revealed that, among 15 varieties tested, none of the cultivar was found immune as well as moderately resistant or tolerant to both the diseases. Five cultivars (JS-93-05, TAMS-98-21, AMS-99-24, NRC-64, JS-335) were reported moderately susceptible and ten (JS-71-05, AMS-99- 16, AMS-92-32, TAMS-38, NRC-67, AMS-99-3, AMS-47, Bragg, AMS-353, AMS-56) were found highly susceptible to root rot and collar rot diseases.

CHAPTER – III
MATERIALS AND METHODS

The present studies entitled **"Studies on Collar Rot *(Sclerotium rolfsii* Sacc.) of Lentil and it's Management."** were carried out at the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

The materials used and methods / techniques followed during the course of studies are narrated as under –

Experimental site

The laboratory and pot culture experiments pertaining to the present research work were conducted in the Department of Plant Pathology, College of Agriculture, IGKV, Raipur (C.G.).

The methods and materials used during the course of present study are given below.

3.1 Materials

3.1.1 Experimental site

The present investigation was carried out at the Department of Plant Pathology and Microbial Biotechnology Laboratory, Department of Biotechnology, College of Agriculture, IGKV, Raipur .

3.1.2 Source of materials

The glassware, chemicals and other materials were obtained from Department of Plant Pathology, College of Agriculture, IGKV, Raipur.

The following instruments were used:-

1. Autoclave for sterilization
2. BOD incubator for incubation
3. Compound microscope
4. Hot air oven for glassware sterilization
5. Forceps, needle, cork borer, inoculation needle
6. Growth chamber
7. Laminar air flow for inoculation

8. Water bath
9. Electronic digital balance
10. Microwave for melting of media.

3.1.3 Source of seeds

Lentil seeds were procured from AICRP on MuLLaRP (All India Coordinated Research Project) on Lentil. IGKV, Raipur.

3.1.4 Chemicals used

Analytical grade chemicals were procured from Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G.).

3.1.5 Cleaning and washing of glassware

All the glassware prior to use were cleaned and washed with detergent followed by rinsing with tap and distilled water. The glasswares were thereafter dried in oven at 60^{o}C for half an hour. All the glasswares were further sterilized in hot air oven at 180^{o}C for 2 hours. Plastic Petri plates were surface sterilized with alcohol, whereas inoculation needle, cork borer, scissors, knife and blade were sterilized by heating over the flame after dipping in 95% alcohol. Sterilization of media was done by autoclaving at 121°C temperature and 15lbs pressure for 20 minutes .

3.1.6 General procedure followed

Three to five replications per treatment were maintained used for all *in vitro* studies. In general, Petri plates were poured with 15-20 ml sterilized melted and cooled potato dextrose agar medium (PDA). This was supplemented with streptomycin, in order to check the bacterial contamination prior to the pouring.

Growth studies were conducted using 5 mm disc (always kept in inverted position) of plate actively growing fungi for inoculation in synthetic media. Three to five replications were maintained under Complete Randomized Block Design (CRD)/ factorial-CRD as per the requirement. The inoculated plates were incubated in BOD incubator at 25 ± 2^{0}C for a period of 3 to 5 days and radial growth of the fungus was recorded. Observations were also recorded number of sclerotia formed after 10 to 15 days incubation.

Table 3.1: Composition of different media used in present investigation

S.No.	Media	Ingredient	Content (g)
1	PDA (Potato Dextrose Agar)	Dextrose	20
		Agar- Agar	20
		Peeled potato	200
		Distilled Water	1000 ml
2	Potato dextrose broth	Dextrose	20
		Peeled potato	200
		Distilled Water	1000 ml
3	Casein hydrolysis	Peptone	5
		Skimmed milk powder	70
		Distilled Water	1000 ml
4	H_2 S production test	Sodium thio-sulphate	0.025
		Ferrous ammonium sulphate	0.2
		Beef extract	3
		Agar	3
		Peptone	30
		Distilled Water	1000 ml
5	Gelatin broth	Yeast extract	3
		Peptone	5
		Gelatin	120
		Distilled Water	1000 ml
6	Lypolytic media	Calcium chloride	0.1
		Sodium chloride	5
		Peptone	10
		Agar	15
		Distilled Water	1000 ml
7	Nitrate reduction (broth)	Potassium nitrate	1
		Beef extract	3
		Peptone	5
		Distilled Water	1000 ml
8	King‘s _B‘ medium	Cyclohexamide	75mg
		Novobiocin	45mg
		Penicillin	75mg
		Dipotassiumhydrogen	1.5
		Phosphate	1.5
		Magnesium sulphate	20
		Protease peptone	20
		Agar	8 ml
		Glycerol	1000 ml
		Distilled Water	
9	*Trichoderma* selective Media	Rose Bengal	0.15
		Potassium nitrate	0.3
		Magnesium sulphate	0.4
		Di potassium phosphate	1.8
		Ammonium nitrate	2
		Glucose	3
		Agar	15
		Distilled water	1000 ml

3.2 Methods

3.2.1. Survey and collection of collar rot of lentil infected plants

An intensive survey was conducted during *rabi* season , 2018-19 and 2019-20, on the incidence of collar rot in lentil growing districts of Chhattisgarh . Symptoms were evident as yellowing collapse. The affected roots showed rotting at the collar region and down ward with the whitish mycelium in earlier stages of infection, rapeseed like sclerotia can be observed attached to mycelium around the collar, at seedling to flowering stage stage were collected from different locations of Chhattisgarh state (Ambikapur, Dhamtari, Bemetara, Bhatapara, Balod, Durg, Dhamdha, Gandai, Kanker, Kawardha, Narayanpur, Patan, Raigarh, Raipur , Rajnandgaon, Saragaon and Sitapur) during Januray to Feburary 2013. Samples of infected roots were collected from farmer‘s fields. Total 10-12 spots were selected randomly for taking root samples representing the whole field. Randomly 50 plants were selected in different locations in a field and numbers of plants infected were counted and the mean disease incidence was expressed in percentage. Wherever required, the complete infected plants were also collected for isolation of the pathogen and other studies. The per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Each sample was kept in paper bag and tied with a rubber band and labelled immediately. Information pertaining to the locality, crop history, etc. was also obtained about the samples. Samples and roots were brought in the laboratory and analysed after collection. The roots of affected plant showing the symptoms of collar rot t were incised and isolation were made for the presence or absence of the causal agent.

3.2.2 Isolation of pathogen from diseased samples

The roots of diseased plant showing symptoms were washed thoroughly with tap water, small pieces of infected roots were cut with the help of sterilized blade. These pieces were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for one minute followed by three washings with sterilized distilled water

to remove traces of $HgCl_2$. The pieces were then transferred aseptically to Petri plates containing PDA. Inoculated Petri plates were incubated at 25 ± 2^oC for three to five days and examined at frequent intervals to see the growth of the fungus developing from different pieces. As and when fungal colony appears they were transferred to PDA slant.

3.2.3 Purification and identification of isolates of *F*

The cultures of were purified by frequent sub culturing by the hyphal tip method and maintained by mass transfer on potato dextrose agar medium at 25 ± 2^0C. These isolates of *Sclerotium rolfsii* were identified by observing the colony char acter, pattern of sclerotial formation and sclerotial size measured with the help of micrometer.

3.3: Morphological and cultural characterization of different isolates of *Sclerotium rolfsaii* Sacc. of lentil

3.3.1 Mycelial growth

Fifteen ml of the sterile PDA medium was poured into sterile Petri dishes and allowed to solidify. A five mm culture disc of *Sclerotium rolfsii* obtained from actively growing region was aseptically placed at the centre of the dish and incubated. The colour of the colony and types of mycelial growth of different isolates were observed after seven days of incubation . The colony characters like, colony diameter and colony colour, colony appearances were observed

3.3.2 Morphological variability

The slides of various isolates were prepared in lactophenol from 10 days old culture for morphological studies. For morphological characteristics *viz.*, hyphal width, size of sclerotia and shape of sclerotia were recorded after ten days of incubation (Dhingra and Sinclair, 1978) . Color, shape, Diameter and weight of 100 sclerotial bodies of individual isolates were recorded.

3.3. 3 Mass multiplication of *Sclerotium rolfsii*.

The *S. rolfsii* was mass multiplied in wheat grain media. Wheat grains were soaked in water for 6 hrs then boiled for little time, drained excess water, air dried and supplemented with 50 g calcium carbonate in 1 kg wheat grains then filled in

6 x 11 inches polythene bags and plugged with non absorbent cotton with the support of one inch diameter PVC ring (length 1.5 inch). These bags were sterilized in autoclave with 1.02 kg/cm^2 pressure for 25-30 minutes. The sterilized bags were inoculated with 2-3 mycelial discs (5 mm) taken from the periphery of the 5 days old culture of *S. rolfsii*. The inoculated bags were incubated in BOD incubator at 25± 2^{0}C for 15 days. Multiplied culture of *S. rolfsii* utilized for development of artificially sick pot of *S. rolfsii*.

3.4: Pathogenicity test

Pathogenicity test of different isolates of *Sclerotium rolfsii* of Ientil (SR-01, SR-02, SR-03, SR-04, SR-05, SR-06, SR-07, SR-08, SR-09,SR-10,SR-11, SR-12, SR-13 ,and SR-14) was done under pot conditions by seed and soil inoculation techniques suggested by (Kataria and Grover, 1976), Soil inoculation technique suggested by Radhakrishanan and Sen (1985) and Sen and Kapoor (1975). Use of following two methods to prove pathogenicity and observation of diseased and healthy plants were recorded 7 to 60 days and PDI was calculated by- PDI = [(number of diseased plants/total number of plants) x 100]

3.4.1: Seed inoculation technique

Apparently healthy surface sterilized lentil seeds (Susceptible variety, K-34) were taken. The seeds were rolled, on 7 days old culture of *Sclerotium rolfsii* grown on PDA contained Petri-plates. Inoculated seeds were sown at 5 cm depth in cemented pots (pre-sterilized and having autoclaved soil) @ of 10 seeds/pot with 3 replications. The un-inoculated apparently healthy seeds served as control. These pots were kept in cage house and watered regularly .

3.4.2: Soil inoculation technique

In vivo, aggressiveness test was conducted in glass house. The sick pots were prepared by using the inoculum of the pathogen @ 25 gm/kg soil. The inoculum of mass multiplied on wheat grain media each isolate was added to the sterilized soil (1% formalin for 15 days). Two kg mixture was placed in 15 cm diameter surface sterilized plastic pots (0.1% mercuric chloride). The pots were left for 5 days for stabilization before sowing. lentil seeds of variety K34 were surface

sterilized with 0.2 % Sodium hypochlorite for one minute followed by three washing with sterile water. Ten seeds were placed in one pot and three replications of each isolates were maintained. Germination per cent, root length, shoot length and per cent mortality was recorded. Percent mortality was calculated by using the following formula :

$$\text{Per cent mortality} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Observation on seed germination was recorded 7 days after sowing and post emergence mortality was recorded up to 60 DAS in above both experiments. When disease was become start, growth of the plant is checked, initially the leaves of infected lentil plants turns light pale in color , plant start drying and finally die. This very character was also observed to confirm the identification of fourteen isolates of *Sclerotium rolfsii* were established from infected plant materials collected during survey of various districts of Chhattisgarh in *Rabi* (2018-19) and (2019-20) (Table 3.1). The fungus was re-isolated from artificially inoculated plants and resulting culture was compared with original one to confirm the identity of the fungus . The cultures were maintained on PDA at 25 ± 1°C and sub-cultured after fifteen days.

Sclerotium rolfsii Sacc. culture on PDA

Mass multiplication of *Sclerotium rolfsii* Sacc.

Mixing of inoculum into soil.

Growing seedlings

Disease expression seen in plant on the left.

Fig. 3.1: Screaning techaniques of collar rot of lentol

3.5 Soil factors affecting development of collar rot disease in lentil

3.5.1 Effect of soil type

This experiment was conducted to know the effect of major soil type found in Chhattisgarh state on development of collar rot in lentil. In order to determine the effect of four soil types *viz.,* (Alfisol (Dorsa), Inceptisol (Matasi),Vertisol (Kanhar) and Entisol (Bhata)) were obtained from adjointing fields of College of Agriculture, Raipur. The inoculum of *S. rolfsii* was allowed to multiply on wheat grain media. The inoculum of *S. rolfsii* was added @ 25g/ kg in each soil type mixed thoroughly and left for 5 days, for establishment of inoculum. Ten surface sterilized seeds of lentil were sown in each pot and data on per cent mortality were recorded after 15 days interval of sowing. Treatment details are given below –

Table 3.2: Major soil type in Chhattisgarh

S.No.	Treatment	Soil	Common name	Scientific name
1	T1	Dorsa	Clayloam	Alfisol
2	T2	Matasi	Sandyloam	Inceptisol
3	T3	Kanhar	Clayey	Vertisol
4	T4	Bhata	Gravely	Entisol

3.5.2 Soil texture

Soil sample was taken in a beaker and 15 ml sodium hexametaphosphate (10 %) was added and stirred for 10 minutes in a 1000 ml beaker. The contents were transferred to 1000 ml measuring cylinder with volume make up. At 5 minutes and 5 hours, temperature and hydrometer reading were taken. Percentage of sand, silt and clay were determined with the help of Bouyoucos hydrometer (Piper, 1950) and the textural classes were determined with the help of textural diagram (International system). In the present study seven textures were under taken viz., sandy, sandy loam, loam, clay, clay loam, silt clay and silt loam. The inoculum of *S. rolfsii* was allowed to multiply on wheat grain media. The inoculum of *S. rolfsii* was added @ 25g/ kg in each soil texture mixed thoroughly and left for 5 days, for establishment of inoculum. Five surface sterilized seeds of lentil were sown in each pot and data on per cent mortality were recorded 15 days after sowing.

3.5.3 Effect of soil pH

In order to determine the effect of different soil pH on development of disease, soil was collected from experimental field of Department of Plant Pathology. Twenty gram of soil was taken and suspended in 40 ml sterile distilled water in 250 ml conical flask and stirred for 30 min. The soil pH was estimated 6.5. The soil pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 by using NaOH and HCL (Kulkarni, 2007). The inoculum of *S. rolfsii* was allowed to multiply on wheat grain media. The inoculum of *S. rolfsii* was added @ 25 g/kg soil, mixed in each pH amended soil and left for 5 days, for establishment of inoculum. Five surface sterilized seeds of lentil were sown in each pot and data on per cent mortality were recorded 15 days of interval after sowing.

3.6.: Collection and characterization of native isolates of *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp.

3.6.1.: Collection and characterization of native isolates of *Trichoderma* spp .

3.6.1.1 Collection of soil samples

Soil samples were collected from different locations of Chhattisgarh from rhizospheric and non rhizospheric region. The plants were gently uprooted using trowel to avoid damage and loss of root system. Loosely adhering soil particles were removed by gentle tapping and thereafter, roots were transferred to sterile polythene bags, brought to the laboratory and stored at 28^0C. The rhizosphere soil was removed by mechanically scraping of root surface.

3.6.1.2. Isolation of *Trichoderma* spp.

The isolation of *Trichoderma* spp. from the collected soil samples was made by soil dilution plate technique (Johnson and Curl, 1972). One gram of soil sample was weighed and transferred in 10 ml sterilized water in test tube and shaken properly to make 10^{-1} dilution 1 ml suspension from this test tube was transferred to next test tube containing 9 ml distilled water by using sterilized pipette and shaken to make 10^{-2} dilution and second upto fifth test tube to make up 10^{-5} dilution. One ml of suspension from the soil dilutions of 10^{-3}, 10^{-4} and 10^{-5} were put in Petri plate containing 20 ml sterilized PDA and gently shaken to spread evenly . The inoculated plates were incubated at 28°C and were observed

for the appearance of *Trichoderma* spp. which was then aseptically transferred to PDA slants on which the growth appears within 1-2 days. The cultures were identified and confirmed as *Trichoderma spp.* through microscopic observation and selective growth on TSM (*Trichoderma* Selective Media) medium .

3.6.1.3. Cultural and Morphological Characterization of the isolates of *Trichoderma* spp.

Cultural characters of all the single spore isolates under investigation were studied on 20 ml sterilized PDA in petri plates. Mycelial disc of approximately 5 mm diameter was transferred aseptically from culture at the centre of each Petri dish and incubated at 28 $\pm 2^0$C for 4 days. The Petri dishes with the culture were observed for radial mycelia growth, colony colour, growth pattern (presence or absence of aerial mycelium or subdued growth etc), appearance, pigmentation secreted metabolite in the substrate medium against white background under sunlight and odour .

3.6.1.4 Microscopic Characterization of the isolates of *Trichoderma* spp.

Conidiophores and conidial morphology, branching pattern, critical for identification to the species level are best observed before the conidia are completely mature. Mounts from the actively growing (fungal growth from the growing colony margin) isolates of *Trichoderma* spp. were prepared in lactophenol cotton blue on glass slide (preferably young tufts where the conidia just begins to develop pigment in actively growing cultures). A strip of cello tape was held in thumb and forefinger and the gum coated surface was placed gently against the sporulating growth of the isolate. The cello tape thus lifted the intact sporulating growth (including conidiophores, phialides, and sporulation) without forming any clump of mycelium and injuring the substrate. Microscopic morphological features of all the selected isolates were observed using a Leica ATC 2000 bi-nocular microscope and micro - 'X' photography was done by using COSLAB DCE-2 attachment at 40X magnification.

3.6.2.: Collection and characterization of native isolates of *Pseudomonas* spp.

Studies were undertaken to identify the fluorescent *Pseudomonas* a bio control agents as potential candidates against *Sclerotium rolfsii* causal agent of dry root rot.

3.6.2.1. Collection of soil samples

Soil samples were collected from different locations of Chhattisgarh (Raipur, Mungeli, Bilaspur, Dhamtari, Baloda bazaar, Bemetara, Kawardha, Griyaband, Jagadalpur, Durg, Rajnandgaon, Korba, Balod, Kanker, Janjgir). Soil samples from the rhizoplane were collected from randomly selected and counted number of plants, which were gently uprooted using trowel to avoid damage and loss of root system. Loosely adhering soil particles were removed by gentle tapping and thereafter, roots were transferred to sterile polythene bags, brought to the laboratory and stored at 28^{o}C. The rhizoplane was removed by mechanically scraping of root surface.

3.6.2.2. Isolation of fluorescent *Pseudomonas* from collected soil sample by serial dilution (Aneja, 1993)

Serial dilutions were prepared for each collected soil sample for isolation of fluorescent *Pseudomonas*. One gram of processed soil was diluted to the strength of 10^{-1} (dilution of the original sample 10 times i.e. 1/1 +9 = 1/10 = 10^{-1}). The soil suspended in the tube was shaken gently but thoroughly to mix soil particles and get them uniformly dispersed. 1ml of the suspension from the first dilutions (10^{-1}) was aseptically transferred to another tube having 10 ml of sterile water marked as (10^{-2}) and thus diluting the original suspension to 100 times (1/10x 1/10 = 1/100 = 10^{-2}). This procedure was repeated till the original sample was diluted to 1000 (10^{-3}) times.

3.6.2.3. Isolation of fluorescent *Pseudomonas* and Designation of isolates

For isolation and enumeration of fluorescent *Pseudomonas*10 µl of soil suspension from the final dilution (10^{-3}) was transferred in sterilized plates. Twenty ml of medium was poured in each plate and gently rotated to ensure uniform distribution on Kings medium ‗B' (KMB) (King's *et al.*, 1954). After solidification the plates were incubated at 28°C for 24 hrs and thereafter examined under on transilluminator against UV light. Colonies with yellow - green and blue-white color pigmentation were marked and recorded.

Individual flourescent colony was picked up with the help of sterilized loop and inoculated on solidified KMB by zig-zag streaking. The plates were

incubated at 28°C for 24 hrs. (colony growing at the last tip of the zig-zag line were transferred to KMB slants).

3.6.2.4. Morphological and Biochemical characterization of the Fluorescent *Pseudomonas* isolates

3.6.2.4.1 Morphological characterization

3.6.2.4.1.1 Gram reaction

This stain is essential for differentiating bacteria in two broad groups; gram positive and gram negative. Gram staining characteristics tire related to structural and chemical properties of the cell wall. On a clean slide, bacterial film was formed by thin spreading a loopfull of the cells on grease free clean micro slide. The film was dried in air for a few minutes. Then the slide was lightly heated at the underside to fix the bacteria on it and stained properly and observed under oil immersion lens of a compound microscope. The gram reactions and the shape of cell was observed and recorded.

3.6.2.4.2 Biochemical characterization

3.6.2.4.2.1 Gelatin hydrolysis test

Gelatin is a protein which dissolves in warm water and exists as a liquid above 25°C and solidifies (gel) when cooled below 25° C. Gelatin hydrolysis was demonstrated by growing test organism in nutrient gelatin / gelatin agar medium. The gelatin hydrolysis was detected by observing liquefaction at low temperature (4°C) after incubation of the test organism in it at 28 ±2° for 48 hrs. Gelatin inoculated tubes that remained liquefied produced gelatinase and showed positive test for gelatin hydrolysis and tubes that remained solid were negative. Gelatin iron agar may also be used for detecting gelatin liquefaction and hydrogen sulphide production.

3.6.2.4.2.2 Nitrate reduction test

Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrogenous gases. The reduction of nitrate may be coupled to anaerobic respiration in some species. The nitrate reduction test was performed using nitrate broth containing following ingredients: peptone 5g, beef extract 3g, potassium nitrate 1g, pH was adjusted to 7 prior to autoclaving. One litre nitrate broth was prepared by adding the component mentioned above and 20ml was distributed in large test tubes. It was then sterilized by autoclaving at 15 lbs pressure at 121°C for 30 minutes. The flasks were inoculated with a fresh culture of the selected isolates and incubated at 28 ±2°C for 48 hrs in an incubator. Five drops each of reagents A (Sulfanillicacid: Himedia make) and B (N,N- dimethyl 1-1 napthylamine: Himedia make) were added to the test tube containing culture to be tested and mixed properly by shaking. A distinct red or pink colour, which should develop within a few minutes indicated nitrate reduction. If the suspension turned pink-red before the addition of Zn powder, the reduction is positive and the test is completed. To the suspensions found colourless after the addition of reagents A and B small amount (—sharp knife point‖) of zinc powder was added to the flasks and after shaking vigorously they were kept at room temperature for 10-15 min. If the medium remain colourless after the addition of Zn powder, the test result is positive. If the medium turns pink after the addition of Zn powder, the result is negative .

3.6.2.4.2.3 Starch Hydrolysis Test

Starch hydrolysis test is used to detect the enzyme amylase, which breaks down starch. Amylase hydrolyses starch (polysaccharide) into maltose, a disaccharide and some monosaccharide such as glucose. Single colony of isolates were streaked on starch agar medium and incubated at 28±2°for 48 hrs. After required incubation, few drops of Lugol‘s Iodine solution were added. A yellow zone around the colony indicated starch hydrolysis, while blue/black area around them indicated or presence of starch.

3.6.2.4.2.4 Casein hydrolysis

Some microorganisms have the ability to degrade the 4\protein casein by producing proteolytic exoenzyme called caseinase which break the peptide bond

CO-NH by introducing water into the molecule, liberating smaller chains of amino acid called peptides which are later broken down into free amino acid by extracellular or intracellular peptidases. Skim milk agar medium was used for casein hydrolysis. After autoclaving the medium was poured in Petri plates the culture was inoculated and incubated at 28 ±2°C or 48 hrs. Formation of clear zone around the colony indicated casein hydrolysis.

3.6.2.4.2.5 Lipolytic activity with Tween 80

The agar medium was prepared with 10.0gm bacteriological peptone, 5.0gm NaCl, 0.1gm $CaCl_2$, 15.0gm agar, pH was set to 6.8 and volume was made up to 1000ml with distilled water. After the medium was autoclaved it was cooled to about 50°C and 5ml of autoclaved Tween 80 was added. The Petri dishes were filled with 25ml of the medium. The inoculated plates were incubated at 28 ±2°C and were examined daily for 10 days.

The presence of a halo around an inoculated site on the Tween medium was viewed with transmitted light; this indicated a positive test and indicated that the *Pseudomonas* isolates produced and esterase. The hydrolysis of the Tween opacity medium is associated with the lipolytic enzyme produced by the respective *Pseudomonas* spp. that liberates fatty acids and bind with the calcium incorporated into the medium. The calcium complex is visible as insoluble crystal around the inoculated site.

3.6.2.4.2.6 Growth at 4°C and 42°C

Pseudomonas isolates were inoculated in Kings B‘ medium, incubated at 4°C and 42°C for 48 hrs and observation was taken.

3.6.2.4.2.7 Antibiotic sensitivity test

A rapid antibiotic sensitivity test was used to distinguish different species of fluorescent *Pseudomonas*. Antibiotic sensitivity studies were performed by the streak plate method.

3.6.2.4.2.8 Triple Sugar Iron agar test

Triple Sugar Iron agar media is used for the differentiation of microbes by ability to determine carbohydrate fermentation and hydrogen sulphide production. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amounts of acids are liberated in butt

(fermentation) than in the slant (respiration). Growing bacteria also form alkaline product from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt. Thus the appearance of the alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and or sucrose (or both), in addition of glucose, produce large amount of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO_2) is detected by the presence of cracks of bubble in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H_2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube.

Triple sugar iron agar medium was prepared by dissolving 16.13gm TSI powder in 1000ml of distilled water and slants were prepared to set in sloped form with a butt about 1 inch long slants were streaked, incubated at 28±2° for 48 hrs and results were observed. The reaction can be summarized as follows-

Table 3.3: Reaction for Triple sugar iron agar test

S. No.	Reaction	Colour“	Description“
1.	Acidic Butt	Yellow	Only dextrose fermented
	Alkaline slant	Red	Glucose and sucrose fermented or
2.	Acid Butt	Yellow	glucose and lactose fermented or all the
	Acid slant	Yellow	three sugar, glucose, lactose and sucrose
3.	Bubbles or cracks	-	fermented Gas production
4.	Black precipitate	-	H_2S gas production

3.6.2.4.2.9 Hydrogen sulphide gas production test

This test was performed in sulphide indole motility (SIM) agar medium. All the ingredients of the medium were dissolved in 1000ml of distilled water, dispensed in tubes and autoclaved. The test organism was stab inoculated and incubated at 28±2℃ for 48 hrs. The tubes were observed for the presence or absence of black coloration along the line of stab inoculation. The black colour indicated positive result. The black colour is due to the production of H_2S from sodium thiosulphate present in the medium that then combines with ferrous ammonium sulphate resulting in the formation of black insoluble ferrous sulphide.

3.6.3.: Collection and characterization of native isolates of *Bacillus* spp.

3.6.3.1. Isolation of *Bacillus* spp.

The isolation of *Bacillus* spp. from the collected soil samples was made by Heat treatment method described by Walker *et al*. (1998).

For enrichment of spores from the soil samples, 1 g of soil sample was suspended in 9 ml sterile distilled water and was incubated in water bath at 80^0C for 10 min. Serial dilutions were made upto 10^{-6} and 100 µl of the suspension was spread on the nutrient agar medium plates enriched with 1% dextrose. The plates were incubated at 28 –30^0C for 24–40 h. The screened colonies were picked

3.6.3.2. Biochemical characterization of *Bacillus*

For the identification of *Bcillus* spp. isolates, following selected biochemical tests were conducted according to Schaad *et al.* (2003).

3.6.3.2.1. Gram staining

Gram staining test is essential for differentiate between gram positive and gram negative Bacteria. Bacterial culture smear was prepared by transferring a loop full freshly prepared bacterial culture suspension on a clean slide and a smear was made. The smear was air dried and heat fixed. The smear was flooded with crystal violet stain solution (primary stain) for one minute. The slide was washed in a gentle stream of tap water for maximum five second to remove unbound crystal violet stain and blotted dry. Then the slides were flooded with Lugol's iodine solution (mordant, fixes the crystal violet to the bacterial cell wall) for one minute and then again the slide was washed with tap water and blotted dry and then flooded with 95 percent ethyl alcohol for 30 seconds to decolorize the slide. The smear was washed in gentle stream of water and counter stained (secondary stain) with safranin for 30 seconds. Slide washed with tap water and blot dry. The slide was observed under compound microscope at different magnification. The Gram negative cells appeared red while Gram positive cells appeared violet colour.

Solutions for Gram reaction:

1. Crystal violate reagent-

Solution A: 2 grams of crystal violet (certified 90 percent of the dye content) and 20ml of ethanol (95percent vol/vol)

Solution B: 0.8 grams of ammonium oxalate, and 80 ml of distilled water

2. **Mordant (grams iodine)-** gram of iodine, grams of potassium iodide, and 300 ml of distilled water,
3. **Decolorizing agent-** 50 ml acetone and 50 ml ethanol (95%)
4. **Counterstain (safranin)**-Working solution: 10 ml of the stock solution (2 g Safranin O and 100 ml of 95 percent ethanol) and 90 ml of distilled water.

3.6.3.2.2. Growth at 45^{o}C temperature

To evaluate the ability of the isolates of *Bacillus* spp. to grow at 45^{o}C, all the *Bacillus* spp. isolates were inoculated in test tubes (in duplicate) containing liquid casein medium [Casein acid hydrolysate- 10 g, Yeast extract- 5 g, K_2HPO_4- 4 g, Dextrose (50% sterile stock solution) 10ml, Distilled water- 1000 ml and pH 5.7]. The inoculated test tubes were incubated at 45^{o}C in a shaker incubator. After five days of incubation the tubes were observed for turbid growth of *Bacillus* spp. in liquid casein medium.

3.6.3.2.3. Growth in 7% NaCl

In this test all isolates of *Bacillus* spp. were inoculated into test tubes (in duplicate) containing liquid nutrient broth plus 7% NaCl and incubated at 28±2^{o}C in a shaker incubator. The tubes were observed daily for growth.

3.6.3.2.4. Growth in PH 5.7

All *Bacillus* spp. were inoculated into test tubes (in duplicates) containing liquid casein medium [Casein acid hydrolysate- 10.0, Yeast extract- 5 g, K_2HPO_4- 4 g, Dextrose (50% sterile stock solution), distilled water- 1000 ml; pH adjusted to pH 5.7) and incubated at 45˚C in shaker incubator. The tubes were observed daily any visible growth of bacteria.

3.6.3.2.5. Utilization of citrate

For this test all isolates of *Bacillus* spp. were streaked in Simmon's citrate agar slants ($MgSO_4$,$7H_2O$- 0.2 g, Na_2H2PO_4- 1 g, K_2HPO_4- 2 g, Sodium citrate- 2 g; NaCl- 5 g, Bromothymol blue- 80 mg, Agar- 20 g, pH adjusted to 6.8-6.9) and un-inoculated slant serve as a control. The slants were incubated at 28±2^{o}C for 48 hours. The presence of bacterial growth in the slants and change of colour from

green to blue was considered as positive for citrate utilization. Unchanged green colour of the medium indicates negative results. Some organisms are capable of growth on citrate and do not produce a colour change. Growth is considered a positive citrate utilization test, even in the absence of a colour change .

3.6.3.2.6. Starch hydrolysis test

Some bacteria possess the ability to produce amylase which is extracellular enzymes (exo-enzymes). The Amylase is secreted out from the bacterial cells and diffuse into the starch agar that break down starch into glucose units. The isolates of *Bacillus* spp. were spot inoculated in culture plates containing nutrient agar supplemented with 0.2% soluble starch. The plates were incubated at 28±2°C for five days. The plates were then flooded with Lugol's iodine solution (Iodine 1.0 g + Potassium Iodine 20 g in 100 ml of water). Hydrolysis of starch was indicated by formation of opaque zones around the bacterial colonies after few minutes.

3.6.3.2.7. Voges -Proskauer (VP) Test

VP test is used to detect acetoin production in bacterial broth culture. All isolates of *Bacillus* spp. were inoculated in test tubes containing VP broth (Buffered peptone- 7 g, Dextrose- 5 g, Dipotassium phosphate- 5 g, pH of the medium adjusted to 6.9). The tubes were incubated at 35^{o}C for 24 hours. After 24 hours of incubation, 0.6 ml of 5% alpha naphthol followed by 0.2 ml of 40% KOH were added to the test tubes and the tubes were shaken gently for several minutes to expose the medium to atmospheric oxygen and then allowed the tubes to remain undisturbed for 10-15 minutes. Formation of red color within 15 to 20 minutes indicated positive result and yellow-brown color indicates a negative result.

3.6.3.2.8. Anaerobic growth in glucose broth

This test was performed to test the ability of the isolates of *Bacillus* spp. to grow under anaerobic condition. The isolates of *Bacillus* spp. were inoculated in test tubes containing glucose broth (Nutrient broth supplemented with 50 ml of 20% dextrose solution). Two sets of test tubes were made with each isolate. One tube was overlaid with 1ml sterile mineral oil (for creating anaerobic condition) and the other tube was without mineral oil (for aerobic control) and then incubated at 28±2°C. Observations were taken up to 14 days for any bacterial growth

(turbidity). Dextrose stock (20%) was prepared and autoclaved separately and added to the autoclaved nutrient broth tubes separately under aseptic condition.

3.4.3.2.9. Acid production from carbohydrates

In this test sugars like arabinose, manitol and xylose which are recommended for the differentiation of different species of *Bacillus* were tested. The test was conducted in Hugh and Leifson medium (peptone- 2.0 g, NaCl- 5.0 g, KH_2PO_4- 0.3 g, Agar- 3.0 g, Bromothymol blue 1% aqueous solution- 3.0 ml and 997 ml distilled water; pH adjusted to 7.1). The medium was heated in a microwave oven for melting of agar and then respected sugar was added to a final concentration of 0.5%. The sugar was mixed thoroughly and poured in test tubes @ 5 ml/tube. The tubes were then sterilized through tyndalization. Tyndallization essentially consists of heating the substance to boiling point or just a little below boiling point and holding it there for 15 minutes, three days in succession. Each isolate was inoculated in tubes (for each sugar) then incubated at 28±2°C and examined daily for 14 days. Acid production was indicated by a change of colour of the medium to yellow. randomly and inoculated in King's B broth and incubated in incubator shaker at 30^0C for 24 h. The isolates were stored in King's B broth with 50% (w/v) glycerol at -80^0C and revived on King's B slants as per requirement.

3.7 Management of collar rot of lentil.

To develop suitable management strategies using cultural, biological and chemical tools in reducing lentil collar rot

3.7.1: Management through cultural method

3.7.1.1: Effect of plant age on collar rot disease development:

To know the effect of age of the crop, an experiment was conducted under glass house condition . Four stages of the lentil crop were taken for susceptible reaction with the collar rot causal pathogen *Sclerotium rolfsii*. These stages of rop were maintained in the plastic pots of 25 cm diameter filled with sterilized soil . In all the stages, inoculum was added to the soil. After 14 days of inoculation, number of plants showing collar rot symptoms due to *S. rolfsii* was recorded. Treatment details are-

Table 3.4: **Plant age on collar rot disease development**

S.N.	Treatment	Plant age	Stage
1.	T_1	10 days	$_1$st
2.	T_2	20 days	$_2$nd
3.	T_3	30 days	$_3$rd
4.	T_4	45 days	$_4$th

3.7.1.2: Effect of different date of sowing on disease incidence

Fifteen surface sterilized seeds of K34 lentil variety were sown at an interval of 10 days in soil filled in pots. The pots are filled with sterilized soil and inoculated by *Sclerotium rolfsii* before the sowing of seeds. The seeds are sown according to given plan of DAS (20th Oct., 30th Oct., 10th Nov., 20thNov. and 30thNov.). Four replications were maintained for each treatment. The pots were irrigated when required. Observations on incidence of wilt disease were taken periodically after 7 days of sowing continued up to 60 days .

3.7.2 Management through biological control

biocontrol agents *viz.,Trichoderma* spp., *Pseudomonas* spp. *and Bacillus spp.* were evaluated to test the antagonism against *Sclerotium rolfsii*

3.7.2.1 Potentiality of bio control agent *Trichoderma* spp. Against *Sclerotium rolfsii*

3.7.2.1.1 Collection of soil samples

Soil samples were collected from different locations of Chhattisgarh (Abhanpur, Ambikapur, Bemetara, Bhatapara, Dhamtari, Dhamdha, Durg, Kanker, Khadsiya, Lohara, Raigarh, Raipur, Rajnandgaon, Sitapur, Thankhamariya, Uattai and Udaypur). Soil samples from the rhizoplane were collected from randomly selected and counted number of plants, which were gently uprooted using trowel to avoid damage and loss of root system. Loosely adhering soil particles were removed by gentle tapping and thereafter, roots were transferred to sterile polythene bags, brought to the laboratory and stored at 28^{o}C. The rhizoplane soil was removed by mechanically scraping of root surface.

3.7.2.1.2 Isolation of *Trichoderma* spp. from collected soil sample

Trichoderma spp. were isolated from the collected soil samples by the following procedure developed for serial dilution method (Johnson and Curl, 1972). using Potato Dextrose Agar (PDA) medium. Rhizoplane soil samples collected were subjected to serial dilutions. One gram of soil was diluted to the strength of 10^{-1} by dissolving it in 10 ml distilled water (DW) (dilution of the original sample to 10 times). The soil suspended in the tube was shaken gently so as to thoroughly mix the soil particles and get them uniformly dispersed. One ml of the suspension from the first dilution ($l0^{-1}$) was transferred aseptically to another tube containing 10 ml DW marked as 10^{-2} and thus diluting the original suspension to 100 times (1/10 x 1/10 = 1/100 = 10^{-2}). This procedure was repeated till the original sample was diluted to 100000 times suspend. Soil once diluted to strength of 10^{-5} was inoculated on to the PDA plates by spread plate technique. The inoculated plates were incubated at 28°C and were observed for the appearance of *Trichoderma* spp. which were then aseptically transferred to PDA slants on which the growth appears within 1-2 days. The cultures were identified and confirmed as *Trichoderma spp.* through microscopic observation and selective growth on TSM (Trichoderma Selective Media) medium.

3.7.2.1.3. Potato Dextrose agar

Required amount of peeled potato was cut into fine pieces. It was boiled in 500 ml of distilled water for 30 minutes and filtered through muslin cloth. Thereafter, 20 g of dextrose and 20 g of Agar-agar were dissolved in 500 ml boiling water. Potato extract was added in boiling mixture and mixed thoroughly by stirring with glass rod. After few minutes of boiling it was transferred to, about 200 ml in each, 500 ml capacity flasks and plugged with non- absorbent cotton. The pH of the medium was adjusted to 7.0 ± 0.2 in the same way as mentioned above and autoclaved at 15 lbs p.s.i. at 121.6°C for 15 minute.

3.7.2.1.4. Potato Dextrose broth

Required amount of peeled potato was cut into fine pieces. It was boiled in 500 ml of distilled water for 30 minutes and filtered through muslin cloth. Thereafter, 20 g of dextrose was dissolved in 500 ml boiling water. Potato extract was added in boiling mixture and mixed thoroughly by stirring with glass rod.

After few minutes of boiling it was transferred to, about 200 ml in each, 500 ml capacity flasks and plugged with non- absorbent cotton. The pH of the medium was adjusted to 7.0 ± 0.2 in the same way as mentioned above and autoclaved at 15 lbs p.s.i. at 121.6°C for 15 minute.

3.7.2.1.5. Slant preparation and PDA plating

The melted potato dextrose agar (PDA) medium was transferred @ 5 ml per culture tube. While transferring care was taken that medium should not touch the inner wall of culture tubes. The culture tubes were sterilized at 15 lbs p.s.i. at 121.6°C for 15 minutes. After sterilization, it was allowed to solidify in slanting position and then stored in refrigerator for further use. Similarily, the sterilized and melted medium was poured aseptically in sterilized petriplates @ 20 ml per petriplate.

3.7.2.1.2. Morphological characterization

All the isolates of *Trichoderma* spp. were subjected to morphological characterization based on their radial growth and colony characteristics.

3.7.2.1.2.1 Radial growth

For measuring the radial growth rate, all the 40 isolates of *Trichoderma* were inoculated in four replications at the center of 85 mm PDA plates. Inoculum was in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates. The plates were incubated at 27°C and the radial growth was measured (in mm) 1 day, 2 days, 3 days, 4 days and 5 days post inoculation.

3.7.2.1.2..2 Colony characteristics

Colony characteristics were observed after five days of incubation of *Trichoderma* isolates of PDA petriplates. The inoculum was taken in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates. The plates were incubated at 27°C and colony type, shape and colour was observed from the top view of culture grown petriplates. However, production of any kind of secondary metabolite was observed from the colour of the colonies from the back side of the culture plate.

3.7.2.1.2.3 Inoculation of liquid media

Five days old culture was used for inoculation. Mycelial discs of 5mm diameter were cut from the margins of the colonies by a sterilized 5mm metal corkborer. A set of three such disc was transferred to each flask of potato dextrose broth by using a sterilized inoculation needle. The inoculated flasks were incubated for 5 days at 24 ± 2ºC.

3.7.2.1.2.4. Recording mycelial dry weight of the *Trichoderma* isolates obtained on liquid media.

The growth of different isolates, obtained on liquid media was measured by dry weight. For this purpose, Whatman filter paper Number 44 was used. The Whatman filter paper was kept in an oven at 60ºC for 48 hrs. and then weighted on an electronic balance. Then the mycelial growth of the *Trichoderma* isolates was poured on the filter paper kept in funnel and was left for filtration for 1 hr. The mycelial mat was retained on the filter paper. This was weighed carefully for calculating the moist weight of mycelium. The Whatman filter paper along with the mycelial growth of different *Trichoderma* isolates was kept in sterilized petriplate and subjected to drying at 60 ºC to get constant weight. The filter paper along with dried mycelium was again weighed and the difference between the final and initial weight of the filter paper denoted the dry wt. of the *Trichoderma* isolates. Four replications of each treatment were maintained.

3.7.2.1.3. Screening of *Trichoderma* spp. isolates

3.7.2.1.3.1. Growth of antagonist and the pathogen in monoculture

To study the growth of antagonists and the test fungus in monoculture, 5 mm mycelial discs of *Trichoderma spp.* florescence *Pseudomonas spp. and Bacillus subtillus* and *Sclerotium rolfsaii* were inoculated centrally on sterilized PDA in Petri-dishes. Then plates were incubated in BOD incubator at 25 + 1^0C. Observations on colony diameter of individual antagonist and the pathogen were recorded after 96 hrs of incubation.

3.7.2.1.3.2.: Growth of antagonist and the pathogen in dual culture

Screening of all antagonists was done against *Sclerotium rolfsii through* dual culture technique developed by (Bell *et al*., 1982) wenty ml sterilized melted PDA medium was poured into sterilized Petri-plates @ 20 ml/plate aseptically, allowed to solidify, then 6mm discs of the fungus and the

antagonistic cut with the help of sterilized cork borer were placed on PDA approximately 4 cm apart each other and incubated in BOD incubator at 25 ± 1ºC for 96 hrs. three repetitions were maintained for each treatment . The per cent growth inhibition was calculated by using the formula:

$$I = C\text{-}T / C \times 100$$

Where,

I = Per cent inhibition

C= Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

3.7.2.1.4. Sclerotial parasitization

Sclerotia parasitization activity of *Trichoderma* spp. was studied under laboratory conditions using soil plating technique. The sclerotia were used from the 10 days old culture of *Sclerotium rolfsii*, isolated from foot rot infected finger millet. The *Trichoderma* inoculum was taken in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates and inoculated in triplicate on fresh PDA plates. The plates were incubated at 27°C for five days. After five days of incubation, 10 grams of sterilized soil was taken and spreaded over the five days grown cultures of *Trichoderma* spp under laminar air flow. 10 numbers of sclerotia were placed over spreaded soil in circular fashion at uniform distance. The plates were carefully sealed without disturbing the sclerotia and incubated at 27°C for ten days. After ten days of incubation, sclerotia were carefully picked individually with the help of forceps and pressed to evaluate the colonization and killing of sclerotia. Observations were recorded for number of sclerotia colonized in each treatment .

3.7.2.1.5. Formulation of *Trichoderma* spp.

The *Trichoderma* spp. isolate were grown in Erlenmeyer flasks each containing 100 ml of potato dextrose broth for 1 week at 25^0C and thoroughly mixed with the medium using magnetic stirrer. After filtration, the spore suspension was adjusted to 5 x 10^8cfu/ml with a haemocytometer. Prior to mixing of mycelial paste with talcum powder (carrier), 20 g of carboxymethylcellulose (CMC) (adhesive) was added to 1 kg of carriers and mixed well. The carriers

were autoclaved at 121^0C for 30 min on two consecutive trays. Then the mycelial paste was mixed thoroughly with the carriers under sterile conditions in a laminar hood. Formulations prepared were spread on sterile filter paper towel in a laminar hood and large clumps were broken. The formulations were allowed to dry for 12–16 hours. Completely dry formulations were crushed in a blender for 1 min, packed in airtight polythene bags and stored at 4^0C for further study.

3.7.2.1.6. *In vivo* efficacy of *Trichoderma* spp. under glass house condition

Pot experiments (CRD) was carried out under green house conditions. The plastic pots (12 cm diameter) filled with sterilized sandy loam soil, were futher inoculated with two week old culture of *Sclerotium rolfsii* (RB4) (prepared on sand maize medium) @ 30g/kg soil and allowed to stabilize for five days.

Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with microbial antagonists viz., *Trichoderma* spp. by using 2 per cent sticker for 1 hour. Eight seeds per pot were sown in 12 cm diameter plastic pots, each containing 400 g of soil. Three replications of each treatment were maintained. Pots without antagonist, seed coating material (untreated) were served as control. Root rot incidence was recorded up to 30 days after sowing. Pots were kept randomly in a green house. Ten treated seeds microbial antagonists were transferred in test tube containing 9 ml sterile distilled water. The test tubes were shaken and dilution series was made. One ml suspension was poured on PDA and fungal conidia / seed was calculated by using the formula: No. of cells or conidia x dilution factor.

Germination was recorded at ten days of sowing. The per cent mortality was observed after 30 days of sowing. Then plants were uprooted for measurement of root length and shoot length.

3.7.2.2.: Potentiality of bio control agent fluorescent *Pseudomonas* spp. against *Sclerotium rolfsii*

Studies were undertaken to identify the fluorescent *Pseudomonas* a bio control agents as potential candidates against *Sclerotium rolfsii* causal agent of dry root rot .

3.7.2.2.1. Collection of soil samples

Soil samples were collected from different locations of Chhattisgarh (Raipur, Mungeli, Bilaspur, Dhamtari, Baloda bazaar, Bemetara, Kawardha, Griyaband, Jagadalpur, Durg, Rajnandgaon, Korba, Balod, Kanker, Janjgir). Soil samples from the rhizoplane were collected from randomly selected and counted number of plants, which were gently uprooted using trowel to avoid damage and loss of root system. Loosely adhering soil particles were removed by gentle tapping and thereafter, roots were transferred to sterile polythene bags, brought to the laboratory and stored at 28^{o}C. The rhizoplane was removed by mechanically scraping of root surface.

3.7.2.2.2 Isolation of fluorescent *Pseudomonas* from collected soil sample by serial dilution (Aneja, 1993)

Serial dilutions were prepared for each collected soil sample for isolation of fluorescent *Pseudomonas*. One gram of processed soil was diluted to the strength of 10^{-1} (dilution of the original sample 10 times i.e. 1/1 +9 = 1/10 = 10^{-1}). The soil suspended in the tube was shaken gently but thoroughly to mix soil particles and get them uniformly dispersed. 1ml of the suspension from the first dilutions (10^{-1}) was aseptically transferred to another tube having 10 ml of sterile water marked as (10^{-2}) and thus diluting the original suspension to 100 times (1/10x 1/10 = 1/100 = 10^{-2}). This procedure was repeated till the original sample was diluted to 1000 (10^{-3}) times. Twenty ml of medium was poured in each plate and gently rotated to ensure uniform distribution on Kings medium _B' (KMB) (King's *etal.*, 1954). After solidification the plates were incubated at 28°C for 24 hrs and thereafter examined under on transilluminator against UV light. Colonies with yellow - green and blue-white color pigmentation were marked and recorded.

Individual flourescent colony was picked up with the help of sterilized loop and inoculated on solidified KMB by zig-zag streaking. The plates were incubated at 28°C for 24 hrs. (colony growing at the last tip of the zig-zag line were transferred to KMB slants).

3.7.2.2.3. Formulation of fluorescent *Pseudomonas*

The fluorescent *Pseudomonas* isolates were grown on King's B (KB) broth for 48 h as shake culture in shaking incubator at 150 rpm and 25±2^{0}C temperature. Talc-powder was taken as carrier to prepare formulation. Ten grams of Carboxy

Methyl Cellulose (CMC) was added to 1 kg of the talc-powder and mixed well. The pH of all materials was adjusted to 7.0 by adding calcium carbonate (10 gm/kg carrier). The talc-powder was autoclaved for 30 min on each of two consecutive days. Four hundred milliliters of the bacterial suspension containing 9 × 10^8 colony forming units (cfu) per ml was added to 1 kg of the talc-powder and mixed well then air dried under sterile conditions. The materials were packed in polythene bag, sealed and incubated at room temperature. (Vidhyasekaran and Muthamilan, 1995)

3.7.2.2.4. Screening for antagonistic activity of fluorescent *Pseudomonas* against *S. rolfsii*

3.7.2.2.4.1. Dual culture interaction

Fluorescent *Pseudomonas* was screened by dual culture method to test the efficacy as bio-control agent against *S. rolfsii*. Fluorescent *Pseudomonas* isolates were multiplied on King's B broth and incubated for 2 days at 28^0C till the fluorescent pigment appeared in the broth. Equal volume of sterilized potato dextrose agar (PDA) and King's B medium were mixed in sterilized Perti plates . The edge of a glass funnel was sterilized by dipping in alcohol followed by flaming. Broth containing young growing cell of fluorescent *Pseudomonas* was dispensed in sterile Petri plate and picked at the edge of the funnel by dipping. Care was taken to remove the excess inoculum by gently shaking the funnel. Inoculation was done by gently touching the edge of the funnel (containing Fluorescent *Pseudomonas*) which encircled the pre-inoculated with *S. rolfsii* on was placed in centre of the plate. Control was also maintained to see the difference. Observation was taken after three days of inoculation.

In vitro antagonistic potential of different isolates of fluorescent *Pseudomonas* was assessed by calculating percentage inhibition growth of pathogen (*S. rolfsii*) over control in presence of fluorescent *Pseudomonas* using the formula:

$$\text{Per cent inhibition} = I = C\text{-}T / C \times 100$$

Where,

I = Per cent inhibition

C = Radial growth of pathogen in control

T= Radial growth of pathogen in presence of fluorescent *Pseudomonas*

3.7.2.2.5. *In vivo* efficacy of fluorescent *Pseudomonas* under glass house condition

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S. rolfsii* (prepared on wheat grain medium) @ 25g/ kg soil and allowed to stabilize for five days. Seeds of chickpea were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with microbial antagonist viz., fluorescent *Pseudomonas* by using 2 per cent CMC sticker for 1 hour. Ten seeds per pot were sown in 15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of each treatment were maintained. Pots without antagonist, seed coating material (untreated) were served as control. Pots were kept randomly in a glass house. Ten treated seeds with microbial antagonists were transferred in test tube containing 9 ml sterile distilled water. The test tubes were shaken and dilution series was made. One ml suspension was poured on PDA and bacterial cells /seed was calculated by using the formula: No. of cells x dilution factor.

Germination was recorded after ten days after sowing. The per cent mortality was taken in 15 days interval upto 45 days after sowing. Then plants were uprooted for measurement of root length and shoot length.

3.7.2.3.: Potentiality of bio control agent *Bacillus* spp. against *Sclerotium rolfsii*

3.7.2.3.1: Isolation of *Bacillus* spp.

The isolation of *Bacillus* spp. from the collected soil samples was made by Heat treatment method described by Walker *et al*. (1998). For enrichment of spores from the soil samples, 1 g of soil sample was suspended in 9 ml sterile distilled water and was incubated in water bath at 80^0C for 10 min. Serial dilutions were made upto 10^{-6} and 100 µl of the suspension was spread on the nutrient agar medium plates enriched with 1% dextrose. The plates were incubated

at 28–30^{o}C for 24–40 h. The screened colonies were picked randomly and inoculated in King's B broth and incubated in incubator shaker at 30^{0}C for 24 h. The isolates were stored in King's B broth with 50% (w/v) glycerol at -80^{0}C and revived on King's B slants as per requirement.

3.7.2.3.2: Formulation of *Bacillus* spp.

The *Bacillus* spp. isolates were grown on King's B (KB) broth for 72 h as shake culture in shaking incubator at 150 rpm and 25±2^{0}C temperature. Talc-powder was taken as carrier to prepare formulation. Ten grams of Carboxy Methyl Cellulose (CMC) was added to 1 kg of the talc-powder and mixed well. The pH of all materials was adjusted to 7.0 by adding calcium carbonate (10 gm/kg carrier). The talc-powder was autoclaved for 30 min on each of two consecutive days. Four hundred milliliters of the bacterial suspension containing 9 × 10^{8} colony forming units (cfu) per ml was added to 1 kg of the talc-powder and mixed well then air dried under sterile conditions. The materials were packed in polythene bag, sealed and incubated at room temperature (25±2^{0}C).

3.7.2.3.3. Screening for antagonistic activity of *Bacillus* spp. against *S. rolfsii*

3.7.2.3.3.1. Dual culture interaction

Bacillus spp. was screened by dual culture method to test the efficacy as bio-control agent against *S. rolfsii*. *Bacillus* spp. isolates were multiplied on King's B broth and incubated for 3 days at 28^{0}C. Equal volume of sterilized potato dextrose agar (PDA) and King's B medium were mixed in sterilized Perti plates. The edge of a glass funnel was sterilized by dipping in alcohol followed by flaming. Broth containing young growing cell of *Bacillus* spp. was dispensed in sterile Petri plate and picked at the edge of the funnel by dipping. Care was taken to remove the excess inoculum by gently shaking the funnel. Inoculation was done by gently touching the edge of the funnel (containing *Bacillus* spp.) which encircled the pre-inoculated with *S. rolfsii* on was placed in centre of the plate. Control was also maintained to see the difference. Observation was taken after three days of inoculation.

In vitro antagonistic potential of different isolates of *Bacillus* spp. was assessed by calculating percentage inhibition growth of pathogen (*S. rolfsii*) over control in presence of *Bacillus* spp. using the formula:

Per cent inhibition = I = C-T / C x 100

Where,

I = Per cent inhibition

C = Radial growth of pathogen in control

T= Radial growth of pathogen in presence of *Bacillus* spp.

3.7.2.3.4. *In vivo* efficacy of *Bacillus* spp. under glass house condition

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S. rolfsii* (prepared on wheat grain medium)@ 25g/ kg soil and allowed to stabilize for five days. Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with microbial antagonist viz., *Bacillus* spp. by using 2 per cent CMC sticker for 1 hour. Ten seeds per pot were sown in 15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of each treatment were maintained. Pots without antagonist, seed coating material (untreated) were served as control. Pots were kept randomly in a glass house. Ten treated seeds with microbial antagonists were transferred in test tube containing 9 ml sterile distilled water. The test tubes were shaken and dilution series was made. One ml suspension was poured on PDA and bacterial cells /seed was calculated by using the formula: No. of cells x dilution factor.

Germination was recorded after ten days after sowing. The per cent mortality was taken in 15 days interval upto 45 days after sowing. Then plants were uprooted for measurement of root length and shoot length.

3.7.2.4. Seed treatment with biocontrol agent

3.7.2.4..a Seed treatment with different doses

Evaluation of biocontrol agent *Trichoderma* spp., fluorescent *Pseudomonas* spp. and *Bacillus* spp., seed treatment with doses (5g, 10g, 15g,

20g, 25g and 30g per kg seed) to find out effective seed dose for control of collar rot of lentil under glass house condition.

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S. rolfsii* (prepared on wheat grain medium) @ 25g/ kg soil and allowed to stabilize for five days. Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with each microbial antagonist viz., *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp. with different seed doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) by using 2 per cent CMC sticker for 1 hour. Ten seeds per pot were sown in 15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of each treatment were maintained. Pots without antagonist, seed coating material (untreated) were served as control . Then plants were uprooted for measurement of root length and shoot length.

3.7.2.4. b Seed treatment and soil application with biocontrol agent.

The experiment was conducted to find out an effective antagonistic treatment (*Trichoderma* spp., fluorescent *Pseudomonas* spp. and *Bacillus* spp.) which enhance germination, shoot length, root length and minimize mortality of seedling by collar rot of lentil.

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S.rofsii* (prepared on wheat grain medium) @ 25g/ kg soil and allowed to stabilize for five days. Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. Seeds of lentil were sown with different treatment *viz*; seed treatment, seed priming, soil treatment, seed treatment + soil treatment, seed priming + soil treatment and control (untreated) with effective talc formulation of each biocontrol agent *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp. Ten seeds per pot were sown in 15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of

each treatment were maintained. Pots without antagonist, seed coating material (untreated) were served as control.

Table 3.5: Types of treatment along with dosages:

Sr. No.	Treatment	Types of treatment	Dosages
1	T1	Seed treatment	10 g/kg seed
2	T2	Seed Priming	10 g/ kg seed soaked for 2 hr
3	T3	Soil treatment	50 g/kg soil
4	T4	Seed + soil treatment	10 g/kg seed +50 g/kg soil
5	T5	Seed Priming + soil treatment	10 g/ kg seed soaked for 2 hr + 50 g/kg soil
6	T6	Control (Untreated)	-

Germination was recorded after ten days after sowing. The per centmortality was observed in 15 days interval upto 45 days after sowing. Then plants were uprooted for measurement of root length and shoot length.

3.7.3 Management through botanicals control

Table 3.6. List of botanicals evaluated against *S. rolfsii*

S.No	Botanical name	Common name	Family	Part used
1	*Azadirachta indica* A. Juss.	Neem	Meliaceae	Leaves
2	*Azadirachta indica* A. Juss.	NSKE*	Meliaceae	Seed
3	(*Aegle marmolus*)	Bel	Asparagaceae	Leaves
4	(*Cymbopogan flexuosus*),	Lemongrass	Lamiaceae	Leaves
5	*Calotropis procera*	Madar	Leguminaceae	Leaves
6	*Lawsonia inermis* L.	Henna/Mehndi	Lythraceae	Leaves
7	*Allium sativum* L.	Garlic	Amaryllidaceae	Bulb
8	*Zingiber officinale* Roscoe.	Ginger	Zingiberaceae	Rhizome
9	*Pongamia pinnata*	Karanj	Asteraceae	Leaves
10	*Eucalyptus oblique* L'Hér.	Eucalyptus	Myrtaceae	Leaves

*****NSKE- Neem seed kernel extract

Neem (*Azadirachta indica* A. Juss.*)*, NSKE*(*Azadirachta indica* A. Juss.*)*, Agave(*Agave tequilan* F.A.C. Weber), Glory flower(*Clerodendrum inerme* (L.) Gaertn), Hongae tree(*Pongamia pinnata* (L.) Panigrahi), Henna/Mehndi(*Lawsonia inermis* L.), Garlic(*Allium sativum* L.), Ginger(*Zingiber officinale* Roscoe.), Coat buttons(*Tridax procumbens* L.), Eucalyptus(*Eucalyptus oblique* L'Hér.)

3.7.3.1. Preparation of cold aqueous extract

Fresh sample of each test plant were collected and washed first in tap water and then in distilled water. 100 g of fresh sample was crushed in a surface sterilized Pestle and Mortar by adding 100 ml sterile distilled water (1:1 w/v). The extract was used as stock solution.

To study the anti-fungal activity of plant extract, the poisoned food technique was followed. Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium respectively, as to get 5, 10 and 15 per cent concentrations. The medium was thoroughly shaken for uniform mixing of the extract and then after adding the botanicals the media was sterilized again. To avoid bacterial contamination, a pinch of Streptomycin sulphate was added at the time of pouring of media in petriplate.

3.7.3.2. *In vitro* evaluation of botanicals against *S. rolfsii*

Twenty ml of medium was poured into each of the 90 mm sterilized Petri plates. Each plate was inoculated with 5 mm mycelial discs taken from the periphery of fungal culture and incubated at 27 ±10C till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the center of the Petri plate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration.

Three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947). The data were analyzed statistically.

3.7.4 Management through Chemicals control

3.7.4.1. *In vitro* evaluation of fungicides against *S. rolfsii* by poison food technique

The efficacy of seven systemic fungicides (Carbendazim 50% WP, Tricyclozole 75% WP, Hexaconazole 5% EC Propiconazole 25% EC, Azoxystrobin 35% EC, Benomyl 50% WP and Thiophanate Methyl 70% WP); four non-systemic fungicides (Mancozeb 75%WP, Thiram 75% WS, Copper oxychloride 50WP and Propineb 70% WP) and six combo fungicides (Metalaxyl 8% +Mancozeb 64% , Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70% + Hexaconazole 5% WP, Propiconazole 13.9% + Difenoconazol 13.9%, Carboxin 37.5% + Thiram 37.5% and Carbendazim 12% + Mancozeb 63% WP) was evaluated *in vitro* at different concentrations of 20, 50, 100,200 and 500 ppm on the growth of *Sclerotium rolfsii* on Potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 1982). The list of fungicides used are given below.

Table 3.7: Trade name, Active ingredient, Formulation and Chemical name of fungicide

S. No.	Trade Name	Active ingredient	Formulation	Chemical name
Systemic Fungicide Group				
1	Bavistin	Carbendazim	50%WP	Methyl 1H-benzimidazol-2-ylcarmate M
2	Sivic	Tricyclazole	75% WP	5-Methyl-1,2,4-triazole(3,4-b)benzothiazole
3	Contaf Plus	Hexaconazole	5% EC	RS-2-(2, 4-D)-1-(1H-1, 2, 4 Trizole-1-yl) hezan 2-ol
4	Tilt	Propiconazole	25% EC	1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-1H-1,2,4-triazole
5	Amister	Azoxystrobin	23%	Methyl(E)-2-(2-(6-(2-cyanopheoxy) pyrimidin-4-yloxy)phenyl)-3-methoxypropenoate
6	Binofit	Benomyl	50% WP	methyl-1-[(butylamino)carbonyl]-H-benzimidazol-2-ylcarbamate [1]
7	Roco	Thiophanate methyl	70% WP	Dimethyl [(1,2-phenylene)bis-(iminocarbonothioyl) bis-[carbamate]
Non-systemic Fungicide Group				
1	Hilthane M45	Mancozeb	75% WP	Manganese ethylene bis Dithiocarbonate plus zinc
2	Thiram	Thiram	75% WS	Tetramethylthiuram disulfide
3	Biltox 50	Copper oxychloride	50 WP	Dicopper chloride trihydroxide
4	Antracol	Propineb	70% WP	Propylenebis(dithiocarbamato) Zinc
Combo Fungicide Group				
1	Ridomil Gold	Metalaxyl+ Mancozeb	4% + 64% WP	Methyl N(2,6dimethylphenyl)-N-(methoxyacetyl)-Dalaninate
2	Nativo	Tebuconzole+ Trifloxystrobin	50%+ 25% WG	
3	Boxer	Captan+ Hexaconazole	70% + 5% WP	(3a*R*,7a*S*)-2 [(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione+ RS-2-(2, 4-D)-1-(1H-1, 2, 4 Trizole-1-yl) hezan 2-ol
4	Taspa	Propiconazole + Difenoconazole	13.9%+ 13.9% EC	1-((2-(2-Chloro-4-(4-chlorophenoxy) phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole

5	Vitavax Power	Carboxin+Thiram	37.5% + 37.5% WP	
6	Saaf	Carbendazim+ Mancozeb	12% + 63% WP	Methyl 1H-benzimidazole-2ylcarbomate+manganesethyl Lene bis– dithiocarbmate plus zinc

The pathogen *S. rolfsii* was grown on PDA medium for 7 days prior to setting up the experiment. The PDA medium was prepared and melted. The required quantity of fungicide was added to the melted medium to obtain the required concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates and suitable check was maintained without addition of fungicides. To avoid bacterial contamination, a pinch of streptomycin sulphate was added to the medium at the time of pouring. A five mm mycelial disc was taken from the periphery of 7 days old colony of *S. rolfsii* and placed in the centre of petriplate. The inoculated plates were incubated at $25 + 2^0$C and four replications were maintained for each treatment. Diameter of the colony was measured when maximum growth of the *S. rolfsii* was reached in any of the treatments and the observations were recorded and per cent inhibition was calculated by using the formula of Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

3.7.4.2 *In vivo* efficacy of fungicides under glass house condition

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S. rofsii* (prepared on wheat grain medium) @ 25g/kg soil and allowed to stabilize for five days. Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with fungicide as par recommended dose. Ten seeds per pot were sown in

15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of each treatment were maintained. Pots without fungicide seed treatment (untreated) were served as control. Collar rot incidence was recorded up to 45 days after sowing. Germination was recorded after ten days after sowing. The per cent mortality was observed after 15 days of sowing. Then plants were uprooted for measurement of root length and shoot length.

3.7.4.3. *In vitro* determination of compatibility in biocontrol agents *Trichoderma* spp., fluorescent *Pseudomonas, Bacillus* spp., with fungicides.

3.7.4.3.1 *In vitro* determination of compatibility of *Trichoderma* spp. with fungicides.

The compatibility of *Trichoderma* spp. with selected fungicides (Hexaconazole 5% EC, Propiconazole 25% EC, Mancozeb 75% WP, Thiram 75% WS, Propineb 70% WP, Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% + Thiram 37.5%) was evaluated *in vitro* at different concentrations (100, 200 and 500 ppm) on Potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 1982).

The biocontrol agent *Trichoderma* spp. was grown on PDA medium for 7 days prior to setting up the experiment. The PDA medium was prepared and melted. The required quantity of fungicide was added to the melted medium to obtain the required concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates and suitable check was maintained without addition of fungicides. To avoid bacterial contamination, a pinch of streptomycin sulphate was added to the medium at the time of pouring. A five mm mycelial disc was taken from the periphery of 7 days old colony of *Trichoderma* spp. and placed in the centre of petriplate. The inoculated plates were incubated at $25 + 2^0$C and three replications were maintained for each treatment. Diameter of the colony was measured when maximum growth of the *Trichoderma* spp. reached in any of the treatments and the observations were recorded and per cent inhibition was calculated by using the formula of Vincent (1947).

$$I = \frac{C- T}{C} \times 100$$

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

3.7.4.3.2. *In vitro* determination of compatibility in fluorescent *Pseudomonas, Bacillus* spp., with fungicides.

The compatibility of bacterial bioagents fluorescent *Pseudomonas*, *Bacillus* spp. with fungicides (Hexaconazole 5% EC, Propiconazole 25% EC, Mancozeb 75% WP, Thiram 75% WS, Propineb 70% WP, Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% + Thiram 37.5%) was evaluated *in vitro* at different concentrations of (100, 200 and 500 ppm) on King's B medium (KBM) using poisoned food technique (Nene and Thapliyal, 1982).

The biocontrol agent fluorescent *Pseudomonas* and *Bacillus* spp. were grown on King's B (KB) medium for 48h and 72 h respectively, prior to setting up the experiment. The King's B medium was prepared and melted. The required quantity of fungicide was added to the melted medium to obtain the required concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates and suitable check was maintained without addition of fungicides. A heavy inoculums from an actively growing bacterial agent was zig-zag streaked on on the respective plates. The inoculated plates were incubated at 25 + 2^0C and three replications were maintained for each treatment. The growth of fluorescent *Pseudomonas* and *Bacillus* spp. in the poisoned plates was recorded as (-) : no growth, (+) : poor growth, (++) : moderate growth, (+++) : good growth.

3.7.4.4. Integration of effective *Trichoderma* spp., fluorescent *Pseudomonas, Bacillus* spp., and fungicide seed treatment *in vivo* pot experiment

The seed dressing talc powder formulation developed form highly effective isolate of *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp. was selected for evaluation along with fungicide Propineb 70% WP. These treatments were compatible among themselves and evaluated alone and in combinations as seed treatment against the pathogen *S. rolfsii*. The experiment was conducted in CRD consisting of 16 treatments.

Table 3.8: Integration of effective *Trichoderma* spp., fluorescent *Pseudomonas spp., Bacillus* spp., and fungicide seed treatment *in vivo* pot experiment

Sr. No.	Treatments
1	T1: *Tricho-32*
2	T2: *Pf*- 26
3	T3: *Bs*-21
4	T4: Propineb 70% WP
5	T5: *Tricho*-9 + *Pf*- 26
6	T6: *Tricho*-9 + *Bs*-21
7	T7: *Tricho*-9 + Propineb70% WP
8	T8: *Pf*- 26 + *Bs*-21
9	T9: *Pf*- 26 + Propineb70% WP
10	T10: *Bs*-21 + Propineb 70% WP
11	T11: *Tricho*-9 + *Pf*- 26 + *Bs*-21
12	T12: *Tricho*-9 + *Pf*- 26 + Propineb 70% WP
13	T13: *Tricho*-9 + *Bs*-21 +Propineb 70% WP
14	T14: *Pf*- 26 + *Bs*-21 + Propineb70% WP
15	T15: *Tricho*-9 + *Pf*- 26 + *Bs*-21 + Propineb70% WP
16	T16: Control (untreated seed)

***Tricho*-9** = *Trichoderma* spp., ***Pf*- 26** = fluorescent *Pseudomonas* and ***Bs*-21** = *Bacillus* spp.

Pot experiments (CRD) was carried out under glass house conditions. The plastic pots (15 cm diameter) filled with sterilized sandy loam soil, were further inoculated with two-week old culture of *S. rofsii* (prepared on wheat grain medium) @ 25g /kg soil and allowed to stabilize for five days. Seeds of lentil were surface sterilized with 2 per cent sodium hypochlorite for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. Seeds were treated with the fungicide at 2 g/kg seed, while talc based formulations *Tricho*-12 at 5 g/ kg and *Pf*- 17 + *Bs*-6 at 10 g/kg of seed separately and for integrated treatment with half doses of the fungicide (1 g/kg) followed by bio-formulations . Ten seeds per pot were sown in 15 cm diameter plastic pots, each containing 2 kg of soil. Three replication of each treatment weremaintained. Pots without fungicide seed treatment (untreated) were served as control. Germination was recorded ten days after sowing. The observation on per cent mortality was taken after 15 days interval upto 45 days after sowing. Then plants were uprooted for measurement of root length and shoot length to record vigour index by using formula; Seedling vigour index = Germination per centage × (Mean shoot length + Mean root length)

3.7.4.5. Evaluation of different antagonists (seed dressers) against lentil disease under field condition:

Field experiments were conducted at Research Farm , CoA, IGKV, Raipur during *Rabi* , 2018 and 2019 in RBD with three replications and eight treatments with a view to evaluate different antagonists against collar rot of disease of lentil. Seeds of lentil cultivar-Bragg were dipped in bioagents/ fungicidal solution for 10 minutes dried in shad and planted 4×3 m sized plot with 30 x 10 cm spacing. Control plots were maintained without seed treatment, without soil application/ drenching of bioagent/fungicides. Per cent disease incidence was recorded in all treatment by counting the number of infected plants.

Table 3. 9: Bio-agent tested against *Sclerotium rolfsaii* seed dresser *in vivo*.

S.No.	Treatment	Treatment Details	Doses
1	T1	Trichoderma herzianum T-6	(@10g/kg seed)
2	T2	Trichoderma herzianum T-28	(@10g/kg seed)
3	T3	Trichoderma viride T-18	(@10g/kg seed)
4	T4	Trichoderma mutant	(@10g/kg seed)
5	T5	Propineb(Antracol)	(@3g/kg seed
6	T6	Hexaconazol+Zineb (Avtar)	(@3g/kg seed)
7	T7	Trichoderma herzianum + Propineb	(@10kg+1.5g/kg seed)
8	T8	Control	

Replications	:	Three
Design	:	R.B.D.
Plot size	:	3.0 m X 4.0 m
Spacing	:	30 cm X 10 cm
Crop and Variety	:	Lentil, K34

Observations

The following observations were recorded at periodic intervals after germination of seeds:

1. Germination of seed after 10 and 20 days after of sowing.
2. Disease incidence at 10, 30 and 60 days after sowing.
3. Plants charecters
4. Observation of Grain Yield (kg/ha).

Experimental materials

Seeds were sown in shallow furrow at a depth of 3-4 cm in each plot. During crop growth harmful pests damaging seed germination and post-

emergences stages were kept under control by using pesticides. .Other cultivation practices like weeding and inter-culturing were followed as per the package of practices to ensure healthy growth of the crop.

3.8. Screening of lentil entries

3.8.1. Field screening

Seeds were procured from AICRP on MULLaRP (All India Coordinated Research Project) on Lentil, Raipur. The field experiment was laid out during *rabi* season 2018-19 and 2019-2020 at the research farm, IGKV Raipur. Total 271 entries were screened against *Sclerotium rolfsii*, out of which 139 entries during 2018-2019 were screened while, 132 entries were screened in 2019-2020. Each test entries was sown in a plot of two rows of 5 meter length 30 cm apart with one row of susceptible check variety JL3 after every two test entry and replicated twice in randomized block Design. Observations on emergence were recorded at ten and twenty DAS. Light irrigation was given just to activate the growth of fungus. Observations on per cent mortality were started from ten days and recorded at five day intervals upto marurity, finally computed as follows.

Table 3.10: IIPR rating scale

S. No.	Reaction	Per cent mortality
1	R- Resistant	0-10
2	MR- Moderately Resistant	11-20
3	MS- Moderately Susceptible	21-30
4	S- Susceptible	31-50
5	HS- Highly Susceptible	51-100

Location severity index (LSI) was calculated by using the formula:

$$\text{LSI} = \frac{\text{Sum of all numerical rating of entries}}{\text{Total numbers of entries}}$$

3.8.2. Evaluation of lentil genotypes against collar rot under glass house conditions:

Similar set of genotypes were also grown in the earthen pots in glass house .Ten plants were maintained in each pot to achieve maximum development of plants. Three replications were maintained in the glass house and pots were irrigated at weekly intervals to provide favorable conditions for the establishment and development of *S. rolfsii*. The fifteen days old mass multiplied culture of *S. rolfsii* @ 25g/ pot was inoculated in the collar region of 25 days old plant.

The observation on per cent mortality were recorded at the interval of seven days and categorization the varieties on the basis of per cent disease incidence and disease scale of IIPR.

3.9 Statistical Analysis

Data entry and processing was carried out using MS-excel sheet. The data were analyzed statistically using CRD and RBD Treatments were compared by mean of critical differences at 5% level of significance.

Skeleton of analysis of variance:

Source of variation	D.F.	S.S.	M.S.S.	T.cal.	F.tab. (5%)
Treatments					
Error					
Total					

Test of significance:

To test the significance difference among the treatment means following formula were used for calculating the critical differences.

$$S.Em\pm = \sqrt{\frac{MSE}{r}}$$

$$C.D. = S.Em \times \sqrt{2} \times \text{‘t’ at error d.f.}$$

Where :

D.F. = Degree of Freedom

S.S. = Sum of squre

M.S.S. = Mean sum of square

The significant different between mean was determined by using critical difference.

S. Em ± = $\sqrt{}$EMss/replication

C.D. = S.Ed. × t 5% at error d.f.

CHAPTER-IV
RESULTS AND DISCUSSION

Among the soil borne diseases of lentil, Collar rot of lentil Lentil (*Lens culinaris M.*) is caused by the ubiquitous soil-borne pathogen *Sclerotium rolfsii* . Collar rot is one of the fast spreading fungal disease of lentil and at seedling stage it causes heavy losses. Therefore, keeping in view, the severity and losses caused by collar rot disease, present investigation entitled **"Studies on Collar Rot *(Sclerotium rolfsii* Sacc.) of Lentil and it's Management."** was carried out in the laboratory as well as in field during 2018-19 and 2019-20 in Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

During the present investigation, field observations were recorded to gather information on the occurrence of disease and screening of lentil entries against collar rot, in research farm, IGKV, Raipur (C.G.). Laboratory and glass house studies on isolation, identification, pathogenicity, symptomatology, soil factors affecting disease development, evaluation of biocontrol agents and fungicides against the pathogen under *in vitro* and *in vivo* condition were carried out. The results thus obtained are presented in different sections under this chapter.

4.1: Survey and collection of collar rot samples in major lentils growing areas of the state

Survey for the occurrence of collae rot disease of lentil was undertaken during *rabi* 2018-2019 and 2019-20 from fourtin locations in seven lentil growing districts of Chhattisgarh state. 7 districts of Chhattisgarh Raipur, Mungeli, Bemetara, Bilaspur, Kawardha, Baloda bazaar and Dhamtari. Observations were recorded from farmer's fields under natural condition.

During the survey, discussions were held with the farmers concerned, regarding occurrence and incidence of the disease. As a result of this discussion, it was revealed that disease appeared in most of the fields wherever , lentil crop was taken continuously for the last 5-6 years. Extensive survey revealed that the disease was prevalent in varying disease incidence in all the districts.

Symptomatic collar rot infected plants were collected from fourtin lentil growing areas of Chhattisgarh state (Raipur IGKV farm- SR1, Agronomy, Field-

SR2, Khurha-SR3, Mungeli, Temari SR4, Chilfi-SR5, Bhtha-Bhurka SR6, Bilaspur, takhatpurSR7, Dhamtari Kurud, Gobra SR8, Baloda bazaar, Simga Surgi-SR9, Bhatapara, Gogiya-SR10, Kawardha, Pandariya- kapadah-SR11, ghuterkudi-SR12, Bemetara Gadamod- SR13, Matia-SR14) for isolation of the causal fungus in the laboratory. Fourtin isolates of *Sclerotium rolfsii* were isolated from collected samples and designated as mention in Table 4.2.

4.1.1: Distribution and incidence of the disease

It is apparent from the data depicted in (Table-4.1 and Fig- 4.1) that lentil collar rot disese incidence was more sever in Raipur, Mungeli, Bemetara, Raipur, Bilaspur districts of Chhattisgarh than other surveyed districts of Chhattisgarh, during different cropping seasons. The average collar rot incidence of lentil (*Sclerotium rolfsaii*.) in different districts varied from 12.57 to 39.97 per cent. In Mungeli district, it was maximum (39.63%) followed by Bemetara (37.63%), Kawardha (32.00%), Raipur (23.73%), Balodabazar (16.89) Bilaspur (16.84%), Dhamtari (12.57%), Least wilt incidence was observed in Dhamtri (12.57%) district of Chhattisgarh.

The disease made its appearance at seedling to maturity stage (10-60 days old crop). Initially the leaves of infected lentil plants turns light pale in color , plant start drying and finally die (Plate 4.8). During survey. Mono-cropping also increase disease incidence was observed. Similarly, a striking correlation was noted between disease incidence and number of irrigations in lentil crop. The disease incidence was significantly higher in fields having less irrigation at as compared to the fields receiving higher number of irrigations. It was also noted that collar rot incidence was more where the crop was cultivated in heavy textured soils of the Chhattisgarh. Highest incidence was found in sandy loam soil followed by clay loam and clay.

Variation was also observed in soil types red, yellow and black soil types were found in one, four and fifty-two locations of Chhattisgarh respectively. Results presented in Table 4.8 indicated that the maximum average disease incidence was recorded in black soil (16.77 per cent) followed by red soil (15.85 per cent). Minimum disease incidence was recorded in yellow soil (9.27 percent).

Observations of disease incidence were also recorded on improved and local lentil varieties. Improved varieties were found in Forty five locations while, local varieties found in twelve locations. Results presented in Table 4.1 and Fig.4.3 indicated that the disease incidence was more in local varieties (19.31 per cent) in comparison to improved varieties (15.40 per cent).

The disease incidence was maximum in untreated seed (19.31 per cent) found in twelve locations as compare to treated seed (15.40 per cent) found in forty five locations. (Fig.4.1)

Results also indicated that the disease incidence was more in irrigated condition (19.45 per cent) found in forty one locations. While, in rainfed conditions in sixteen location disease incidence recorded was 7.94 per cent (Fig. 4.5). The disease made its appearance at seedling to maturity stage (10-60 days old crop). Initially the leaves of infected lentil plants turns light pale in color , plant start drying and finally die (Plate -4.2). During survey. Mono-cropping also increase disease incidence was observed. Similarly, a striking correlation was noted between disease incidence and number of irrigations in lentil crop. The disease incidence was significantly higher in fields having less irrigation at as compared to the fields receiving higher number of irrigations. It was also noted that collar rot incidence was more where the crop was cultivated in heavy textured soils of the Chhattisgarh. Highest incidence was found in sandy loam soil followed by clay loam and clay.

Gosh *et al.*(2013) conducted survey in 2010-2011 *rabi* cropping season to obtain information on the distribution and incidence of lentil diseases in respect to soil type, cultivar used, seed treatment in central and southern parts of India. Collar rot diseases were found at all of the sites and incidence ranged from 7.1% - 10.5% respectively irrespective of cultivar type and locations. Kadam *et al.* (2011) conducted field surveys in the Marathwada region of Maharashtra to determine the prevalence of diseases on groundnuts, the pathogen associated with the disease and the performance of different groundnut cultivars against collar rot in the field. The results showed that maximum disease incidence occurred in Renapur Tahsil (17.8%), followed by Udgir (16.7%), Ausa (14.7%) and Latur (14.3%). The lowest disease incidence was recorded in Nilanga (8.9 %).

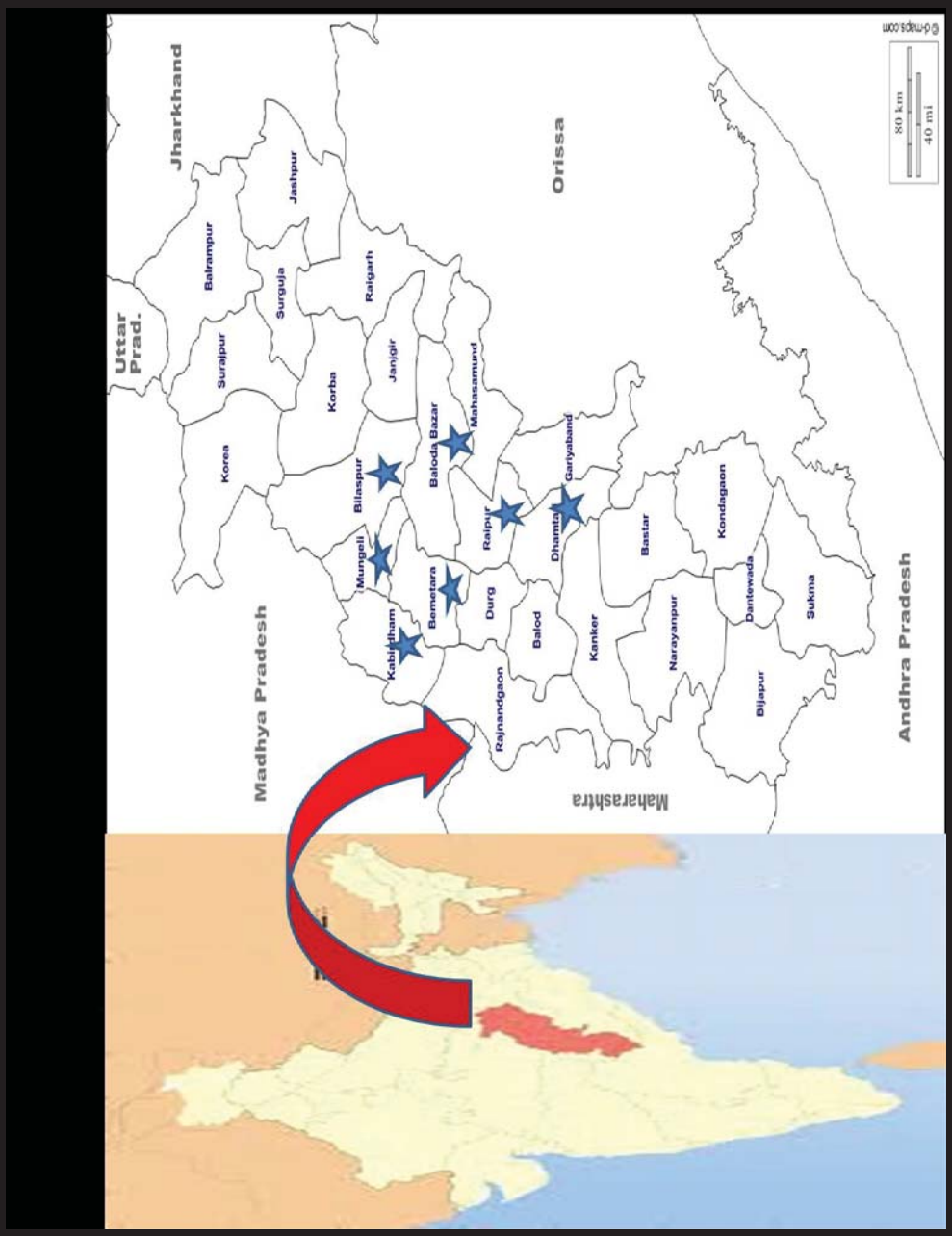


Fig. 4.1 Survey and collection of the infected plant sample

Table 4.1: Survey and collection of the infected plant sample

S. No.	District	Block	Village/ Location	Soil type	Seed type	Seed Treated/ Untreated	Rainfed/ Irrigated
1	Raipur	Raipur	IGKV, Farm	Black	Improved	Treated	Irrigated
2			Agronomy, Field	Black	Improved (Experimental seed)	Untreated	Irrigated
3		Abhanpur	Khurha	Black	Local	Untreated	Rainfed
4	Mungeli	Mungeli	Temari	Black	Local	Untreated	Irrigated
5		Lormi	Chilfi	Black	Local	Untreated	Rainfed
6			Bhtha-Bhurka	Black	Local	Untreated	Rainfed
7	Bilaspur	Bilaspur	takhatpur	Black	Local	Untreated	Irrigated
8	Dhamtari	Kurud	Gobra	Black	Local	Treated	Rainfed
9	Baloda bazar	Simga	Surgi	Black	Local	Treated	Irrigated
10		Bhatapara	Gogiya	Black	Local	Untreated	Irrigated
11	Kawardha	Pandariya	kapadah	Black	Local	Treated	Rainfed
12			ghuterkudi	Black	Local	Treated	Rainfed
13	Bemetara	Bemetara	Gadamod	Black	Local	Untreated	Irrigated
14			Matia	Black	Local	Untreated	Irrigated

Table 4.2: Per cent disease incidence of lentil Collar rot of lentil fields in various districts of Chhattisgarh

S. NO.	Isolates	District	Village /Location	*Average PDI 2018-2019	2019-2020	**Mean
1	SR-1	Raipur	IGKV, Farm	9.51	12.44	10.97
2	SR-2		Agronomy, Field	40.97	37.15	39.06
3	SR-3		Khurha	21.47	20.87	21.17
4	SR-4		Temari	50.83	47.58	49.20
5	SR-5	Mungeli	Chilfi	26.74	31.48	29.11
6	SR-6		Bhtha-Bhurka	43.42	39.76	41.59
7	SR-7		takhatpur	17.89	21.35	19.62
8	SR-8	Bilaspur	Gobra	11.64	16.48	14.06
9	SR-9	Dhamtari	Surgi	10.56	14.58	12.57
10	SR-10	Baloda bazar	Gogiya	15.12	18.65	16.885
11	SR-11		kapadah	25.74	29.54	27.64
12	SR-12	Kawardha	ghuterkudi	36.36		36.36
13	SR-13		Gadamod	23.12	22.85	22.98
14	SR-14	Bemetara	Matia	35.72	39.54	37.63

* Average of three villages **Mean of total wilt incidence of two year (2018-19 and 2019-20)

Table 4.3 Per cent disease incidence of lentil collar rot fields invarious districts of Chhattisgarh

District	Per cent Disease Incidence
Raipur	23.73
Mungeli	39.97
Bilaspur	16.84
Dhamtari	12.57
Baloda bazar	16.89
Kawardha	32.00
Bemetara	37.63

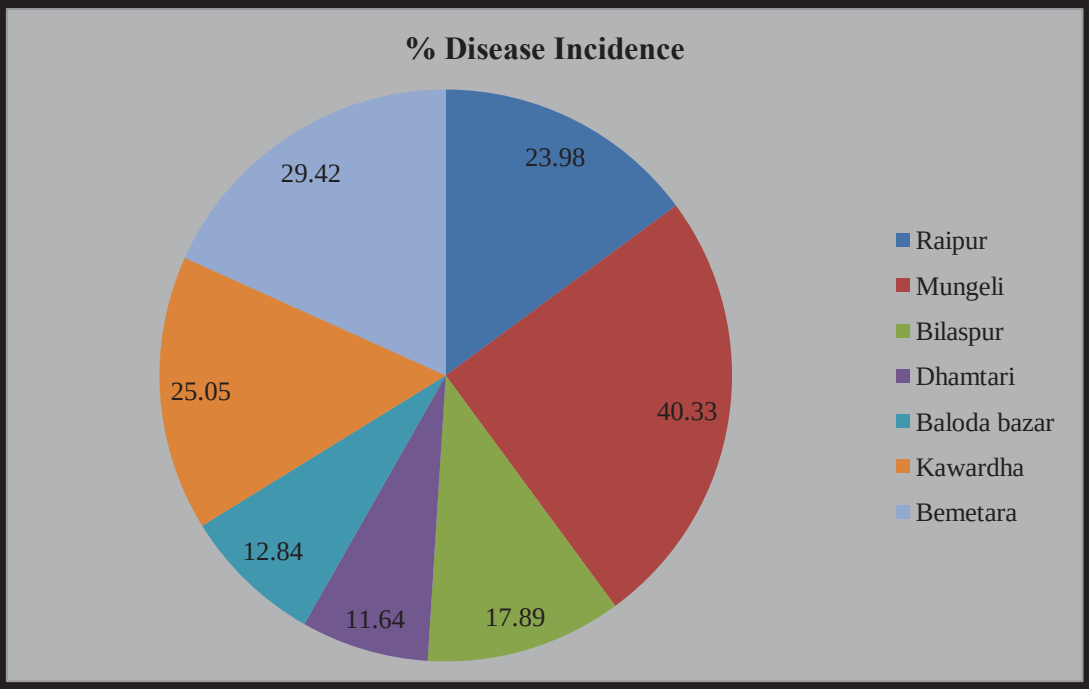


Fig.-4.2. Per cnt disease of lentil of collar rot field in various district of Chhattisgarh

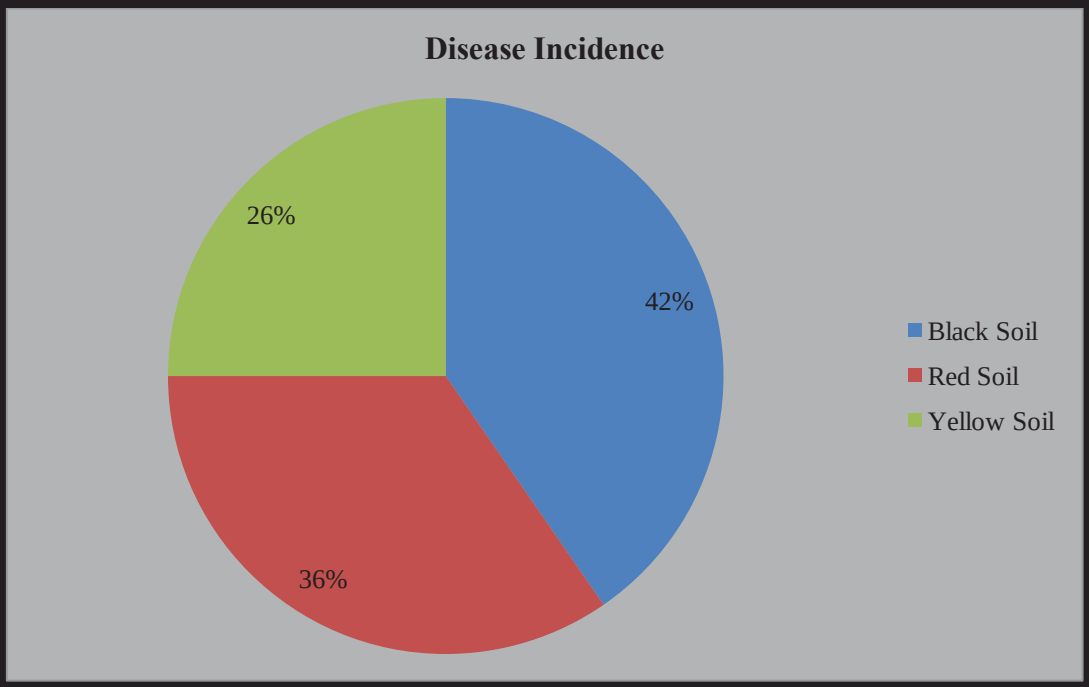


Fig. 4.3 : Disease incidence in different soil types of Chhattisgarh

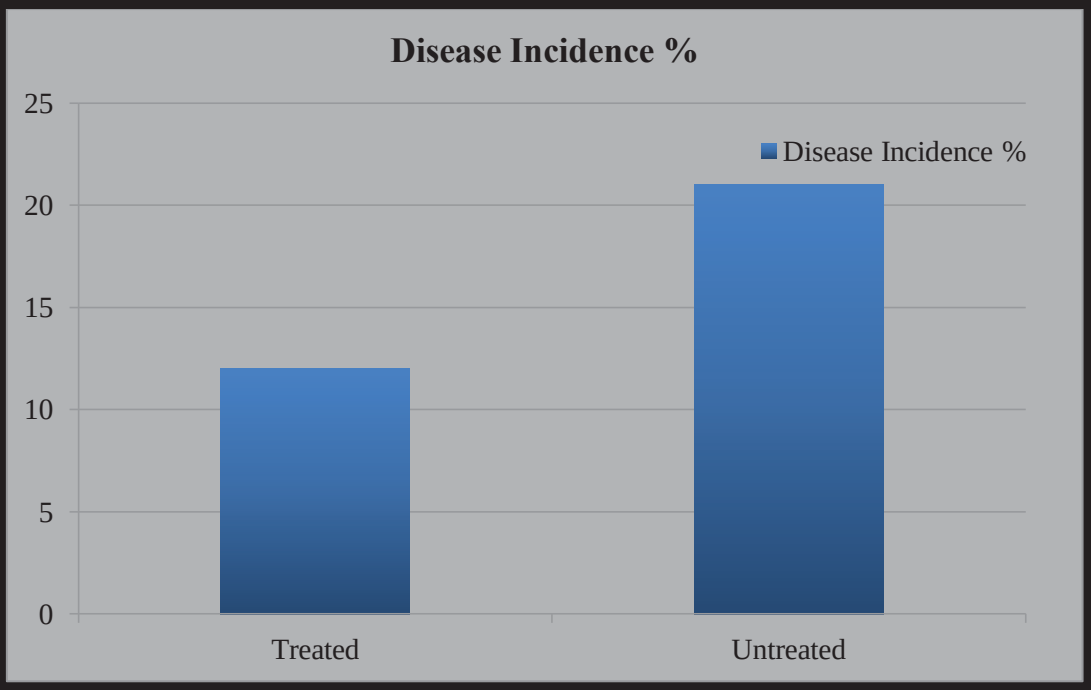


Fig. 4.4 : Disease incidence in treated and untreated seed in Chhattisgarh

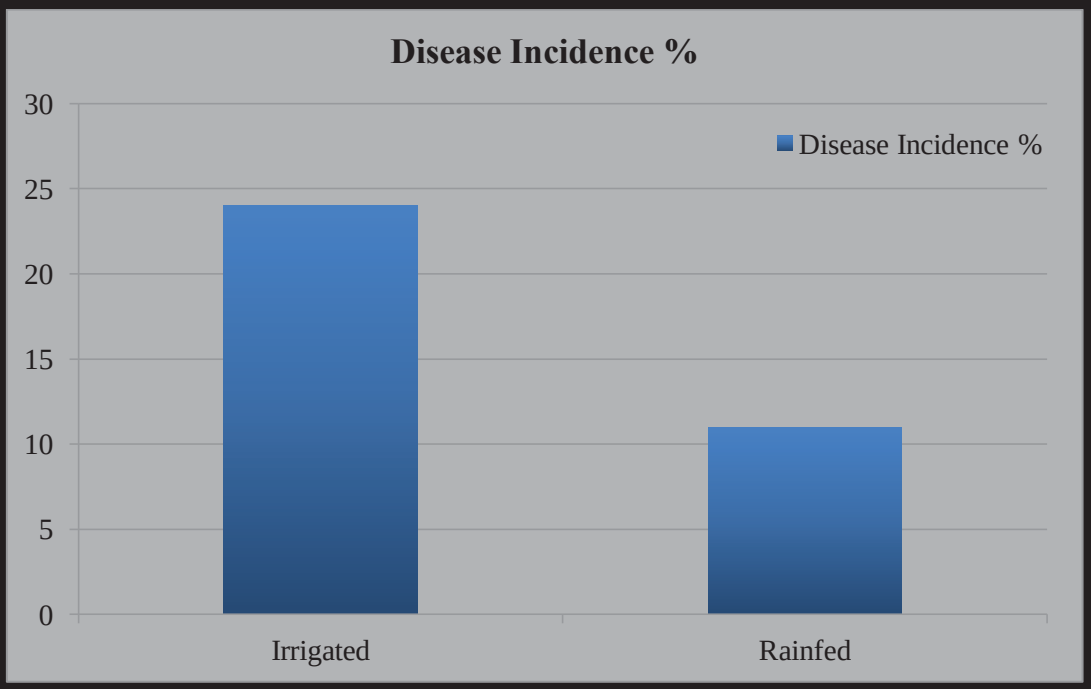


Fig. 4.5: Disease incidence in Irrigated and Rainfed Condition in Chhattisgarh

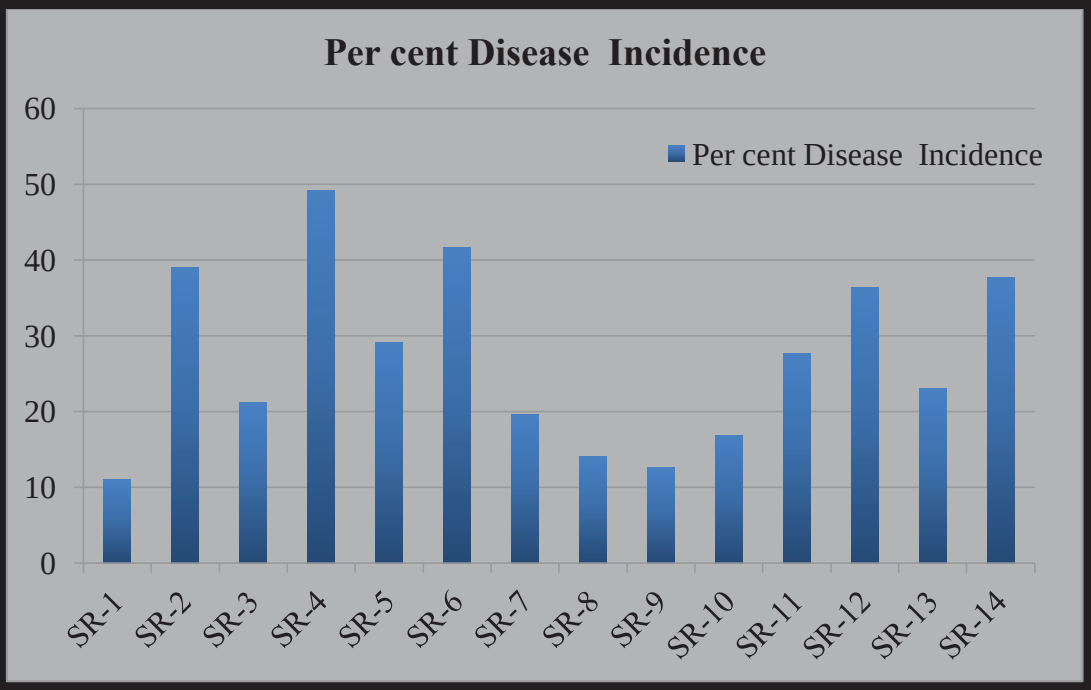


Fig. 4.6: Per cent disease incidence of lentil collar rot of different isolates of Chhattisgarh

Fig.4.7: Survey of lentil affected field

4.2.: Symptomatology

Following symptoms of collar rot disease was observed in lentil under natural field condition. (Fig.4.8)

(I) Drying plants whose foliage turns slightly yellow scattered throughout the field was observed.

(J) Affected young seedling turned yellow and collapsed but the older seedlings dried without collapsing.

(K) No clear cut drooping of leaves was seen.

(L) Uprooted seedling showed rotting at the collar region and downwards .

(M) The collar portion was covered with whitish mycelia strands.

(N) White mycelia growth also seen on the tap root of a completely dried seedling, rapseed-like sclerotia were observed attached to mycelia growth around the collar region.

Fig. 4.8. Field symptoms of collar rot of disease of lentil (a); Sclerotia production on collar rot region (b); isolated pure culture of *S. rolfsii* (c); Sclerotia formation in pure culture (d)

Seed inoculation

Soil inoculation

Healthy plant in control

Fig.4.9: Pathogenity test

4.3: Isolation, purification, characterization and pathogenicity of the pathogen

Various cultures of *Sclerotium rolfsaii* were obtained from the samples collected from different lentil growing areas of Chhattisgarh Raipur, Mungeli, Bemetara, Bilaspur, Kawardha, Baloda bazaar and Dhamtari. The cultures of *Sclerocium rolfsii* from Raipur was designated as SR-01 while the different *Sclerocium rolfsii* isolates were designated as (Raipur Agronomy, Field)-SR2, (Khurha)-SR3, (Mungeli, Temari)- SR4, (Chilfi)-SR5, (Bhtha-Bhurka)- SR6, (Bilaspur, takhatpur)-SR7, (Dhamtari Kurud, Gobra)- SR8, (Baloda bazaar, Simga Surgi)-SR9, (Bhatapara, Gogiya)-SR10, (Kawardha, Pandariya- kapadah)-SR11, (ghuterkudi)-SR12, (Bemetara Gadamod)- SR13, (Matia)0-SR14. for isolation and purification of the causal fungus in the laboratory. Fortin isolates of *Sclerotium rolfsii* were isolated from collected samples and designated as mention in Table 4.2.

The cultures were purified by single hypal tip method and were maintained on PDA at 25 ± 2^{0}C. Based on mycological characters the pathogen identified as *Sclerotium rolfsii* Sacc. First the fungal mycelium was silky white in colour and later turned to dull white with radial spreading giving fan like appearance. Microscopic examination of the fungal culture revealed that the mycelium was hyaline, thin walled, septate and profusely branched with clamp connections. When the fungus attained maturity, small mycelial knots were formed later turned to mustard seed like sclerotia. Initially sclerotia were deep brown or brownish black shiny, hard, spherical to irregular in shape. At maturity, the sclerotia showed honey dew like liquid material. The sclerotial bodies were concave on the side attached to the mycelium and was easily detachable from the mycelium. The sclerotia were bigger in size measuring about 1.5 to 2 mm in diameter. The colony character, morphological characters of mycelium and sclerotia were in agreement with earlier reports (Mirza and Aslam 1993, Anitha Chowdary, 1997). Thus the fungus under present investigation was identified as *Sclerotium rolfsii*. These isolates were further under taken for study.

Table 4.4: Isolates of *Sclerotium rolfsii*, collected from different lentil growing locations of Chhattisgarh.

S.No.	District	Location	Isolate
1	Raipur	IGKV, Farm	SR1
2		Agronomy, Field	SR2
3		Khurha	SR3
4	Mungeli	Temari	SR4
5		Chilfi	SR5
6		Bhtha-Bhurka	SR6
7	Bilaspur	takhatpur	SR7
8	Dhamtari	Gobra	SR8
9	Baloda bazar	Surgi	SR9
10		Gogiya	SR10
11	Kawardha	kapadah	SR11
12		ghuterkudi	SR12
13	Bemetara	Gadamod	SR13
14		Matia	SR14

4.4. Cultural and morphological characterization of different isolates of *Sclerotium rolfsii* Sacc. of lentil

Variation in cultural and morphological characteristics were observed in all the isolates of *S. rolfsii* and result is represented in Table 4.5.

4.4.1. Mycelial growth

The data presented in Table 4.3 indicated that the significant variations were observed in mycelial growth of *S. rolfsii* isolates collected from different location of Chhattisgarh.

Isolates of *S. rofsii* fungus categorized in to three classes on the basis of complete radial mycelium growth. Isolates wise completion of radial growth was recorded at the interval of 24h, 48h, 72h and 96h and then classified into 3 groups: fast growing (diameter >80-90mm), moderately fast growing (70-79 mm) and slow growing (< 70). First group comprised of 09 isolates SR1 (IGKV Farm), SR3 (khurha, abhanpur), SR4 (mungeli), SR6 (bhatha-bhurka,mungeli), SR7 (takhatpur, bilaspur), SR9 (surghi, sigma,balodabazat), SR10 (gogiy, bhatapara), SR11 (kapadah, pandariya kawardha), SR13 (gadamod, bemetara) were fast growing (diameter >80-90 mm). Second group include moderately fast growing (70-79 mm) 03 isolates, SR2 (Agronomic field, Raipur), SR5 (chilfi, lormi, mungeli), SR14

(Matia, bemetara), as mycelial growth. The third group consist 01 isolates SR8 (Gobra, Kurud, Dhamtari) were slow growing (< 70). Similar type of finding was also reported by Sharma *et al*. (2002) and Akram *et al*. (2015). Reddi Kumar *et al*. (2014) stated that ISR-1 and ISR-2 isolates of *S. rolfsii* were very fast growing, which covered entire Petriplate (9.0 cm) within 96 hrs of incubation. Bagwan (2011) reported significant variation of growth in 59 isolates of *S. rolfsii* and categorized the isolates in to three groups fast growing, medium growing and slow growing. Hussain *et al.*(2010) observed morphological variation among twelve isolates of *S. rolfsii* Sacc.in which three isolates (AT-1, AT-2, RW-2 were fast growing, three (SR-1, CH-1, DL-2) intermediate and others exhibited slow radial colony growth.

4.4.2 Hyphal width

Based on the hyphal width of isolates, the pathogen was categorized in three groups. First group comprised of 02 isolate SR9 (Surghi, simga, Balodabajar), and SR10 (Gogiya, Bhatapara), with hyphal width ranging between 7-9 µm. Second group include 9-11µm in 09 isolates SR1 (IGKV Farm), SR2 (Agronomy field,Raipur), SR3 (Khurha,Abhanpur,Raipur), SR5 (Chilfi,Lormi,Mungeli), SR6 (Bhatha-Bhurka, lonrmi, Mungeli), SR7 (Takhatpur, Bilaspur), SR11 (Kapadah, Pandaria, Kawardha), SR13 (Gadamod, Bemetara) SR14 (Matia, Bemetara) as hyphal width and third group consist of 03 isolates SR4 (Temari, Mungeli), SR8 (Dobra, Kurud, Dhamtari), SR12 (Ghutaekudi), with hyphal width of more than 11 µm. However, maximum and minimum hyphal width observed in SR12 (Ghuterkudi, pandaria, Kawardha) (12.00 µm) and SR10 (Gigia, Bhatapara, Balodabazar) (7.2 µm) respectively. Gupta *et al*. (2012) observed a wide range of hyphal diameter in *R. bataticola* and classified in three groups very thin (5.2-6.5 µm), thin (6.6-7.9µm) size and thick 76 (8.0-9.3µm). These observations coincide with the observations of Sobti and Sharma (1992) who reported that the width of hyphae varied from 4.16-8.48µm.

4.4.3 Distance between septa

Based on the distance between septa of isolates, the pathogen *S. rolfsii* was categorized in four groups. First group comprised of 01 isolate SR10 (Gogia,

Bhatapara balodabazar), distance between septa with ranging between 13-18 µm. Second group include 18-23 µm in 04 isolates SR7 (Takhatpur,Bilaspur), SR8 (Gobra,Kurud,Dhamtari), SR13 (Gadamod,bemetara), SR14 (Matia, Bemetara) as distance between septa and third group Include 24-26 µm consist of 06 isolates SR1 (IGKV,Farm, Raipur), SR02 (Agronomy field,Raipur), SR4 (Temari,Mungeli), SR5 (Chilfi,), SR6 (Bhatha-Bhurka), SR9 (Surgi). Fourth group include 27-31 µm in 2 isolates, SR3 (Khurha), SR11 (Kapadah), SR12 (Ghuterkudi) as septa distance in hypha of pathogen.

4.4.4 Type of mycelial growth

Based on myceial growth pattern on PDA after five days of incubation, the forty isolates were placed in two groups. The first group includes SR8 (Gobra), SR9 (Surghi, Sigma), SR13 (Gadamod), SR14 (Matia) isolates having fluffy mycelia growth. Whereas SR1 (IGKV Farm) SR2 (Agronomic Field) SR3 (Khurha), SR4 (Temari), SR5 (Chilfi), SR6 (Bhatha-Bhurka), SR7 (Takhatpur), SR10 (Gogiya), SR11 (Kapadah) and SR12 (Ghuterkudi) isolates comprised of second group with compact mycelial growth pattern. Similar studies were conducted by Rasu *et al.* (2013) and reported that out of 17 isolates of *S. rolfsii* tested for their cultural morphology, most of them were observed with compact colonies and few were fluffy colonies . Bagwan (2011) also reported that out of 59 isolates, colonies of 35 isolates were fuffy, whereas 24 were compact .

4.4.5 Number of sclerotia

The data presented in Table 4.6 and Fig 4.11 indicated that the significant variations were observed in sclerotia production among the *S. rolfsii* isolates. Highest number of sclerotia production per plate was noted in SR9 (Surghi) (258.33) followed by SR7 (Takhatpur) (203) which were at par with each other. Least sclerotia production per plate was found in SR4 (Temari) (67.66) followed by SR11 (Kapadah), SR12 (Ghuterkudi). Present results were compared with finding of Bagwan (2011) who observed that twelve isolates produced a large number of sclerotia (>500 sclerotia/plate) but smaller in size (0.5-1.4), while 9 isolates produced relatively fewer sclerotia (140-286 sclerotia/plate) but larger in size (2.1-2.5 mm).

Table 4.5: Variability in morphology of sclerotia produced by different isolates of *S. rolfsii.*

S.No.	Isolate	Location	Mycelial growth (mm)*				Hyphal width(µm)**	Distance between septa (µm)**	Types of mycelia growth
			24 Hr	48 Hr	72 Hr	96 Hr			
1	SR1	Raipur	14.5	34.5	61	82.33	9.7	25.23	Compact
2	SR2		12.66	32.66	55.83	73.16	9.9	24.05	Compact
3	SR3		13.34	35.33	65.66	84.66	10.9	27.12	Compact
4	SR4	Mungeli	12.66	32.66	64.33	83.33	11.5	25.26	Compact
5	SR5		10.66	30	51	74.66	9.2	25.44	Compact
6	SR6		16.33	38.67	68.66	90	9.3	24.56	Compact
7	SR7	Bilaspur	18.67	40.33	72.66	87.66	10	19.84	Compact
8	SR8	Dhamtari	12.5	30	54.33	75.66	11.5	20.84	Fluffy
9	SR9	Baloda bazar	12.83	34.16	56.16	81	8	24.29	Fluffy
10	SR10		17.34	41.34	74.33	90	7.2	18.09	Compact
11	SR11	Kawardha	13.34	35.33	65.66	84.66	10.9	27.12	Compact
12	SR12								
13	SR13	Bemetara	18.83	33.83	63.83	90	9.8	19.28	Fluffy
14	SR14		14.66	32.34	50.66	73.66	9.3	19.33	Fluffy

Average of three replicatio
** Average of five replication

4.4.6. Sclerotial weight

Sclerotial weight was taken after 30 days of incubation. Data (Table 4.6) also showed that, the maximum weight of the 100 sclerotia was found in isolate SR10 (Gogoa) (0.242mg) followed by SR3 (Khurha) (0.241 mg) and SR9(Surghi) (0.172 mg) isolate. Lowest weight of 100 sclerotia was found in isolate SR12 (Ghuterkudi) (0.06 mg) followed by isolate SR4 (Temari) (0.10 mg).

4.4.7. Sclerotial size

The average size of 10 sclerotia in the fortin isolates varied from 0.4 to 1.6 mm in diameter. Maximum sclerotial size was found in isolate SR5 (Chilfi) that is 1.6 mm followed by SR10 (Gogia) (1.5 mm) and SR4 (Alekhuta), SR6 (Chatod), SR27 (Bilaspur), SR40 (Kodukupa) (1.5). Whereas least sclerotia size observed in isolate SR12 (Ghuterkudi), SR4 (Temari), SR7 (Takhatpur), SR9 (Surghi), SR8 (Gobra), SR6 (Bhata-Bhurka) (0.8 mm). Similar findings given by Bagwan (2010)

who reported that the average size of sclerotia of most of the isolates varied from 2.1 to 2.5 mm in diameter. Rasu *et al*. (2013) reported that the isolates varied with the average sclerotial size of 1 to 1.2 mm in dia, whereas the largest sclerotia were produced by SrJ1, SrSB5, SrTO2 and SrGN2 (1.5 to 1.6 mm in dia).

4.4.8 Sclerotial shape

Observations of sclerotial shape the isolates were classified in two groups that is Spherical and Ellipsoidal. Spherical shape sclerotia recorded in 11 isolates SR1 (IGKV Farm), SR2 (Agronomy Field), SR4 (Temari), SR5 (Chilfi), SR6 (Bhatha-burka), SR7 (Takhatpur), SR8 (Gobra), SR10 (Gogia), SR11 (Kapadah), SR12(Ghuterkudi), SR13 (Gadamod), whereas, shape Ellipsoidal sclerotia were found in 3 isolates SR3 (Agronomy field), SR9 (Surghi), SR14 (Matia). Present results were coincide with the findings of Reddi *et al.* (2014) that shape of sclerotia among the isolates of *S. rolfsii* varied in spherical and irregular shape.

4.4.9 Sclerotial colour

Based on pigmentation of the sclerotia, isolates were assigned to three different colour groups, light brown, reddish brown and dark brown. Isolate SR4 (Temari), SR11 (Kapadah), SR12 (Ghuterkudi), were observed to have light brown colour, and isolate SR1 (IGKV Farm), SR3 (Khurha), SR8 (Gobra), SR9 (Surghi), SR13 (Gadamod), SR14(Matia) had reddish brown colour, whereas the sclerotia of isolates SR2 (Agronomy Field), SR5 (Chilfi), SR6 (Bhatha- Bhurka), SR7 (Takhatpur), SR10 (Gogiya) were dark brown in colour. Similar finding were reported by Bagwan (2011), Rasu *et al*. (2013) and Reddi *et al*. (2014) that mostly dark to light brown sclerotia formed in *S. rolfsii* isolates.

4.4.10 Arrangement of sclerotia

The pattern of sclerotial production was also studied and based on their arrangement on the culture medium, the isolates were divided into two main categories. It was noted that the isolates had a particular pattern of formation of sclerotia in culture. In the first category,9 isolate SR1 (IGKV Farm), SR2 (Agronomy Field), SR5 (Chilfi), SR6 (Bhatha-Bhurka), SR8 (Gobra), SR9 (Srghi), SR10 (Gogia), SR11(Kapadah), SR12 (Ghutrekudi) produced sclerotia in irregular

manner scattered all over the culture and rim of the plate. In the second category, 5 isolate SR3 (Khurha), SR4 (Temari), SR7 (Takhatpur), SR13 (Gadamod) and SR14 (Matia) produced sclerotia in group.

Akram *et al.* (2015) found variation among isolates of *Sclerotium rolfsii* on the basis of their mycelial growth rate, colony morphology, size and pattern and distribution, number, weight, size and arrangement of the sclerotia. Reddi *et al.* (2014) observed that the sclerotia were scattered all over the plate singly or joined together, preferably at the periphery and /or centre of the Pertri plate.

Table 4.6: Variability in morphology of sclerotia produced by different isolates of *S. rolfsii.*

S.No.	Location	Isolates	No. of Sclerotia (12 DAI)	Sclerotial Weight (mg)*	Size of Sclerotia (mm)**	Shape of sclerotia	Colour of Sclerotia	Arrangement of sclerotia
3	Khurha	SR3	212	0.133	1.2	Spherical	Reddish Brown	Scattered
4	Temari	SR4	135	0.159	1.5	Spherical	Dark Brown	Scattered
5	Chilfi	SR5	112.33	0.241	1.2	Ellipsoidal	Reddish Brown	Group
6	Bhtha-Bhurka	SR6	67.66	0.1	0.4	Spherical	Light Brown	Group
7	takhatpur	SR7	172	0.123	1.6	Spherical	Dark Brown	Scattered
8	Gobra	SR8	155	0.13	0.8	Spherical	Dark Brown	Scattered
9	Surgi	SR9	203	0.124	0.6	Spherical	Dark Brown	Group
10	Gogiya	SR10	143.66	0.117	0.7	Spherical	Reddish Brown	Scattered
11	kapadah	SR11	258.33	0.172	0.7	Ellipsoidal	Reddish Brown	Scattered
12	ghuterkudi	SR12	76.33	0.242	1.5	Spherical	Dark Brown	Scattered
13	Gadamod	SR13	78.66	0.113	1	Spherical	Light Brown	Scattered
14	Matia	SR14	184	0.045	0.4	Spherical	Light Brown	Scattered

*Average of 100 sclerotia

** Average of 10 sclerotia

Plate:4.10: (a) morphological characterization of different isolates of *Sclerotium rolfsii*.Sacc. of lentil

Plate 4. 10: (b) morphological characterization of different isolates of *Sclerotium rolfsii*.Sacc. of lentil

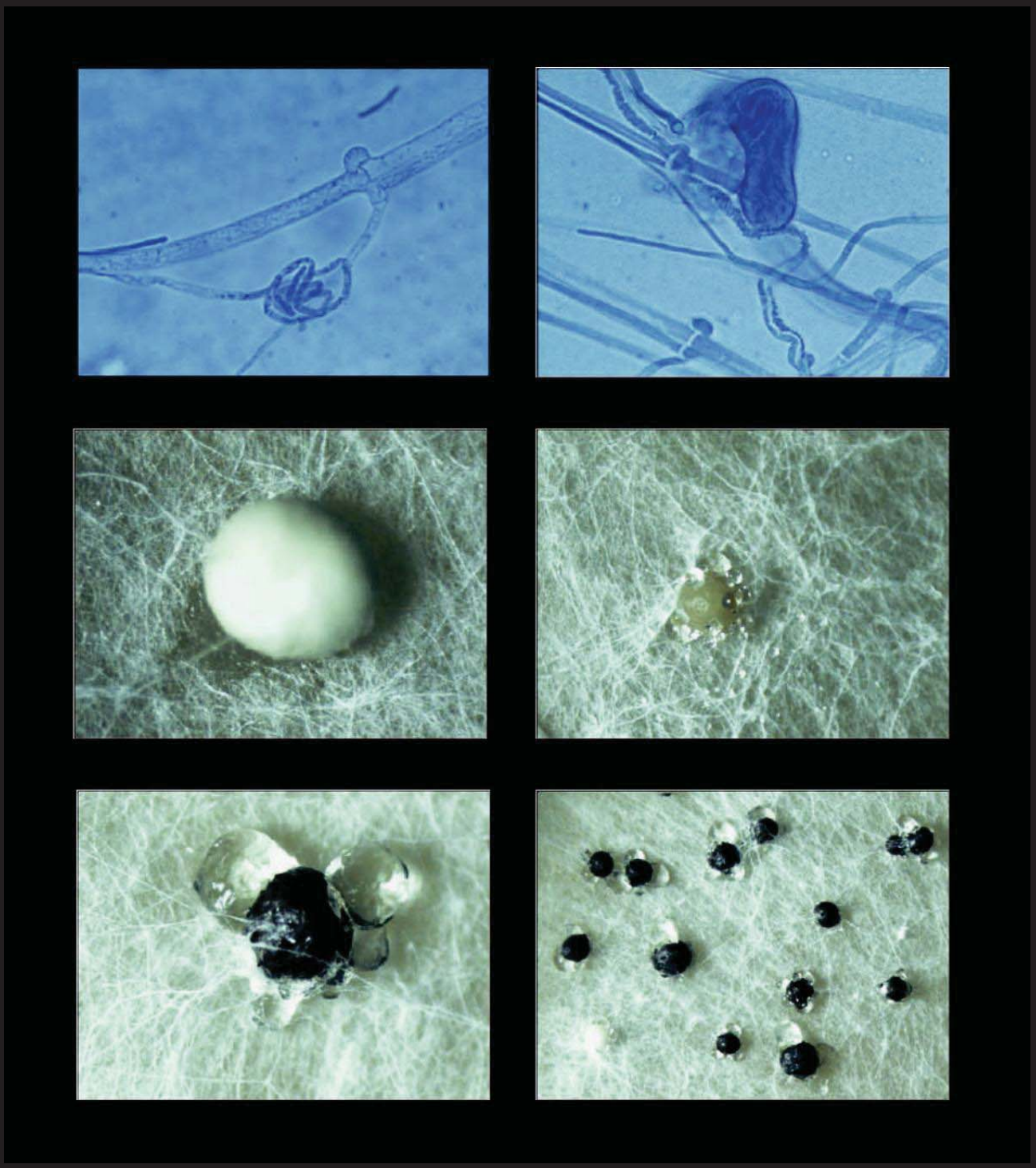


Fig.4.11(c) Morphology and sclerotia formation of *S. rolfsii*

4.5 Pathogenicity test

All the isolates were found to pathogenic under artificially inoculated condition. Artificially inoculated plants expressed typical symptoms of collar rot. The seedling was turned yellow collapsed and showed rotting at the collar region. The collar portion was covered with whitish mycelia strands. Morphologically on re-isolation of fungus confirmed the presence of *S.rolfsii*.

The fungus of each isolate isolated from seeds and diseased portion of lentil plants, was purified by hyphal tip technique and was tested for its pathogenicity. The pathogenicity of the isolated fungus was proved by seed inoculation and soil inoculation techniques. The pathogen of each isolate multiplied on sterilized wheat grains (Plate 4.9) and used as seed and soil inoculation (20g/pot). A mixture of garden soil: Farm yard manure (3:1) was sterilized in an autoclave at 1.3 kg per square centimeters pressure at 121°C temperature for twohours. The inoculated soil was filled in the fresh pots of 20 cm face diameter size. The pots were kept in the cage house for seven days and were irrigated with distilled water to allow establishment of the pathogen. Pots with un-inoculated sterilize soil was kept as control. Apparently healthy lentil seeds of variety 'K-34' were surface sterilized (0.1 per cent sodium hypo-chloride solution for two minutes) Followed by two rinsing in sterilized distilled water (SDW) and were sown in inoculated pots @15 seed / pot, keeping four pots as four replications. For comparison seeds were sown in sterilized soil without pathogen (un-inoculated control). Per cent disease incidence of Pathogencity test of *Sclerotium rolfsii* is presented (Fig-4.9).

Highest per cent disease incidence was observed in soil inoculation technique in isolate SR4 (70.00) Followed by soil inoculation in isolate SR-3 (68.33). While minimum percent disease incidence was observed in soil inoculation technique in isolate SR9 (36.67%). Re-isolations from these diseased seedlings yielded the culture of the fungus of each isolate and identical to original one. The re-isolation culture was again found to produce the disease .

4.5.1 Aggressivess of *S. rolfsii* isolates

4.5.1.1 *In vivo* pot experiment

Aggressiveness of all the isolates was tested by sick pot method in glass house condition. Fortin isolates of *S. rolfsii* were screened to study most virulent strains for collar rot.

The data presented in Table 4.7 revealed that the isolates are comparatively variable in their virulence. The disease response on cultivar" K-34 of different isolates varied with average morality per cent of 66.66 to 100 %. Highest mortality was observed in SR 4 (Temari) which was significantly superior than other isolates, followed by SR14 (Matia) (96.29 %), SR5 (Chilfi) (89.68 %), SR3 (Khurha) (85.71%), SR8 (Chilfi) (85.18 %), SR11 (Kapadah) (83.33 %) and SR12 (Ghuterkudi) (81.48%). However, lowest mortality 66.66 % was observed in isolate SR2 (Agronomic field, Raipur) followed by SR13 (Gogia Bhatapara) (69.29%), SR6 (Bhatha-Bhurka) (69.64%) and SR9 (Surghi) (73.54%).

Effect of *S. rolfsii* isolates on germination, root and shoot length of lentil under sick pot soil was studied and the data presented in Table 4.7 revealed that *S. rolfsii* isolates significantly affect the germination, root and shoot length of lentil. Isolate SR4 was found significantly superior in reducing the germination per cent (63.33 per cent) over all the other isolates, followed by SR1 (IGKV, Farm), SR5(Temari) and SR6 (Bhatha-Bhurka) that is 70 per cent. However, maximum germination per cent was recorded in SR12 (Ghuterkudi) that is 90.00 per cent followed by SR8 (89.00), SR13 (Gadamod) and SR10 (Gogiya) (83.33%)

Root length was found to vary from 10.33 cm to 15.6615.66 cm. Minimum root lenght 10.33 cm was found in isolate SR1 (IGKV,Farm) followed by SR10 (Gogiya), SR9 (Surghi), SR 3 (Khurha), (11.66 cm) and SR12(Ghuterkudi), SR7 (Takhatpur) (12.33 cm) which exhibited significant difference with isolates SR6 (Bhatha-Bhurka), SR11 (Kapadah), SR14 (Matia) was 14.11 cm.

Shoot length was found to vary from 13.20 cm to 21.66 cm. Minimum shoot lenght 13.20 cm was found in isolate SR 1 (IGKV Field) followed by SR9 (Surghi)(15.66 cm), SR2 (Agronomy field raipur), SR12 (Guterkudi) and SR11 (Kapadah) (17.66 cm) which were at par with each other. While, the maximum shoot length was observed in SR8 (Gobra) (21.66 cm) followed by SR4 (Temari)

(21.00 cm), SR13 (Gadamod) and SR5 (Chilfi) (20.33 cm) which were at par with each other.

In agreement to above finding, Bagwan (2011) studied pathogenic variability among 59 isolates of *S. rolfsii* collected from groundnut growing areas and only nine isolates were found to be highly virulent causing more than 60% mortality of plants due to stem rot .

The result indicated that the isolate SR4 (Temari, Mungeli) is most virulent and caused 100 per cent mortality. Therefore, isolate SR4 was used for mass multiplication on wheat grain medium to make sick soil for further screening studies.

Table 4.7: Aggressiveness of *S. rolfsii* isolates causing collar rot of lentil under glass house condition.

S. No.	Isolates	Germination (%)*	Root Length(cm)**	Shoot length(cm)**	Per cent mortality***
1	SR1	66.66 (8.2)	10.33	14.66	75.39 (60.40)
2	SR2	76.66 (8.8)	14.33	15.66	100.0 (90.00)
3	SR3	70.00 (8.4)	11.66	17.66	85.71 (67.76)
4	SR4	63.33 (8.0)	14.33	21	69.04 (56.55)
5	SR5	66.66 (8.2)	14.66	20.33	89.68 (74.54)
6	SR6	70.00 (8.4)	12.66	18.33	69.64 (56.62)
7	SR7	70.00 (8.4)	12.33	19.66	80.95 (64.39)
8	SR8	90.00 (9.5)	13.66	21.66	85.18 (67.61)
9	SR9	76.66 (8.7)	11	15.66	73.54 (59.06)
10	SR10	83.33 (9.1)	10.66	18.66	96.29 (83.50)
11	SR11	80.00 (9.0)	13.33	17.66	83.33 (66.17)
12	SR12	90.00 (9.5)	12.33	17	81.48 (65.23)
13	SR13	86.66 (9.3)	15.66	20.66	80.47 (64.20)
14	SR14	80.00 (9.0)	14	19.33	66.66 (54.80)

+ Figures in parentheses are square root transformation ** Average of five replication

@ Figures in parentheses are angular transformation

4.6. Soil factors affecting development of collar rot disease in lentil

4.6.1 Effect of soil type on collar rot of lentil

An experiment was conducted to know the effect of soil type on collar rot disease development caused by *S. rolfsii* in lentil. Result showed significant difference and data are presented in Table 4.8 and Fig.4.14.

Four different soil types are found in Chhattisgarh region that were taken for study. Significantly least mortality per cent (19.17 %) was recorded in Matasi. However, highest mortality was observed in Kanhar 57.41 %. The soil type, Dorsa (35.25 %) and Bhata (40.14 %) were statistically at par among themselves.

Table 4.8: Effect of soil type on development of collar rot disease in lentil

S.N.	Soil type	Percent mortality*
		Mean
1	Kanhar	57.41(49.26)
2	Bhata	40.14(39.31)
3	Dorsa	35.25(36.42)
4	Matasi	19.17(25.97)
	SEm	**1.86**
	CD(5%)	**5.86**

Figures in parentheses are angular transformation

Soil type also significantly influenced the germination per cent, root length and shoot length in lentil when inoculated with *S. rolfsii* and data are presented in Table 4.9 and Fig.4.15

Highest germination was observed in Kanhar (92.67 per cent) followed by Dorsa and Bhata 88.65 per cent, which were found to be at par with each other. While, the lowest germination per cent was observed in Matasi that is 83.94 per cent. Maximum root length was observed in Kanhar (36.19 cm) and Bhatha (35.16 cm) followed by Dorsa (33.78 cm) which were at par with each other. Minimum root length was recorded in Dorsa that is 33.78 cm. Maximum shoot length was observed in Khanar (26.85) followed by Bhata (24.70 cm) which were at par with each other. Minimum shoot length was recorded in Matasi (23.39 cm) and Dorsa (23.45 cm). Soil type also affects the vigour index of plant. The vigour index is multiplication of germination percentages and seedling length of the final day count. Maximum vigour index was found in Kanhar that is 5841.91 where as minimum vigour index was recorded in Matasi (4709.03)

Table 4.9: Effect of soil type on germination and vigour index of lentil in inoculated soil with *S. rolfsii.*

S.No	Soil Type	Germination (%)	Root Length (cm) *	Soot Length (cm) *
		Mean	Mean	**Mean**
1	Kanhar	92.67(9.63)	36.19	26.85
2	Bhata	88.65(9.42)	35.16	24.70
3	Matasi	83.94(9.16)	32.71	23.39
4	Dorsa	90.33(9.50)	33.78	23.45
	SEm	**1.80**	**0.88**	**1.31**
	CD(5%)	**5.68**	**2.78**	**4.14**

* Average of 3 replication
Figures in parentheses are square root transformation

Nene (1979) reported vertisol soil seems to favour the disease more than alfisol soil. The mortality was high in vertisol which was significantly influenced by soil type when compared to alfisol. Chauhan *et al.* (2002) reported high wilt incidence in vertisol as it contain high clay content (64%). Hussain *et al*. (2006a) studied the effect of soil type (clay, clay loam, sandy loam and sandy) on the incidence of collar rot disease in lentil caused by *S. rolfsii* and found that the mortality of seedling was higher in clayey soil. Krishna and Krishnappa, (1996) reported *Sclerotium rolfsai,* f.sp. *ciceri* spore was high in vertisol (1300 CFU/g soil) as compared to alfisol (1153 CFU/g soil). Total mortality was observed on 20-24 days after sowing in vertisol while the wilt incidence was only 73.4% in alfisol.

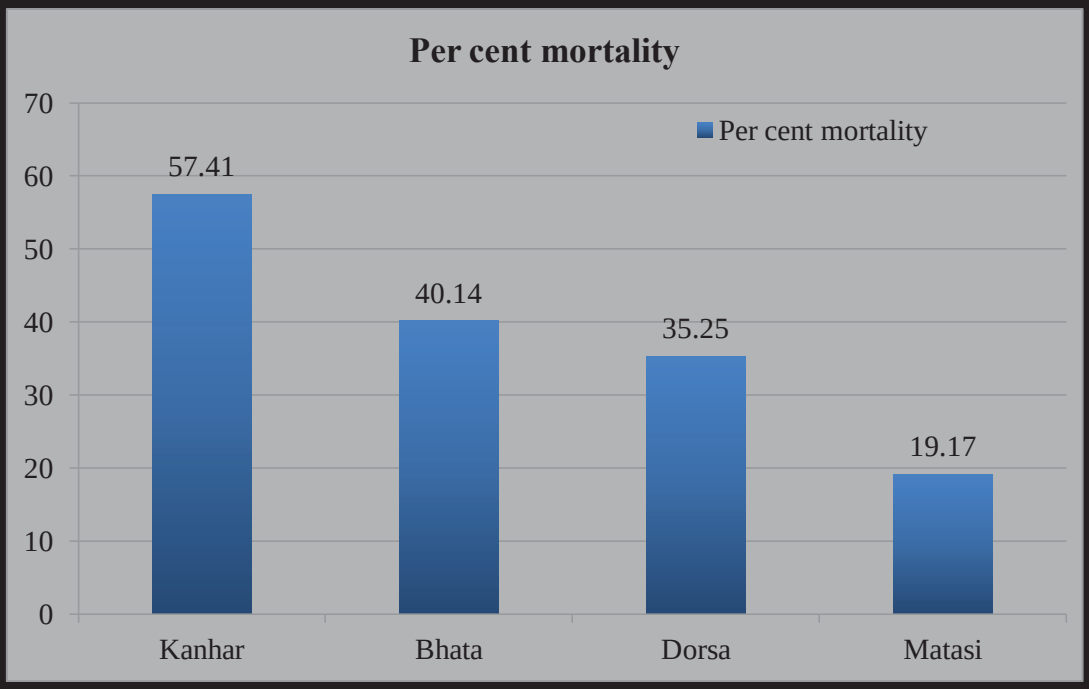


Fig.4.12 (a): Per cent mortality of lentil due to collar rot under glass house condition in different soil type

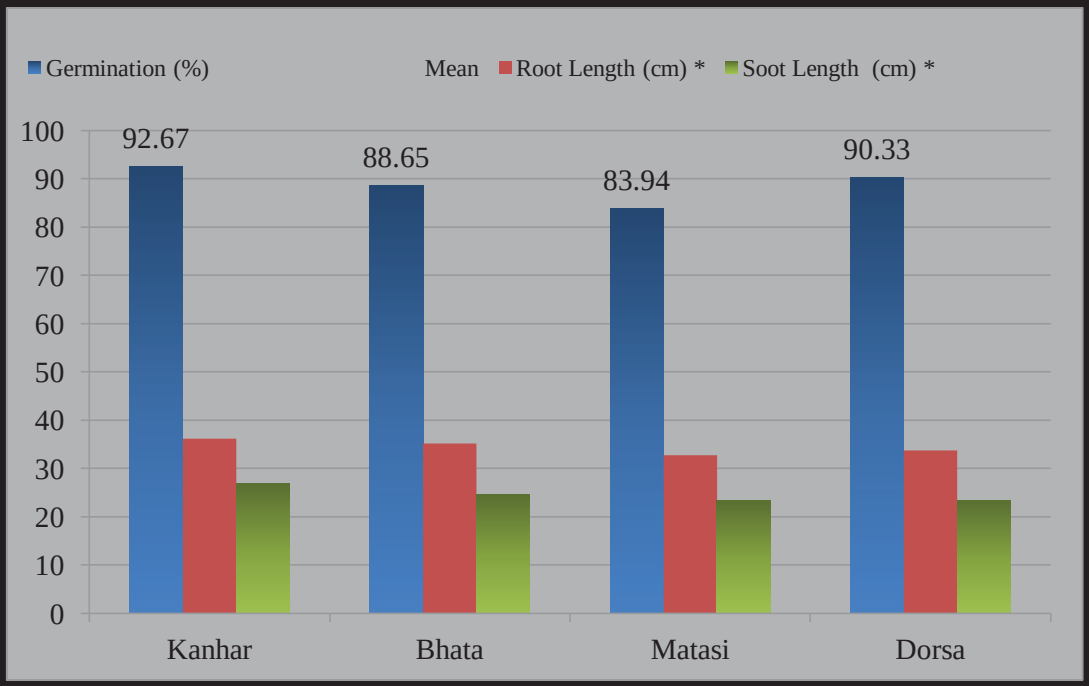


Fig.4.13 : Effect of soil type on germination, root and shoot length of lentil in inoculated soil with *S. rolfsii*

Fig 4.14 Effect of four soil types *viz.*, (Alfisol (Dorsa), Inceptisol (Matasi),Vertisol (Kanhar) and Entisol (Bhata))

4.6.2 Effect of soil texture on collar rot of lentil

An experiment was conducted to know the effect soil texture on collar rot disease development caused by *S.rolfsii* in lentil. Result showed significant difference among the treatmentsand data are presented in Table 4.10, Fig.4.17

Significantly least mortality per cent (23.14%) was recorded in sandy loam texture followed by sandy texture with mortality per cent of 26.82 % which were at par with each other. However, highest mortality was observed in clay texture soil 62.96 %, followed by Clay loam 46.59 %.

Table 4.10: Effect of different soil texture on development of collar rot disease in lentil.

S.No.	Soil texture	Per cent mortility*
1	Sandy	26.82(31.19)
2	Sandy loam	23.14(28.75)
3	Loam	30.83(33.73)
4	Clay	62.96(52.51)
5	Clay loam	46.59(43.04)
6	Silt clay	37.48(37.75)
7	Silt loam	32.91(35.01)
	SEm±	1.24
	CD (5%)	3.82

* Average of three replication
Figures in parentheses are angular transformation

Soil texture also significantly influenced the germination per cent, root length and shoot length in lentil when inoculated with *S.rolfsii*, was studied and data are presented in Table 4.11 and Fig.4.17 It is clear from the data which showed highest germination in sandy textured soil, (100.0 per cent) followed by Sandy loam (96.22%) and Silt loam (95.55%) which were significantly superior with other treatments. Loam , Silt clay and Clay loam soil supported lentil seed to germinate by 93.11, 93.33 and 86.55 per cent, respectively. While, lowest germination per cent was observed in Clay (85.11%). Maximum root length was observed in Sandy soil (22.27 cm) which significantly superior than other treatments. Minimum root length was recorded in Clay soil (14.07 cm). The root length Silt loam (19.47 cm), Silt clay (19.00 cm), Sandy loam (18.60 cm) and Loam (20.47) were statistically at par among themselves. Maximum shoot length was recorded in Clay soil (19.27 cm) followed by LSandy loam (15.67 cm), Clay

loam (16.60 cm) Silt clay and Silt loam (17.20 cm) which were at par with each other. Minimum shoot length was observed in Clay soil (19.27 cm). Soil type also affects the vigour index of plant. Maximum vigour index was found in Sandy soil (4054) where as minimum vigour index was in Clay loam (2837.56).

Table 4.11: Effect of soil texture on germination and vigour index of lentil in inoculated soil with *S. rolfsii.*

S.No.	Soil texture	Germination (%)	Root Lengh (cm)	Shoot Lengh (cm)	Vigour Index
1	Sandy	100.00(10.00)	22.27	18.27	4054
2	Sandy loam	96.22(9.81)	18.60	15.67	3297.45
3	Loam	93.11(9.65)	20.47	17.27	3513.97
4	Clay	85.11(9.23)	14.07	19.27	2837.56
5	Clay loam	86.55(9.30)	16.93	16.60	2902.02
6	Silt clay	93.33(9.66)	19.00	17.20	3378.54
7	Silt loam	95.55(9.77)	19.47	17.20	3503.81
SEm±		**1.35**	**1.35**	**1.15**	
CD (5%)		**4.17**	**4.18**	**3.53**	

1.Average of three replication
1. Average of five replication
Figures in parentheses are square root transformation

In agreement to above findings, Hussain *et al*. (2006) found that in clayey soil, seedling death was 94% whereas in clay loam, sandy loamy and sandy soils, it was 82, 78 and 60%, respectively. Seedling death in sandy soil was significantly less than that noted in all other types of soil . Mahato *et al*. (2017) reported that incidence of collar rot varied with different soil textures. Maximum incidence (100%) was observed in sandy clay loam followed by sandy clay (88.33%), silt clay loam (56.67%), Clay loam (43.33%) and minimum disease incidence recorded in silt loam (36.67 %). Results reveled that Sandy clay loam soil was highly favorable for collar rot disease. Banyal *et al.* (2008) also reported that lighter soils were more favourable to the collar rot disease of tomato caused by *Sclerotium rolfsii* than the heavy textured soil .

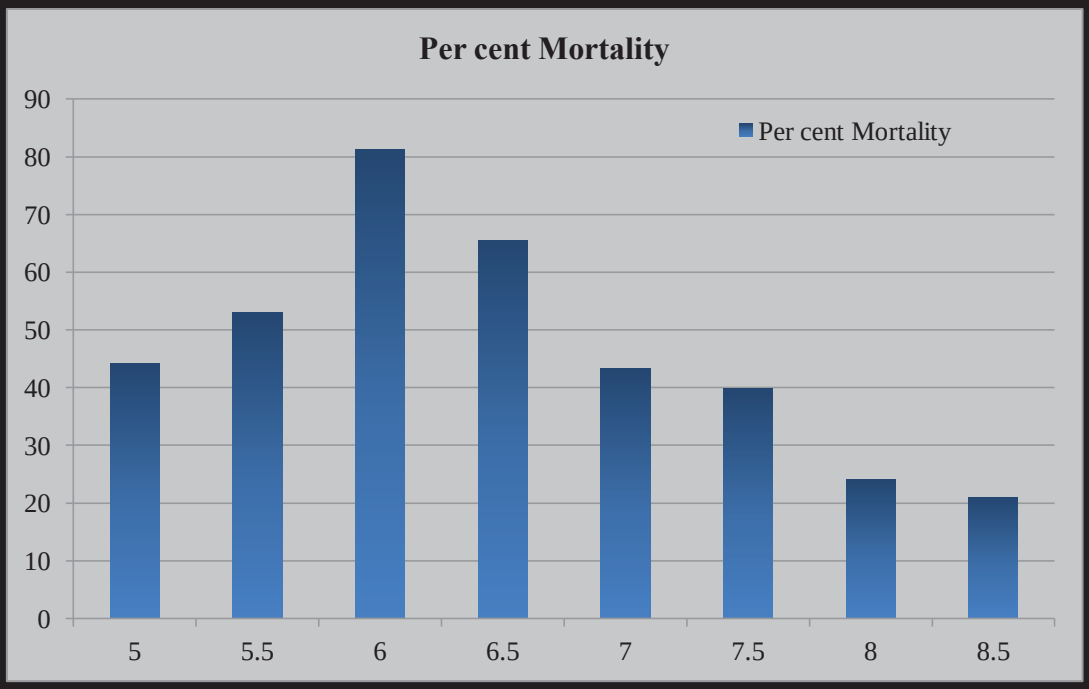


Fig.4.15 : Per cent mortality of lentil due to collar rot under glass house condition at different soil pH

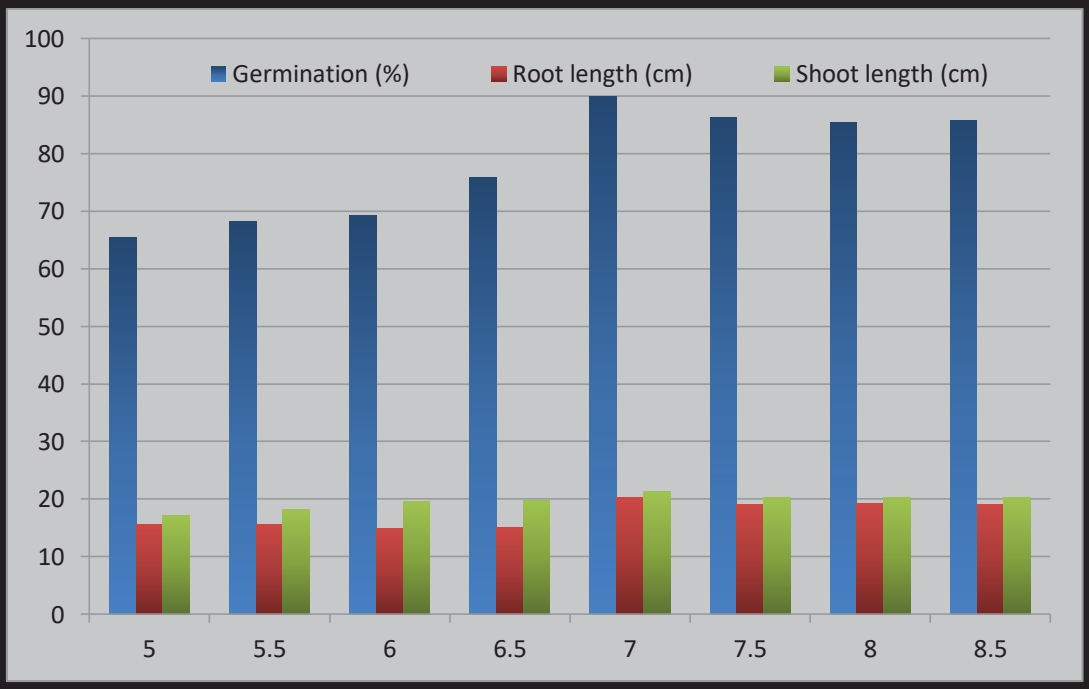


Fig.4.16 : Effect of soil pH on germination, root and shoot length of chickpea in inoculated soil with *S. rolfsii*

4.6.3 Effect of different pH on collar rot of lentil.

An experiment was conducted to know which soil pH is favorable or unfavorable for collar rot disease development caused by *S. rolfsii* in lentil as described in materials and methods. Result indicated significant difference and presented in Table 4.12 and Fig. 4.15

Significantly least mortality per cent (20.97%) was recorded at pH 8.5 followed by pH 8 (24.02 %), pH 7.5 (39.81 %), pH 7 (43.33 %), pH 5 (44.21 %) and pH 5.5 (52.99 %) which were next in order. However, significantly highest mortality was observed at pH 6.0 that is, 81.25 % followed by pH 6.5 (65.56). All the treatment differ significantly in terms of mortality per cent with each other , except pH 5, pH 5.5, pH 7 and pH 7.5.

Table 4.112: Effect of different pH on development collar rot disease in lentil.

S. No.	pH	Per cent mortality
1	5	44.21(41.68)
2	5.5	52.99(46.71)
3	6	81.25(64.34)
4	6.5	65.56(54.07)
5	7	43.33(41.17)
6	7.5	39.81(39.12)
7	8	24.02(29.35)
8	8.5	20.97(27.25)
SEm±		**1.61**
CD (5%)		**5.05**

* Average of three replication

Figures in parentheses are angular transformation

Soil pH also significantly influenced the germination per cent, root length and shoot length and vigour index of lentil when inoculated with *S. rolfsii* (SR4) and data are presented in Table 4.13

Highest germination was observed in pH 7.0 that is 90.00 per cent, followed by pH 7.5 with 86.22 per cent which were at par with each other. Whereas lowest germination was observed in pH 5 that 65.44 per cent followed by pH 5.5 (68.22 per cent) which were at par with each other. Maximum root length was observed in pH 7.0 (20.20 cm) followed by pH 8.0, pH 8.5 (19.07 cm) and pH 7 (18.60 cm) which were at par, but significantly better than other treatments. Minimum root length was recorded in pH 5 that is 14.87 and pH 5.0

(15.53 cm). Maximum shoot length was observed in pH 7.0 (21.27 cm) followed by pH 8.5. (20.33 cm), pH 8.0 (20.27 cm), pH 7.5(20.20 cm) and pH 6.5 (19.80 cm) which were at par with each other. Whereas, minimum shoot length was recorded in pH 5.0 (17.20 cm). Highest vigour index was found in pH 7 that is 3771.00 whereas least was in pH 5 that is 2141.85.

Similar findings were given by Mahato *et al*. (2017) who reported that highest incidence (100%) was recorded at 6.5 and 7.0 pH level and lowest incidence (51.67%) was recorded at 8.0 pH level. Prasad *et al*. (1986) found the pH range of 5.0 to 7.0 as best for sclerotial formation at different temperatures i*n vitro*. Wide range of pH with optimum near 6.0 for the growth of various isolates of *Sclerotium rolfsii* have been reported by Aycock (1966), Narasimhan (1969) and Sharma and Kaushal (1979). Banyal *et al*.(2008) reported that maximum death of tomato caused by *Sclerotium rolfsii* at 6.5-7.5 pH level in green house experiments.

Table 4.13: Effect of different pH on germination and vigour index of lentil in inoculated soil with *S. rolfsii.*

S. No.	pH	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
1	5	65.44(8.09)	15.53	17.20	2141.85
2	5.5	68.22(8.26)	15.67	18.27	2315.38
3	6	69.33(8.33)	14.87	19.60	2389.8
4	6.5	75.78(8.71)	15.13	19.80	2650.13
5	7	90.00(9.49)	20.20	21.27	3771
6	7.5	86.22(9.29)	19.07	20.20	3385.85
7	8	85.44(9.24)	19.20	20.27	3372.31
8	8.5	85.78(9.26)	19.07	20.33	3379.73
SEm±		**1.60**	**1.31**	**1.05**	
CD (5%)		**5.03**	**4.12**	**3.30**	

1.Average of three replication

2 Average of five replication

Figures in parentheses are square root transformation

4.7. collection and characterization of native isolates of Trichoderma spp., Pseudomonas spp., and Bacillus spp.:

4.7.1: Isolation and collection of *Trichoderma* isolates

Trichoderma spp. were isolated from the soil samples by serial dilution method using Potato Dextrose Agar (PDA) medium. The cultures were identified and confirmed as *Trichoderma* spp. through microscopic observation. In the present investigation, seventeen isolates of *Trichoderma* spp. were collected from different locations of Chhattisgarh and designated in Table 4.14

Table 4.50: Isolates of *Trichoderma* spp. collected from different locations of Chhattisgarh.

Table 4.14: Details of location of soil sample collection, presence and code of *Trichoderma* isolates

S.No.	Location	Block	District	Code of isolate
1	Raipur	Raipur	IGKV, Farm	T_1
				T_2
2			IPM, Field	T_3
3			Aarang	T_4
4		Arang	Arang	T_5
5		Abhanpur	Khurha	T_6
6	Mungeli	Mungeli	Temari	T_7
7			Navapara	T_8
8		Lormi	Chilfi	T_9
9			Bhtha-Bhurka	T_{10}
10			Sipahi	T_{11}
11	Bilaspur	Bilaspur	takhatpur	T_{12}
12		Bilaspur	Devri khurd	T_{13}
14	Dhamtari	Kurud	Gobra	T_{14}
15	Baloda bazar	Simga	Surgi	T_{15}
16		Bhatapara	Gogiya	T_{16}
17			Sandyi	T_{17}
18			Mohba	T_{18}
19			Padumtari	T_{19}
20	Kawardha	Kawardha	Newari farm	T_{20}
21		Pandariya	kapadah	T_{21}
22			ghuterkudi	T_{22}
23		Pandariya	Sangona	T_{23}
24	Bemetara	Bemetara	Bemetara	T_{24}
25			Matia	T_{25}
26			mohbhate	T_{26}
27			Gadamod	T_{27}
28	Mahasamund			T_{28}

29	Griyaband	Gariyaband	Panduka	T_{29}
30		Mainpur	birighat	T_{30}
31	Jagadalpur	Kumharavan		T_{31}
32	Balod	Balod		T_{32}
33	Kanker	Kanker		T_{33}
34		Kanker		T_{34}
35	Janjgir	Janjgir		T_{35}
36	Durg	Durg	Anjora	T_{36}
37		Dhamda	Dhamda	T_{37}
38	Rajnandgaon	Rajnandgaon	Surgi	T_{38}
39			Mohba	T_{39}
40	Korba	Korba	KVK Farm	T_{40}

4.7.2 Cultural and Morphological characterization of *Trichoderma* isolates

The Petri dishes with *Trichoderma* culture were observed for colony colour, growth pattern (presence or absence of aerial mycelium or subdued growth), appearance, pustule, pigmentation and odour. Table 4.59 exhibit primary characterization on the basis of cultural and morphological characteristics (Plate 16).

4.7.2.1 Colony colour: Fifteen*Trichoderma* isolates can be categorized in 5 groups (Table 4.59). Yellow green colour of colony was observed in isolate T1 and T40, whitish green in isolates T4, T12, T20, T22 and T33, green in isolate T7, T14, T29 and T31a whereas, light green in isolates T19, T23, T31b and T36.

4.7.2.2 Growth pattern: *Trichoderma* isolates in terms of growth pattern are grouped in two, that is, effused and subdued. Isolates T4, T12, T22 and T23 exhibited effused growth pattern whereas, isolates T1, T7,T19, T20, T29, T31a, T31b,T33, T36 and T40 showed subdued pattern, as presented in Table 4.59.

4.7.2.3 Appearance: Colony appearance is grouped in 5 categories (Table 4.59). Uniform ringed velvety exhibited by isolate T1, velvety at periphery by isolate T4, T14and T20, velvety ringed by several isolate namely, T7, T19, T29, T31a, T31b, T33 and T40 whereas, scattered appearance by isolates T12, T22, T23 and T36.

4.7.2.4 Pustules: One of the macroscopic featuresof *Trichoderma* is the ability to form pustules. Formation of pustules by Trichoderma is grouped into four categories, absence of pustules was found in isolate T1, T7, T14, T19, T20, T29, T31a, T31b, T33 and T40. Scattered pustules were observed in isolate T4, compact pattern pustules in T12 and T23 whereas, minute pustules were observed in T22 and T36 (Table 4.59).

4.7.2.5 Pigments: Pigmentation of *Trichoderma* isolates were studied on PDA plates. The production of diffusible yellowish orange pigments were found in T1, T7, T12, T19, T22, T29 and T40 isolates, whereas, in T4, T14, T20, T23, T31a, T31b, T33 and T36 isolates no pigmentation were found (Table 4.59).

4.7.2.6 Odour: No odour was found to produce from *Trichoderma* isolates (Table 4.59).

Table 4.15: Morphological characteristics of *Trichoderma* isolates based on key characters

Isolates	Colony colour	Growth pattern	Appearance	Pustule	Pigments	Odour
T1	Yellow green	Subdued	Uniform ringed velvety	Absent	Yellowish orange	Absent
T4	Whitish green	Effused	Velvety at periphery	Scattered	Absent	Absent
T7	Green	Subdued	Velvety ringed	Absent	Yellowish orange	Absent
T12	Whitish green	Effused	Scattered	Compact	Yellowish orange	Absent
T14	Green	Subdued	Velvety ringed at periphery	Absent	Absent	Absent
T19	Light green	Subdued	Velvety ringed	Absent	Yellowish orange	Absent
T20	Whitish green	Subdued	Velvety ringed at periphery	Absent	Absent	Absent
T22	Whitish green	Effused	Scattered	Minute	Yellowish orange	Absent
T23	Light green	Effused	Scattered	Compact	Absent	Absent
T29	Green	Subdued	Velvety ringed	Absent	Yellowish orange	Absent
T31	Green	Subdued	Velvetyringed	Absent	Absent	Absent
T32	Light green	Subdued	Velvety ringed	Absent	Absent	Absent
T33	Whitish green	Subdued	Velvety ringed	Absent	Absent	Absent
T36	Light green	Subdued	Scattered	Minute	Absent	Absent
T40	Yellow green	Subdued	Velvety ringed	Absent	Yellowish orange	Absent

4.7.2.7. Colony characteristics on Potato dextrose Agar (PDA) medium :

All the isolates of *Trichoderma* were inoculated in three replications at the center of 85 mm PDA plates in the form of 5 mm mycelial discs taken from margin of colonies 5 days old culture, grown on PDA plates. Colony characteristics were observed for their growth type, margin, colony colour etc after 5 days incubation at 25ºC. Production of any type of secondary metabolite or antibiotic was also observed by colour production seen on the back side of petriplate after 5 days of growth. Isolates of *Trichoderma* spp. grew as regular circular growth on potato dextrose agar medium. However, there were clear differences in colour and type of colonies of different isolates of *Trichoderma* spp. Sporulation was not present in some of the isolates, which was revealed by absence of pigmentation in colony growth. However, in some of the isolates, there was massive sporulation, marked by dark green colour ring. Colonies were found of different colours like light green, dark green-white, light green-

white, yellow green and white in different isolates. Colonies represented varied growth type pattern of aerial, sub-aerial and submerged-aerial type. However, most of the colonies were of aerial and sub-aerial type. On observing the growth of isolates from the back side of petriplates, it was witnessed that two isolates (T_{23} and T_{38}) were producing the yellow colour pigmentation which might be due to production of antibiotic compound. Out of the two isolates T_{23} and T_{38}, *Trichoderma* isolate T_{23} was producing completely yellow colour in petriplate growth which was observed not only from back side of petriplate but also from the front side growth in petriplate. This shows its high capacity to produce antibiotics. Colony characteristics of different isolates of *Trichoderma* spp. have been given in Table 4.16. Isolates for their colony characteristics have been shown in Figure 4.17(a,b) as front view and 2(b) as down side view of petriplate.

4.7.2.8 Radial growth on PDA medium :

All the isolates of *Trichoderma* spp. were inoculated as 5 mm mycelia disc taken from 5 days old culture plates to fresh PDA plates and incubated at 25ºC. Radial growth (colony diameter) was recorded initially after 48 hours of incubation period and then after every 24 hours until full petriplate growth. Colony diameter

of different isolates of *Trichoderma* spp. has been provided in Table 5. All the 40 isolates of *Trichoderma* spp., except T_{33} isolate, accomplished full petriplate growth of 85 mm within 72 hours of incubation period. However, isolate T_{33} took more than 90 hours to complete full petriplate growth. Based on observations recorded after 24 hrs and 48 hrs of incubation period, isolates were observed as medium to fast growing nature and there was presence of noticeable difference in their growth rate. After 24 hours and 48 hours of incubation period average radial growth was observed in range of 6.0 to 23.7 mm and 26 mm to 72 mm, respectively. After 48 hours of incubation period, maximum radial growth of 72 mm was observed in isolate T_{23}. However, minimum radial growth of 26 mm was observed in T_{38} isolate of *Trichoderma* after 48 hours of incubation period. The data presented shows significant differences in their growth rate after same incubation period. Detailed data for each isolate after different incubation period has been given in Table 5. Colony growth of different isolates after three days of incubation period at 25ºC has been shown in Figure 4.18(a,b) Graphical representation for radial growth of different isolates.

Table 4.16: Colony characteristics of different isolates of *Trichoderma* spp. after 5days of incubation period at 25ºC

S.No.	Code of *Trichoderma* spp.	Colony characteristics			
		Shape	Colour [a]	Type	Colour [b]
1	T_1	Circular	Light green	Aerial	White
2	T_2	Circular	Dark green-white	Sub-aerial	White
3	T_3	Circular	Dark green-white	Sub-aerial	White
4	T_4	Circular	Dark green-white	Sub-aerial	White
5	T_5	Circular	Dark green-white	Sub-aerial	White
6	T_6	Circular	Light green-white	Aerial	White
7	T_7	Circular	Light green-white	Aerial	White
8	T_8	Circular	Light green-white	Aerial	White
9	T_9	Circular	Light green-white	Sub-aerial	White
10	T_{10}	Circular	Light green-white	Aerial	White
11	T_{11}	Circular	Dark green-white	Aerial	White
12	T_{12}	Circular	Light green-white	Aerial	White
13	T_{13}	Circular	Dark green-white	Aerial	White
14	T_{14}	Circular	Dark green-white	Aerial	White
15	T_{15}	Circular	White	Sub-aerial	White
16	T_{16}	Circular	Dark green-white	Aerial	White
17	T_{17}	Circular	Dark green-white	Aerial	White
18	T_{18}	Circular	Light green-white	Sub-aerial	White
19	T_{19}	Circular	Light green-white	Sub-aerial	White
20	T_{20}	Circular	Light green-white	Sub-aerial	White
21	T_{21}	Circular	Light green-white	Aerial	White
22	T_{22}	Circular	Dark green-white	Aerial	White
23	T_{23}	Circular	Yellow-green	Submerged-aerial	Yellow
24	T_{24}	Circular	Green	Submerged	White
25	T_{25}	Circular	White	Sub-aerial	White
26	T_{26}	Circular	Light green-white	Aerial	White
27	T_{27}	Circular	White	Aerial	White
28	T_{28}	Circular	Light green-white	Aerial	White
29	T_{29}	Circular	White	Aerial	White
30	T_{30}	Circular	White	Aerial	White
31	T_{31}	Circular	Light green	Aerial	White
32	T_{32}	Circular	Light green-white	Aerial	White
33	T_{33}	Circular	White	Submerged	White
34	T_{34}	Circular	Light green-white	Aerial	White
35	T_{35}	Circular	Light green-white	Sub-aerial	White
36	T_{36}	Circular	Dark green-white	Aerial	White
37	T_{37}	Circular	White	Sub-aerial	White
38	T_{38}	Circular	Light green-white	Aerial	Yellow
39	T_{39}	Circular	Light green-white	Sub-aerial	White
40	T_{40}	Circular	Light green-white	Aerial	White

[a]= colony colour from the front side growth of colony, [b]= colony colour from the back side view

Fig. 4.17 (a): Colony characteristics of different isolates of *Trichoderma* spp. on potato dextrose agar medium after 5 days of incubation (T1 to T20 in Fro

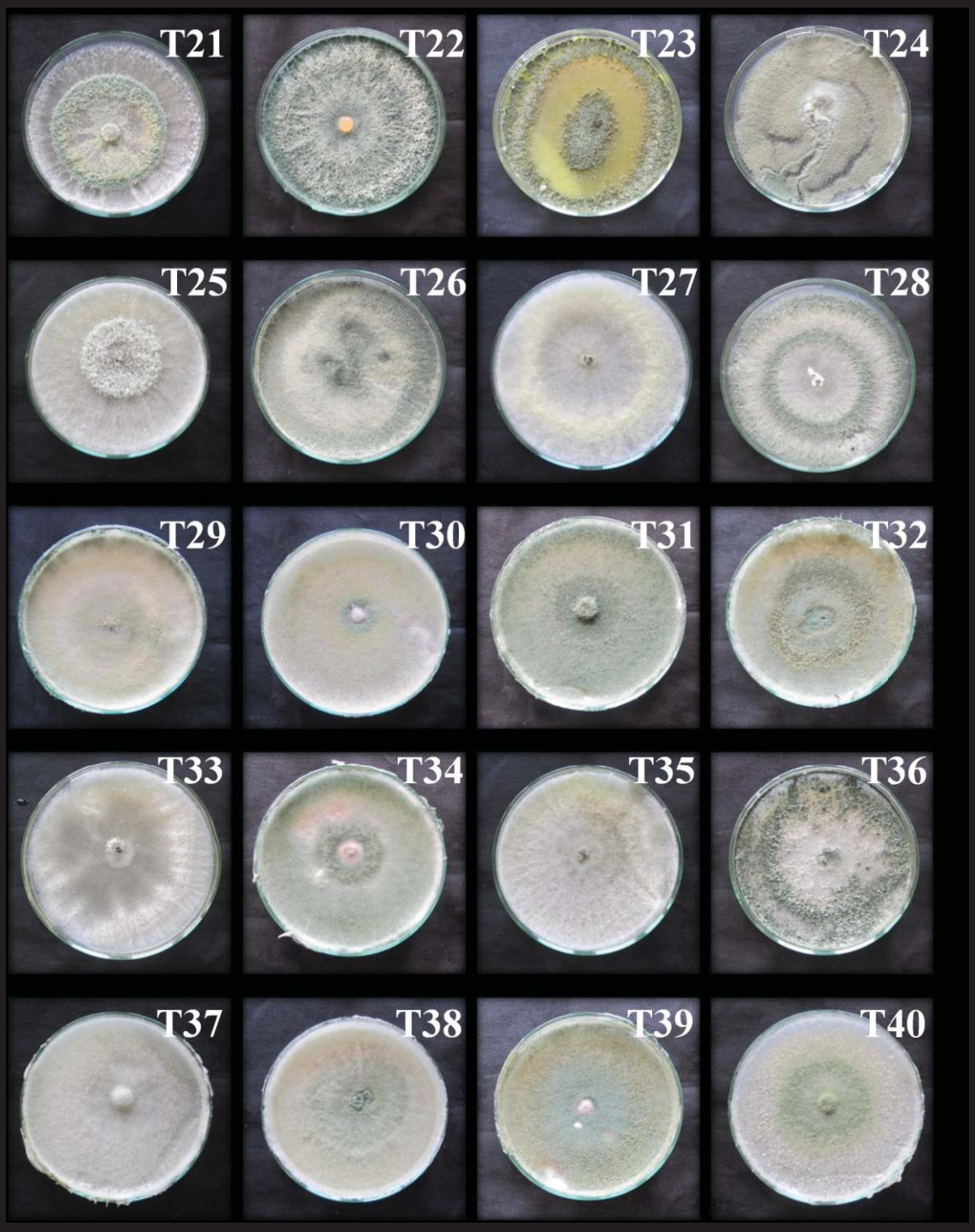


Fig. 4.17(b): Colony characteristics of different isolates of *Trichoderma* spp. on potato dextrose agar medium after 5 days of incubation (T21 to T40 in Front side view)

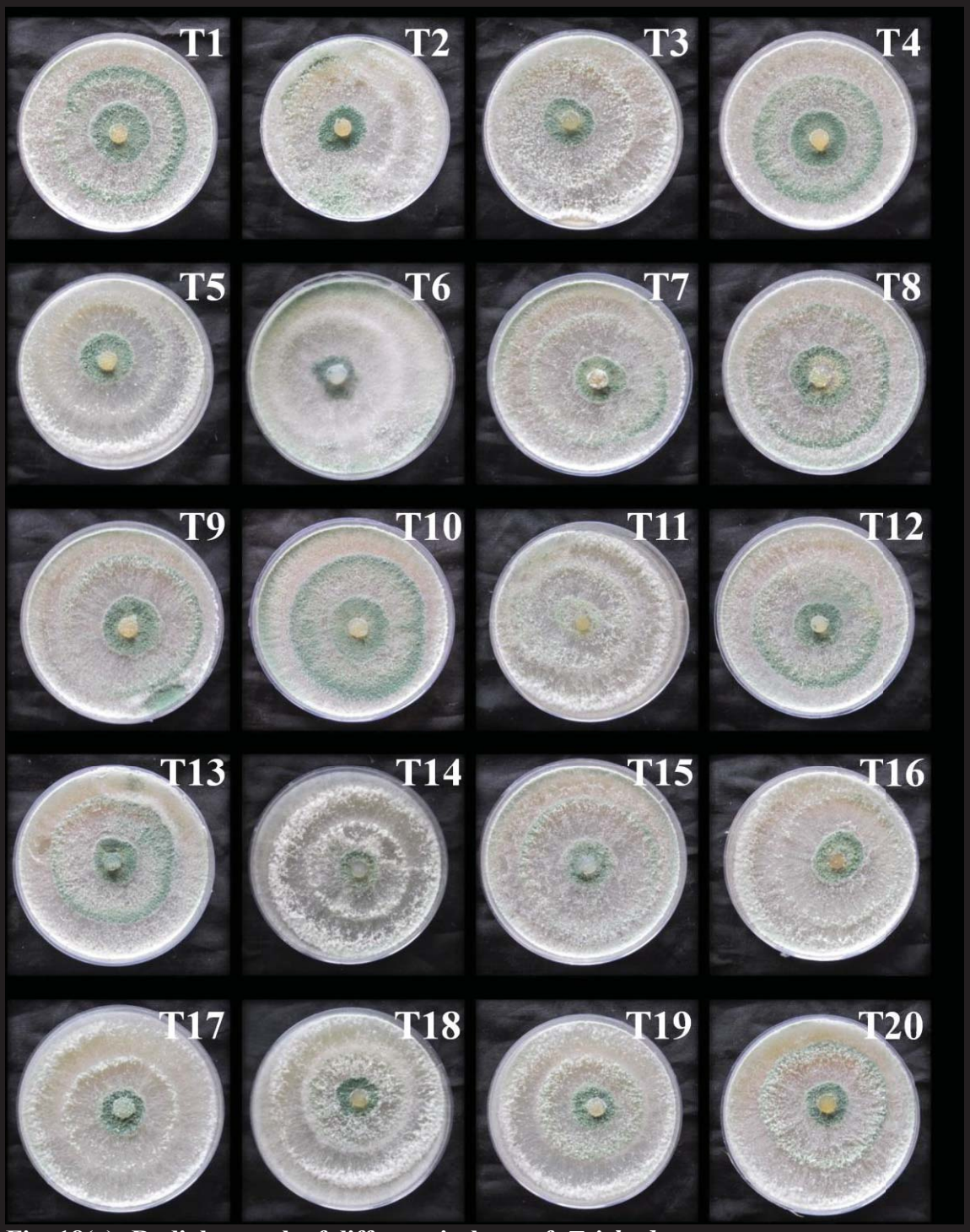


Fig. 18(a): Radial growth of different isolates of *Trichoderma* spp. on potato dextrose agar medium after 3 days of incubation at 25ºC (T1 to T20)

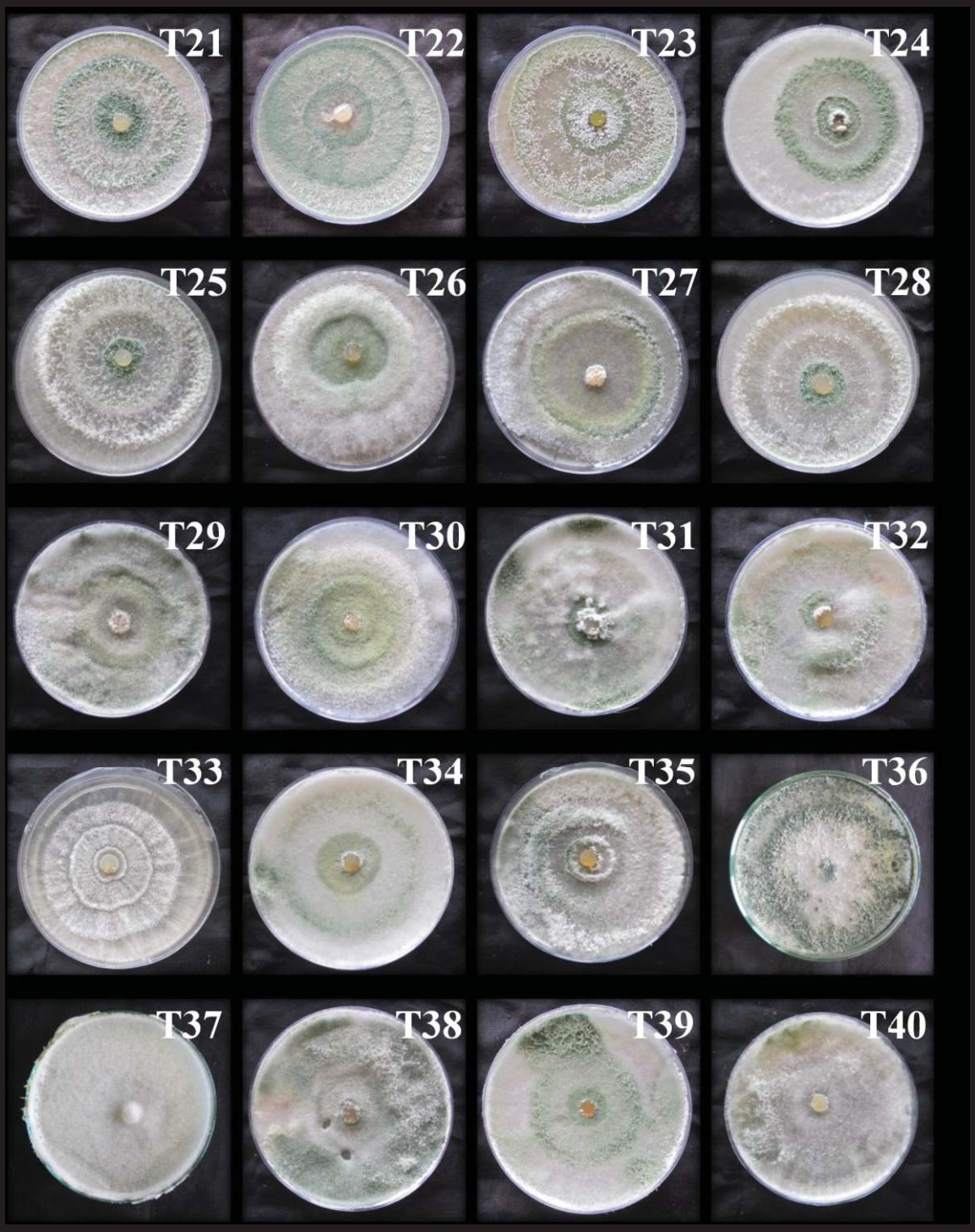


Fig. 18(b): Radial growth of different isolates of *Trichoderma* spp. on potato dextrose agar medium after 3 days of incubation at 25°C (T21 to T40)

Table 4.17: Average radial growth of different isolates of *Trichoderma* spp. after different incubation period at 25ºC

S.No.	Code of *Trichoderma* spp.	Avg. radial growth of *Trichoderma* (in mm)[a]		
		24 hrs	48 hrs	72 hrs
1	T_1	20.0	56.0	85.0
2	T_2	13.0	50.0	85.0
3	T_3	14.0	54.3	85.0
4	T_4	17.0	58.0	85.0
5	T_5	12.0	50.0	85.0
6	T_6	17.0	56.0	85.0
7	T_7	15.0	56.0	85.0
8	T_8	20.0	58.0	85.0
9	T_9	17.7	60.0	85.0
10	T_{10}	22.0	60.0	85.0
11	T_{11}	12.0	44.0	85.0
12	T_{12}	14.0	54.0	85.0
13	T_{13}	13.0	48.3	85.0
14	T_{14}	10.0	40.0	85.0
15	T_{15}	14.0	54.0	85.0
16	T_{16}	14.7	52.3	85.0
17	T_{17}	12.0	46.0	85.0
18	T_{18}	11.7	40.0	85.0
19	T_{19}	11.0	48.0	85.0
20	T_{20}	11.7	50.0	85.0
21	T_{21}	18.0	56.0	85.0
22	T_{22}	21.0	66.0	85.0
23	T_{23}	14.0	72.0	85.0
24	T_{24}	12.0	52.0	85.0
25	T_{25}	11.0	40.0	85.0
26	T_{26}	6.0	34.0	65.7
27	T_{27}	19.3	50.0	85.0
28	T_{28}	14.0	40.0	85.0
29	T_{29}	10.0	38.0	85.0
30	T_{30}	12.0	46.0	85.0
31	T_{31}	14.0	60.0	85.0
32	T_{32}	23.7	66.0	85.0
33	T_{33}	11.3	28.0	54.0
34	T_{34}	15.0	70.0	85.0
35	T_{35}	14.7	48.0	85.0
36	T_{36}	18.0	57.0	85.0
37	T_{37}	20.0	66.0	85.0
38	T_{38}	10.7	26.0	85.0
39	T_{39}	9.7	45.7	85.0
40	T_{40}	16.0	64.3	85.0
	S.Em±	0.72	0.82	0.19
	C.D. (5%)	2.10	2.39	0.55

Table 4.18: Average moist and dry mycelial weight of different isolates of *Trichoderma* spp.

S. no.	Code of *Trichoderma* spp.	Moist weight (g.)[a]	Dry weight (g.)[a]
1	T_1	11.9	2.3
2	T_2	8.3	2.2
3	T_3	9.6	2.3
4	T_4	9.3	2.5
5	T_5	10.5	2.9
6	T_6	11.5	3.2
7	T_7	6	2.1
8	T_8	9.1	2.7
9	T_9	11.9	2.1
10	T_{10}	10.5	1.6
11	T_{11}	3.3	1.4
12	T_{12}	11.1	2.5
13	T_{13}	12.2	3.6
14	T_{14}	10.3	2.8
15	T_{15}	5.4	2.3
16	T_{16}	8.7	1.9
17	T_{17}	8.2	1.9
18	T_{18}	9.5	2.8
19	T_{19}	9.9	3.3
20	T_{20}	6.5	1.9
21	T_{21}	6.8	1.5
22	T_{22}	8.6	3.2
23	T_{23}	4.4	1.8
24	T_{24}	6.3	2
25	T_{25}	9.7	2.7
26	T_{26}	5.4	1.7
27	T_{27}	7.8	1.8
28	T_{28}	7	1.8
29	T_{29}	6.1	1.5
30	T_{30}	4.6	1.5
31	T_{31}	6.7	3
32	T_{32}	5.5	1.4
33	T_{33}	5.8	1.3
34	T_{34}	6.6	2
35	T_{35}	4.4	1.3
36	T_{36}	7	1.8
37	T_{37}	5.2	1.6
38	T_{38}	2.6	1.2
39	T_{39}	5.4	1.6
40	T_{40}	4.3	1.5
S.Em±		0.72	0.37
C.D. (5%)		2.11	1.07

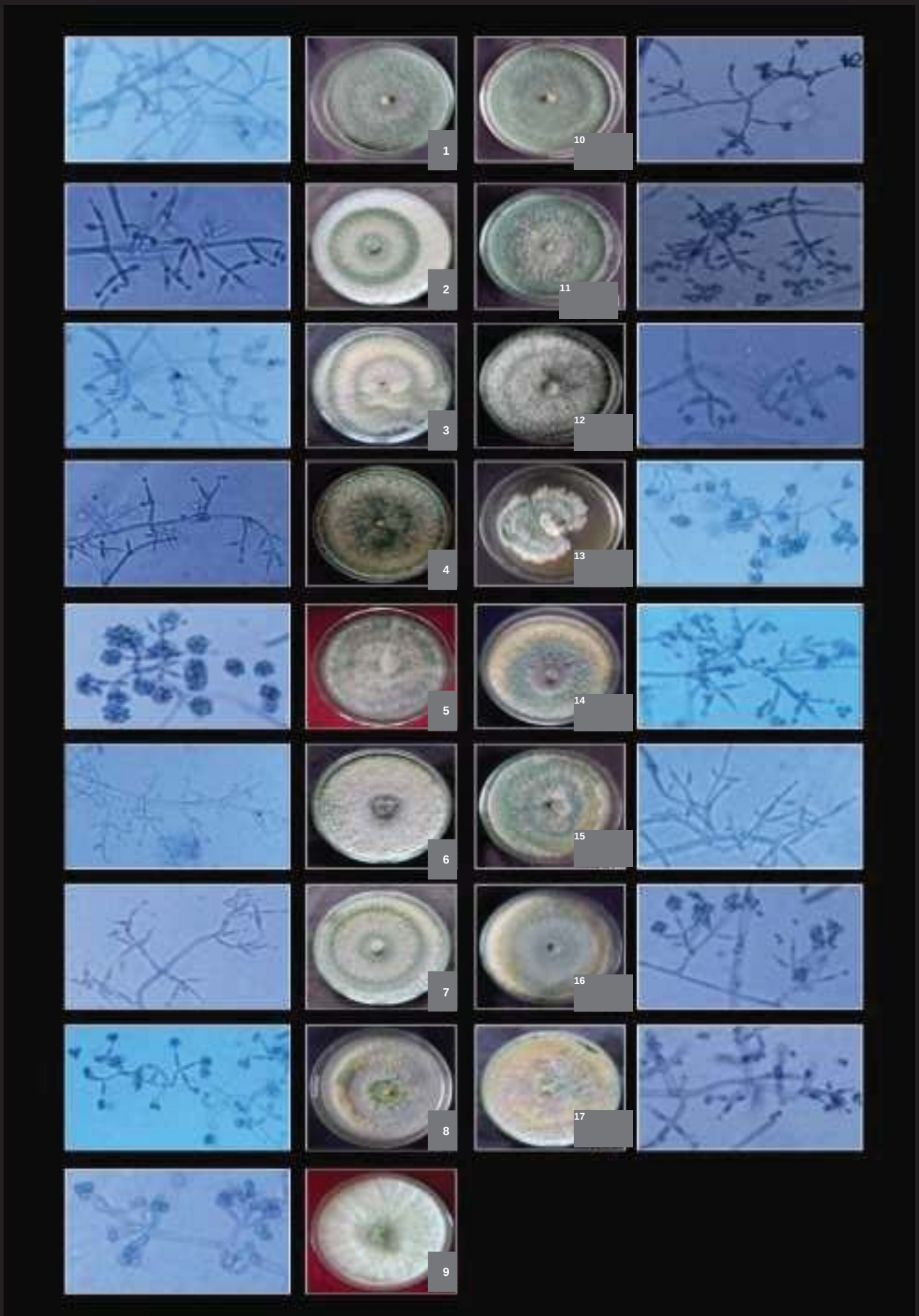


Fig 4.19(a) Culture and phialide morphology of different *Trichoderma* isolates

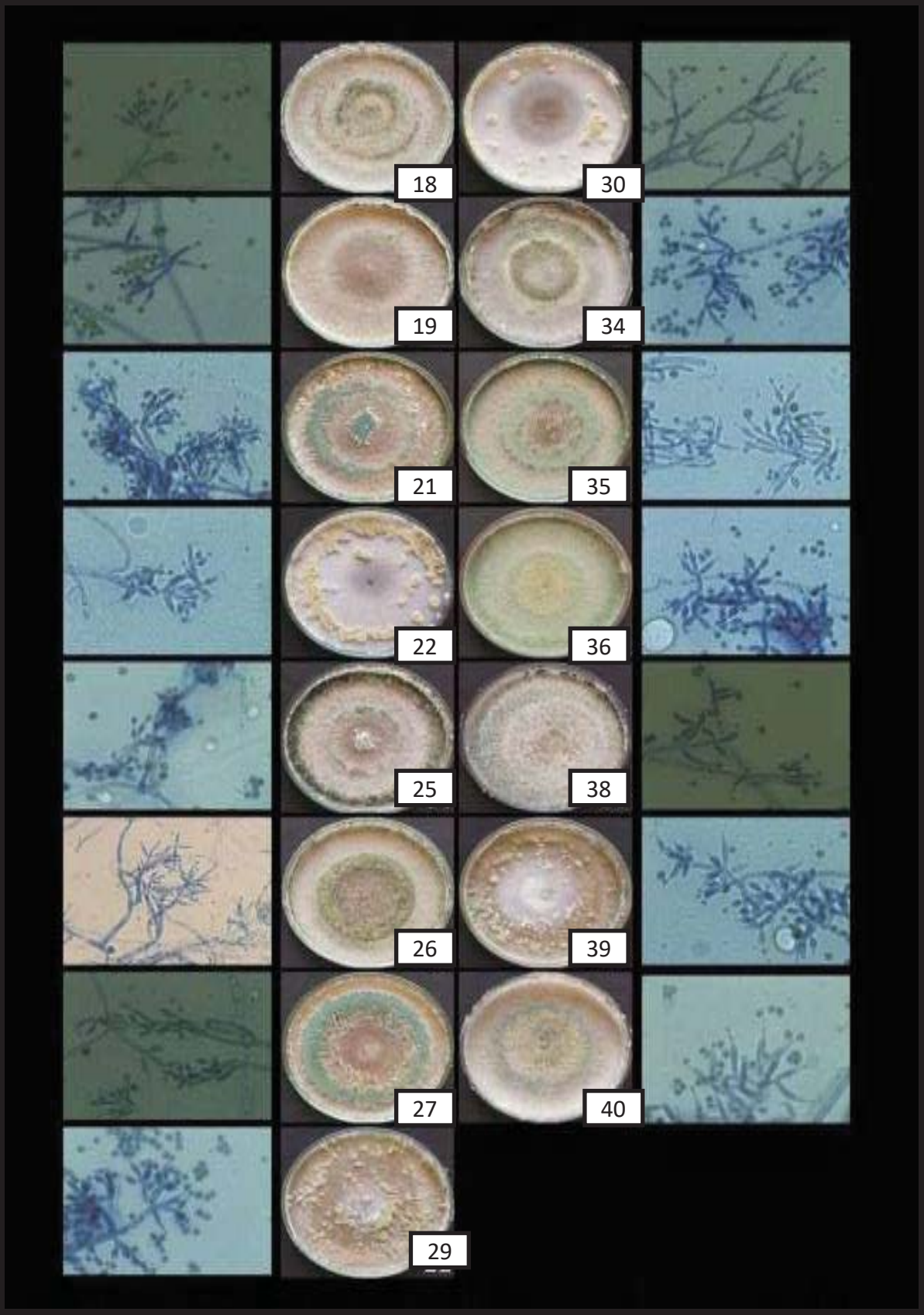


Fig 4.19 (b)Culture and phialide morphology of different *Trichodrma* spp. isolate

4.7.3: Isolation of fluorescent *Pseudomonas*

Fluorescent *Pseudomonas* were isolated from the soil samples by serial dilution method using Kings 'B' medium, thereafter, examined under UV light. Colonies with yellow - green and blue-white color pigmentation were marked and recorded. Twenty- four fluorescent *Pseudomonas* isolates were isolated from collected soil sample from different location of Chhattisgarh and designated (Fig 4.33).

They were further, screened for following biochemical test gelatin Hydrolysis test, nitrate test,starch hydrolysis test, casein hydrolysis test, lypolytic test, growth test at 4°C and 42° C and antibiotic sensitivity test, triple sugar iron agar test and hydrogen sulphide test .

4.7.3.1 Biochemical tests for characterization of fluorescent *Pseudomonas*

4.7.3.1.1 Gelatin hydrolysis test

In the present investigation twenty two isolates were categorized in two group based on liquification/hydrolysis of gelatine media nineteen isolates releaved positive whereas, three isolates were negative to gelatin hydrolysis test (Table 4.65). Positive result means liquification of media, P2, P3, P8, P14, P19, P20, P22, P23, P25, P27-, and P29 isolates may be considered as *Pseudomonasfluorescens* or *Pseudomonas aeruginosa.* Negative result means solidification of media and may considered as *Pseudomonas putida* (P4, P7 and P29). Gelatin is a protein resulted from collagen hydrolysis. Gelatinase breakdown protein to polypeptide, peptide, and fatty acid so that they could be absorbed by organism cell membrane. This character to hydrolyze gelatin was also indicated by several *P. fluorescens* showing positive test to hydrolyze gelatin (Arwiyanto *et al*., 2007).

4.7.3.1.2 Nitrate test

On the basis of nitrate utilization nineteen isolates were assign positive (+) (P2, P3, P4, P8, P14, P18, P19, P20, P22, P23, P25, P27, P29,) and may considered as *P. fluorescens* whereas three showed negative (-) response (P7, P and P29) unable to utilized nitrate and regarded as *P. putida* (Table 4.65).

4.7.3.1.3 Starch Hydrolysis Test

In starch hydrolysis test isolates were grouped in two categories, negative (-) giving blue colour and positive (+) with no colour change. This was grouped on

the basis of blue colour formation when iodine indicator was added to the Petri plates. Nineteen isolates (P2, P4, P7, P8, P14, P18, P19, P20, P22, P23, and P29,) exhibited negative (-) response which confirms the presence of *P. fluorescens* whereas three isolates (P3, P25 and P27) showed positive result (Table 4.19).

The positive result of starch hydrolysis test was mentioned by clear zone surrounding the bacterial colony. The zone showed that starch in the media could be hydrolyzed because of an enzymatic reaction, *i.e*., amylase, secreted by the antagonist (Karkalas, 1985). The hydrolyzed starch could absorb purple colour from Iodine because of an interaction between polysaccharide and Iodine. Karkalas (1985) also mentioned that amylase is an enzyme that could disperse glycoside in starch and change it. The ability of fluorescent *Pseudomonas* in hydrolyzing starch was a specific character of most gram negative bacteria, supported by Arwiyanto *et al*. (2007) that fluorescent *Pseudomonas* is a gram negative bacteria and can hydrolyze starch.

4.7.3.1.4 Casein hydrolysis

In casein hydrolysis test seventeen isolates (P2, P3, P8, P14,P18, P19, P20, P22, P25 and P29) exhibited positive (+) response and they may be considered as *P. fluorescens* or *P. aeruginosa* whereas, five isolates (P4, P7, P23 P27 and P29) showed negative result may assign as *P. putida* (Table 4.19). Some microorganisms have the ability to degrade (isolate shown positive results) the protein casein by producing proteolytic exoenzyme called caseinase which break the peptide bond CO-NH by introducing water into the molecule, liberating smaller chains of amino acid called peptides which are later broken down into free amino acid by extracellular or intracellular peptidases.

4.7.3.1.5 Lypolytic activity

In lypolytic test four isolates produce positive (+) to test (P2, P3, P7 and P40). These isolates may be considered as *P. fluorescens* or *P. aeruginosa* whereas, rest of the eighteen isolates (P4, P8, P14, P18, P19, P20, P22, P23, P25, P27 and P29) exhibited negative (-) response (Table 4.19). In negative result there was no clear zone around the bacterial colony growth. The activity of lypolysis is related to enzyme production that could disintegrate fat. In this test, the media was

supplemented with Tween 80 (polyhydroxybutirate) as indicator of lypase enzyme formation. The enzyme formed by lypolysis microorganisms could change fat to fatty acid and glycerol (Abouseoud *et al.*, 2007).

4.7.3.1.6 Growth at 4°C and 42°C

Isolates when inoculated in King‘s B medium and incubated at 4°C for 48 hours. Nine isolates (P2, P3, P4, P7, P8, P14, and P29) showed growth therefore they may be considered as *P. fluorescens* whereas, rest of the isolates (P18 and P27) may be *P. aeruginosa* (Table 4.65).

Also when these same set of the isolated were subject to 42°C , no growth was observed at in five isolates (P8, P19, P22, P27 and P29) they may considered as *P. fluorescens* or *P. putida* whereas, isolates resulted in growth may be assign as *P. aeruginosa* (Table 4.19).

4.7.3.1.7 Antibiotic sensitivity test

Antibiotic sensitivity test was used to distinguish different species of fluorescent *Pseudomonas.* Isolates (P2, P4, P3, P7, P8, P14, P18, P19, P20, P22, P23, P25, P27 and P29) resistance to kanamycin may be considered as *P. aeruginosa*, except P41 which exhibited sensitivity towards kanamycin can assign as fluorescent *Pseudomonas* (Plate 29). On other hand in carbenicillin test isolates showed resistance response may be considered as fluorescent *Pseudomonas* or, *P. putida* (P2, P3, P4, P18, P19, P20, P22, P25, and P29) while isolates sensitive to carbenicillin may be *P. aeruginosa* (P7, P8, P14, P23, P27 and P40)(Table 4.19).

4.7.3.1.8 Triple sugar iron agar test

Triple Sugar Iron agar media is used for the differentiation of microbes by ability to determine carbohydrate fermentation and hydrogen sulphide production. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amounts of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline product from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt. Thus the appearance of the alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and or sucrose. Nineteen isolates (P4, P3, P7, P8, P14, P18, P19, P20, P22, P25, P27 and P29)

produced red colour after on slant and butt after incubation whereas isolate (P2 and P23) produced yellow colour on slant (Table 4.19).

4.7.3.1.9 Hydrogen sulphide test

The slants were observed for the presence or absence of black coloration along the line of stab inoculation. The black colour indicated positive result. The black colour is due to the production of H_2S from sodium thiosulphate present in the medium that then combines with ferrous ammonium sulphate resulting in the formation of black insoluble ferrous sulphide. This was found in ten isolates (P3, P4, P8, P14, P18 and P19) (Table 4.19).

Table 4.19: Biochemical tests for characterization of fluorescent *Pseudomonas*

S.No.	Isolate	Gelatine hydrolysis test	Nitrate test	Starch hydrolysis test	Casein hydrolysis test	Lypolytic test	4 ° C	42° C
1.	P2	+	+	-	+	+	+	+
2.	P3	+	+	+	+	+	+	+
3.	P4	-	+	-	-	-	+	+
4.	P7	-	-	-	-	+	+	-
5.	P8	+	+	-	+	-	+	+
6.	P14	+	+	-	+	-	+	+
7.	P18	+	+	-	+	-	-	+
8.	P19	+	+	-	+	-	-	-
9.	P20	+	+	-	+	-	-	+
10.	P22	+	+	-	+	-	-	-
11.	P23	+	+	-	-	-	-	+
12.	P25	+	+	+	+	-	-	+
13.	P27	+	+	+	-	-	-	-
14.	P29	+	+	-	+	-	+	+
15.	P33	+	+	-	+	-	-	+
16.	P34	+	-	-	+	-	+	+
17.	P35	+	+	-	+	-	+	+
18.	P36	+	+	-	+	-	-	+
19.	P38	+	+	-	+	-	-	+
20.	P39	+	+	-	+	-	-	+
21.	P40	+	+	-	+	+	-	+
22.	P41	-	-	-	-	-	-	-

- (Negative) and + (positive)

Table 4.20: Antibiotic sensitivity test to confirm the identity of fluorescent *Pseudomonas*

S.No.	Isolate	Mycelial growth (mm)	
		Kanamycin	Carbenicillin
1.	P2	6	4
2.	P3	23	5
3.	P4	3	5
4.	P7	5	2
5.	P8	24	1
6.	P14	4	0
7.	P18	8	4
8.	P19	25	7.5
9.	P20	31.5	4
10.	P22	28	10
11.	P23	5	0
12.	P25	22.5	3
13.	P27	5	0
14.	P29	21	5
15.	P33	21	3
16.	P34	9	5
17.	P35	22	11
18.	P36	17	21
19.	P38	22.5	6
20.	P39	32.5	4
21.	P40	32	1
22.	P41	0	6

Table 4.21: Biochemical test to confirm the identity of fluorescent *Pseudomonas* throughTriple Sugar Iron Agar test and Hydrogen Sulphide Test

S.No.	Isolate	Triple Sugar Iron Agar Test		Hydrogen Sulphide Test
		Slant/Butt colour red	Slant/Butt colour yellow	Slant/Butt colour black
1.	P2	-	+	-
2.	P3	+	-	+
3.	P4	+	-	+
4.	P7	+	-	-
5.	P8	+	-	+
6.	P14	-	-	+
7.	P18	+	-	+
8.	P19	+	-	+
9.	P20	+	-	-
10.	P22	+	-	-
11.	P23	-	+	-
12.	P25	+	-	-
13.	P27	+	-	-
14.	P29	+	-	-
15.	P33	+	-	-
16.	P34	+	-	-
17.	P35	+	-	+
18.	P36	+	-	+
19.	P38	+	-	-
20.	P39	+	-	+
21.	P40	+	-	+
22.	P41	+	-	-

- (Negative) and + (positive)

Table- 4.22: Biochemical characterization of isolates of fluorescent *Pseudomonas* spp.

Isolates	Oxidase	KOH test	Levan production	Arginine dihydrolysis	Growth test at 45°C	Gelatin liquefication	Extraction of pigment with water	Tentative identification
FP-1	+	+	-	+	-	+	+	*P. fluorescens*
FP-2	+	+	-	+	-	+	+	*P. fluorescens*
FP-3	+	+	-	+	-	-	+	*P. putida*
FP-4	+	+	-	+	-	+	+	*P. fluorescens*
FP-5	+	+	-	+	-	+	+	*P. fluorescens*
FP-6	+	+	-	+	-	+	+	*P. fluorescens*
FP-7	+	+	-	+	-	+	+	*P. fluorescens*
FP-8	+	+	-	+	-	+	+	*P. fluorescens*
FP-9	+	+	-	+	-	+	+	*P. fluorescens*
FP-10	+	+	-	+	-	+	+	*P. fluorescens*
FP-11	+	+	-	+	-	+	+	*P. fluorescens*
FP-12	+	+	-	+	-	+	+	*P. fluorescens*
FP-13	+	+	-	+	-	++	+	*P. fluorescens*
FP-14	+	+	-	+	-	+	+	*P. fluorescens*
FP-15	+	+	-	+	-	+	+	*P. fluorescens*
FP-16	+	+	-	+	-	+	+	*P. fluorescens*
FP-17	+	+	-	+	-	+++	+	*P. fluorescens*
FP-18	+	+	-	+	-	+++	+	*P. fluorescens*
FP-19	+	+	-	+	-	+++	+	*P. fluorescens*
FP-20	+	+	-	+	-	+++	+	*P. fluorescens*
FP-21	+	+	-	+	-	+++	+	*P. fluorescens*

+ = positive; - = negative

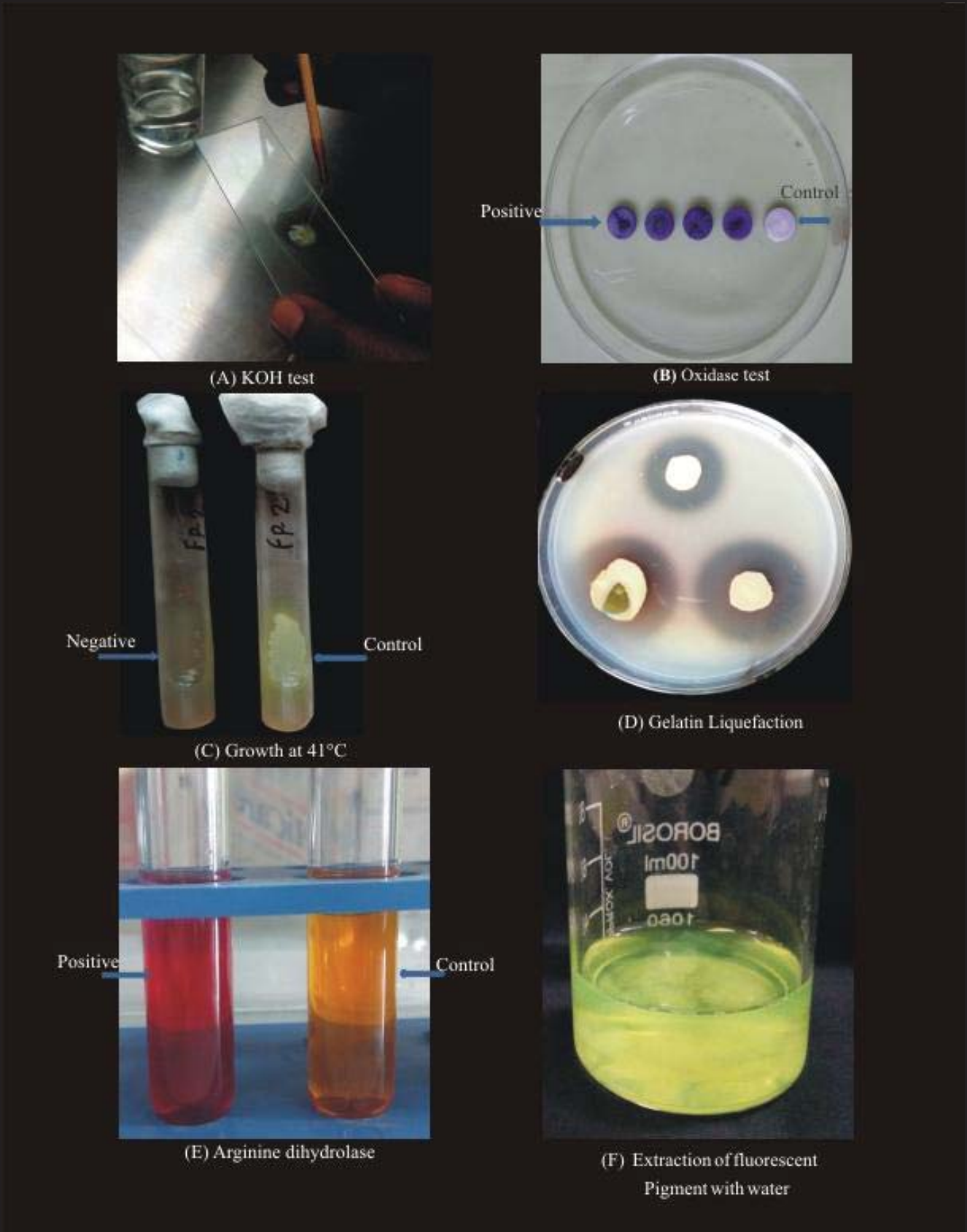


Fig.4.20 Biochemical charecterisation of isolates of fluorescent *Psudomonas spp.*

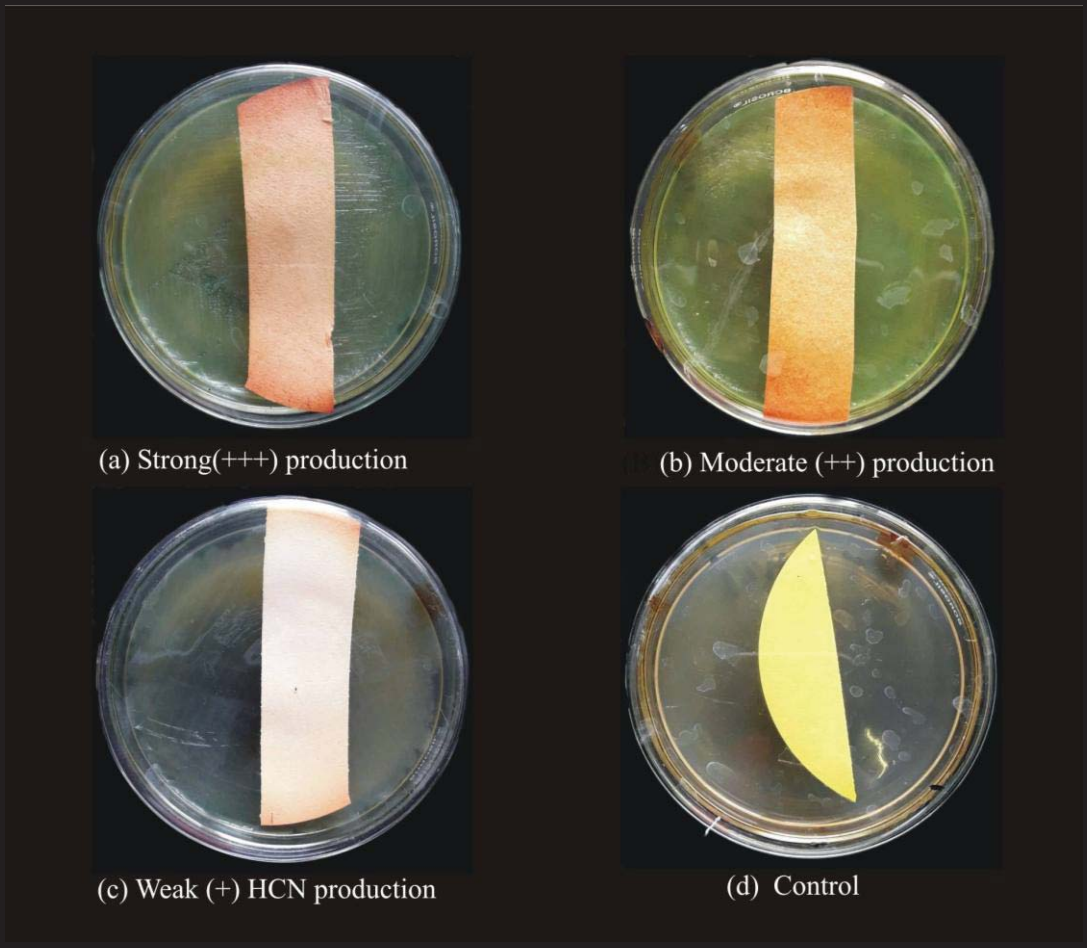


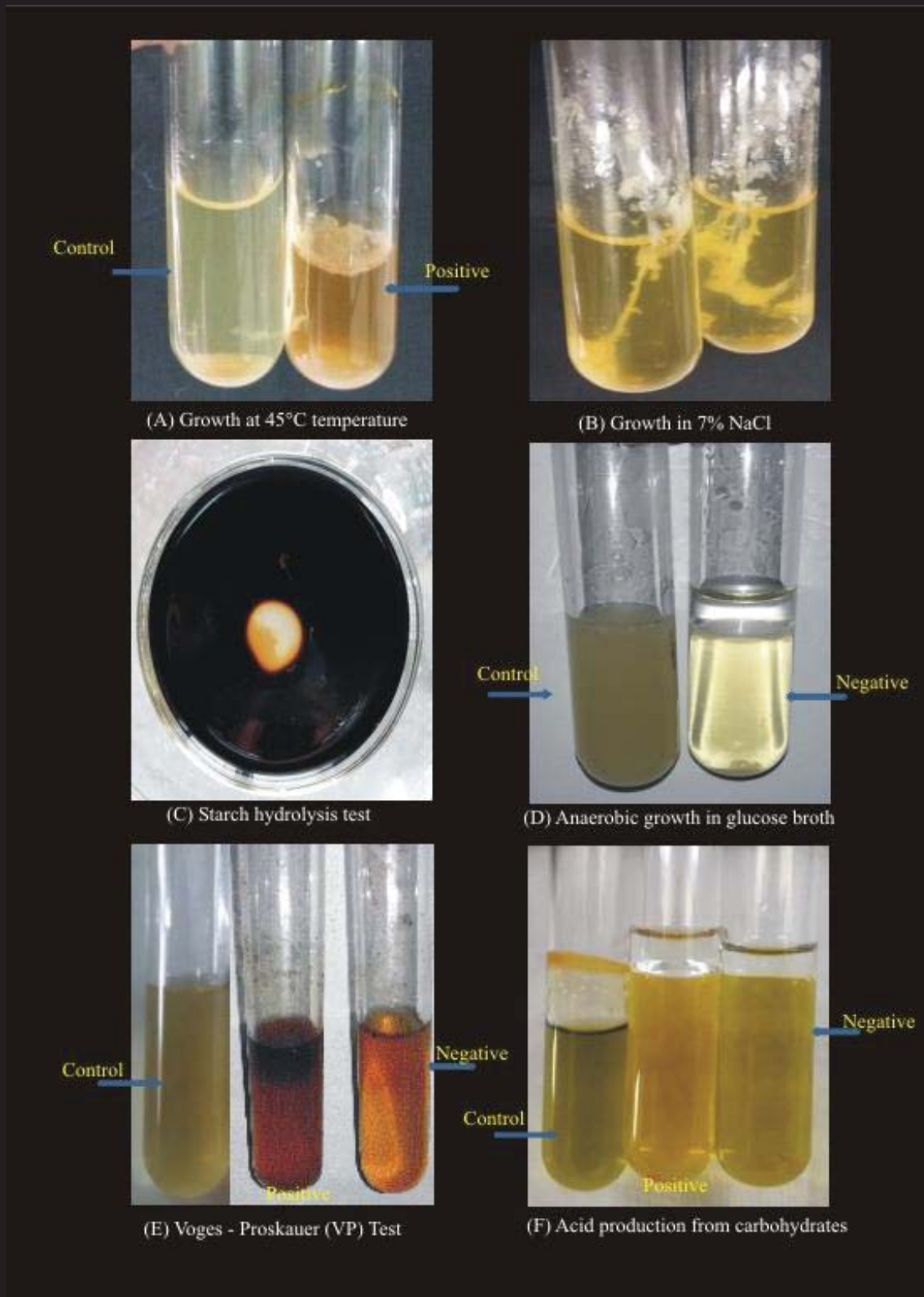
Fig. 4.20(a): HCN Production test of Flurescent Pseudomonas

4.7.4. Isolation of *Bacillus* spp.

Bacillus spp. were isolated from the soil samples by serial dilution method using nutrient agar medium, thereafter, examined on the basis of morphological and cultural characteristic. Selected colonies inoculated in King's B broth and incubated in incubator shaker at 30^0C for 24 hours. 24 *Bacillus* spp. isolates were isolated from the collected soil sample of different location of Chhattisgarh and designated (Table 4.37).

Table 4.23. Biochemical test of *Bacillus* spp.

isolates	Gram reaction	Growth at 45°C	Growth at PH 5.7	Growth In 7% Nacl	Utilization Of Citrate	Anaerobic Growth In Glucose Browth	Acid Form				Vp Test	Starch Hydrolysis	Tentative identification
							Dextrose	Arabinose	Mannitol	Xylose			
BC-1	+	+	+	+	+	-	V	+	+	+	+	+	B. subtilis
BC-2	+	+	+	+	+	-	V	+	+	+	+	+	B. subtilis
BC-3	+	+	+	+	+	-	+	+	+	+	+	+	B. subtilis
BC-4	+	+	+	-	+	-	V	+	+	+	+	+	ND
BC-5	+	-	-	-	+	-	V	+	+	+	+	+	ND
BC-6	+	+	+	+	+	-	V	+	+	+	-	+	ND
BC-7	+	+	+	+	+	-	V	+	+	+	+	+	B. subtilis
BC-8	+	+	+	+	+	-	+	+	+	+	+	+	B. subtilis
BC-9	+	+	+	+	+	-	V	+	+	+	-	+	ND
BC-10	+	+	+	+	+	-	V	+	+	+	+	+	B. subtilis
BC-11	+	+	+	+	+	-	V	+	+	+	-	+	ND
BC-12	+	+	+	+	+	-	+	+	+	+	+	+	B. subtilis
BC-13	+	+	+	+	+	-	V	+	+	+	+	+	B. subtilis
BC-14	+	+	+	-	+	-	V	+	+	+	+	+	B. subtilis
BC-15	+	+	+	-	+	-	V	+	+	+	-	+	ND
BC-16	+	+	+	+	+	-	V	+	+	+	-	+	B. subtilis
BC-17	+	+	+	-	+	-	+	+	+	+	+	+	ND
BC-18	+	+	+	-	+	-	+	+	+	+	+	+	B. subtilis
BC-19	+	+	+	-	+	-	V	+	+	+	-	+	B. subtilis
BC-20	+	-	+	-	+	-	V	+	+	+	+	+	B. subtilis
BC-21	+	+	+	-	+	-	V	+	+	+	-	+	ND
BC-22	+	+	+	+	+	-	V	+	+	+	+	+	ND
BC- 23	+	+	+	+	+	-	+	+	+	+	-	+	ND
BC- 24	+	+	+	+	+	-	+	+	+	+	+	+	ND



Fig,4.21 Biochemical Characterization of *Bacillus* spp.

4.7.6: Management of collar rot of lentil

4.7.6.1.: Management throught *in vivo* method

4.7.6.1.1: Effect of plant age on collar rot disease development

Effect of plant age on collar rot disease development was studied and results are presented in the table 4.24, figure 4.22. The results revealed that, there was significant difference in mortality percentage among the different age of the plant.

Significantly highest per cent mortality of 90 per cent was recorded in 10 days old plant. This was followed by stage 20 days old plant with 80 per cent mortality. Plant age of 30 days and 45 days recorded 60 per cent and 40 per cent mortality respectively. They were significantly lower from 10 days old plant. A considerable difference in mortality was also found between 20 days and 45 days old plant with significantly different mortality percentage. In the present study, it was observed that *Sclerotium rolfsii* can infect all the age of the lentil crop. The most susceptible age of the crop can be identified based on plant age which shows the maximum mortality. It was found that, 10 days old plant showed maximum mortality (90%) and it was identified as the most susceptible stage. Similar findings were reported by Singh and Dwivedi (1988) that, Barley seedlings were found most susceptible to the attack of *Sclerotium rolfsii* during first fifteen days of the growth and the percent infection of the plant reduced with aging. Similarly while studying with lentil diseases, Hussain (2006) and his associated reported that disease was more severe on one week old seedlings (58.7 to 100%) than older plants (19.3 to 86.3%).

Table 4.24: Effect of plant age on collar rot disease development.

S.No.	Treatment	Age of crop (Days)	Mortility* %
1	T1	10 days	90(76.70)**
2	T2	20 days	80(66.89)
3	T3	30 days	60(51.03)
4	T4	45 days	40(38.93)
	Sem+-		6.63
	CD at 0.05		20.66

*= Average of four replications
**= Figures in parenthesis are arcsine transformed value

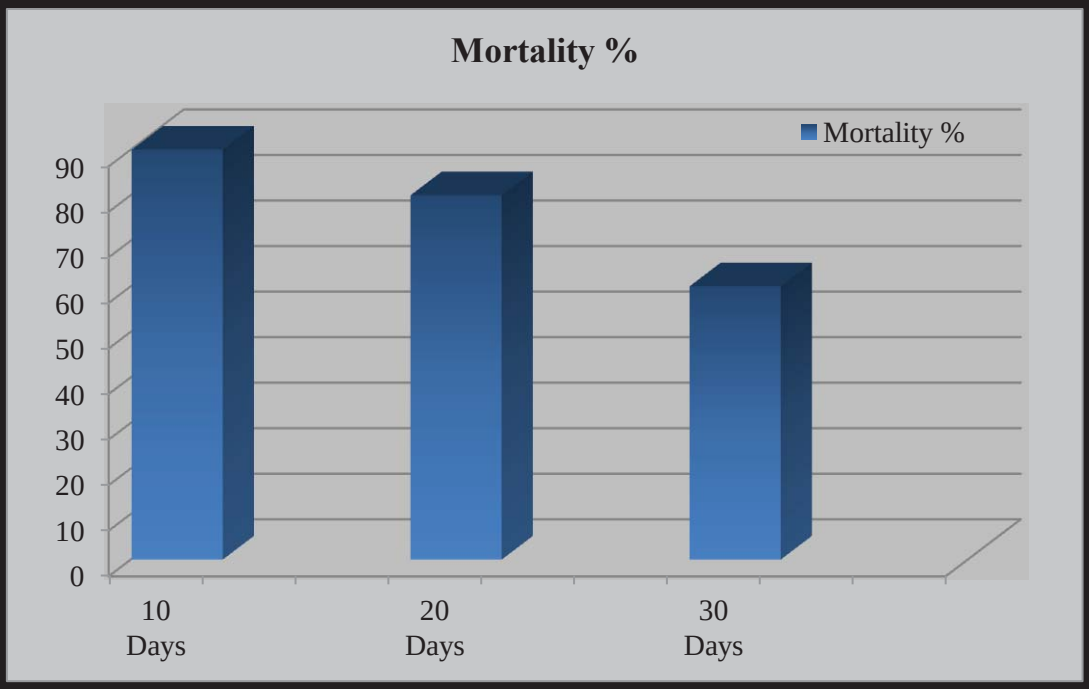


Fig.4.22(a): Effect of plant age on collar rot disease development

Fig 4.22(b) Effect of plant age on collar rot disease development

4.7.6.1.2: Effect of different date of sowing on Collar rot disease development

The result existing in Table-4.25 shows that incidence of wilt was recorded higher in early sown crop that is 20th Oct. in both the year of testing. Maximum mean disease incidence 62.90 per cent was recorded in 20th Oct sown crop followed by 30th Oct sown crop (54.67%). The minimum wilt disease incidence was recorded 23.25 per cent in the late sown crop 30November in both years.

Table4.25: Effect of different date of sowing on disease incidence of Collar rot of lentil

S.No.	Date of Sowing	Per cent Disease Incidence* 2018	2019	Mean Per cent Disease Incidence**
1	20th Oct.	63.25(52.82)	62.25(52.09)	62.9
2	30th Oct.	54.75(47.73)	54.58(47.63)	54.67
3	10th Nov.	42.42(40.63)	43.75(41.41)	43.09
4	20th Nov.	31.08(33.88)	32.17(34.54)	31.63
5	30th Nov.	22.25(28.12)	24.25(29.5)	23.25
	CV%	**3.62**	**4.13**	
	S.Em.±	**0.77**	**0.9**	
	C.D. at 5%	**2.39**	**2.76**	

*Mean of four replications

Figures in parentheses are angular transformed values .

** Mean of two year disease incidence (2018-19 and 2019-10)

4.7.6.1.3. : Effect of Tillage practices for disease control

The result existing in Table-26. and Fig.-23. shows that incidence of wilt was recorded higher in zero tillage in both the year of testing. Maximum mean disease incidence 37.12 per cent was recorded in lentil crop followed by Conventional tillage (31.60%). The minimum collar rot disease incidence was recorded 16.73 per cent in the minimum tillage in both years.

4.26: Effect of Tillage practices for disease control

S. NO.	Treatment	Tillage Practices	Per cent disease incidence
1	T1	Utera	26.85
2	T2	Zero	37.12
3	T3	Minimum	16.73
4	T4	Conventional	31.60
		Sem	**4.46**
		CD	**13.03**

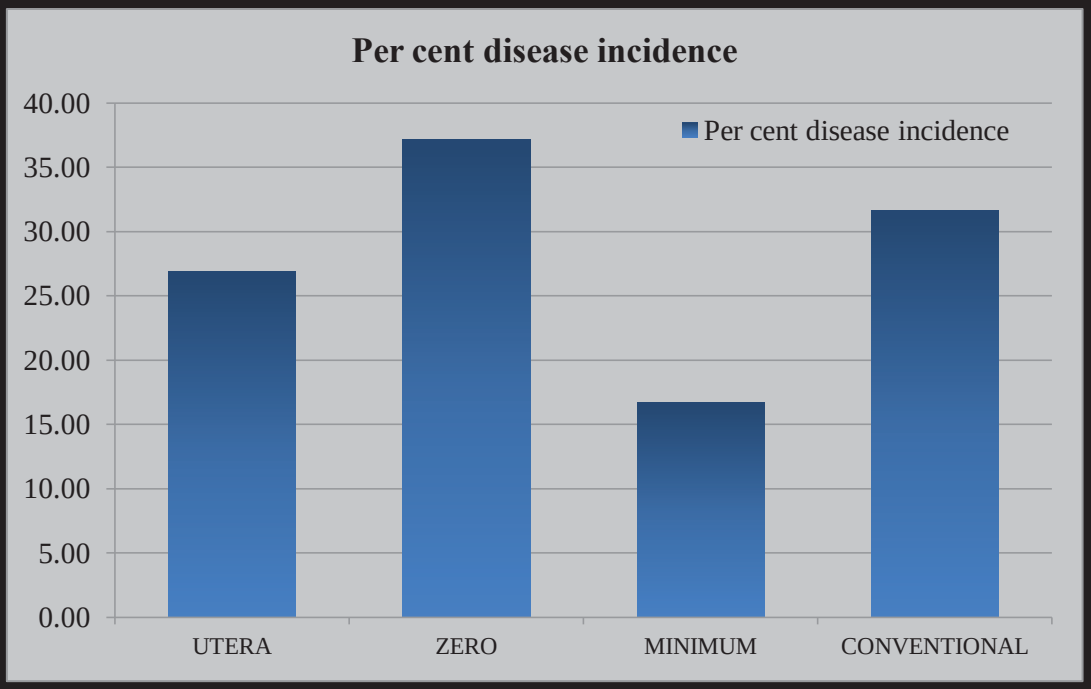


Fig. 4.23 Effect of Tillage practices for disease control

Fig4.23(a): Effect of Tillage practices for disease control

4.7.6.1.4: Effect of seed primimg for disease control

The result existing in Table-4.27 and Fig.-4.24. shows that incidence of collar rot was recorded higher in no seed priming in both the year of testing. Maximum mean disease incidence 14.40 per cent was recorded in lentil crop. The minimum collar rot disease incidence was recorded 12.68 per cent in the seed priming in both years.

4.27: Effect of seed primimg for disease control

S.No.	Treatment	Priming	Per cent Disease Incidence
1	T2	Seed Priming	12.68
2	T2	No Seed Priming	14.40
		SEm±	4.61
		CD (0.05)	14.48

4.8: Efficacy of biocontrol agents i.e. *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp. againt *S. rolfsii*.

4.8.1 Isolation of Trichoderma isolates

4.8.1.1 Screening for antagonistic activity of *Trichoderma* spp against *S. rolfsii*

*Trichoderma*spp. were isolated from the soil samples by serial dilution method using Potato Dextrose Agar (PDA) medium. The cultures were identified and confirmed as *Trichodermaspp.* through microscopic observation. In the present investigation, fifteen isolates of *Trichoderma* spp. were collected from different locations of Chhattisgarh given in Table 4.28. Out of fifteen *Trichoderma* isolates, seven isolates belongs to central region, three from northern and eastern region while, only one isolates each was isolated from western and southern region.

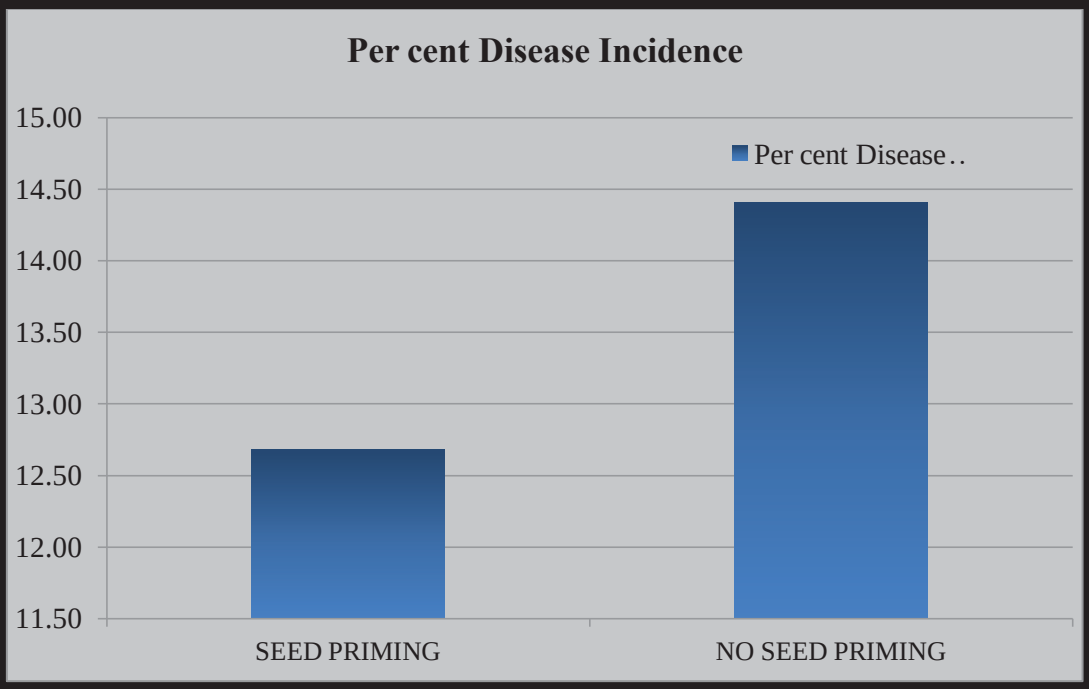


Fig.4.24: Effect of seed primimg for disease control

Table 4.28: Details of location of soil sample collection, presence and code of *Trichoderma* isolates

S.No.	Location	Block	District	Code of isolate
1	Raipur	Raipur	IGKV, Farm	T_1
				T_2
2			IPM, Field	T_3
3			Aarang	T_4
4		Arang	Arang	T_5
5		Abhanpur	Khurha	T_6
6	Mungeli	Mungeli	Temari	T_7
7			Navapara	T_8
8		Lormi	Chilfi	T_9
9			Bhtha-Bhurka	T_{10}
10			Sipahi	T_{11}
11	Bilaspur	Bilaspur	takhatpur	T_{12}
12		Bilaspur	Devri khurd	T_{13}
14	Dhamtari	Kurud	Gobra	T_{14}
15	Baloda bazaar	Simga	Surgi	T_{15}
16		Bhatapara	Gogiya	T_{16}
17			Sandyi	T_{17}
18			Mohba	T_{18}
19			Padumtari	T_{19}
20	Kawardha	Kawardha	Newari farm	T_{20}
21		Pandariya	kapadah	T_{21}
22			ghuterkudi	T_{22}
23		Pandariya	Sangona	T_{23}
24	Bemetara	Bemetara	Bemetara	T_{24}
25			Matia	T_{25}
26			mohbhate	T_{26}
27			Gadamod	T_{27}
28	Mahasamund			T_{28}
29	Griyaband	Gariyaband	Panduka	T_{29}
30		Mainpur	birighat	T_{30}
31	Jagadalpur	Kumharavan		T_{31}
32	Balod	Balod		T_{32}
33	Kanker	Kanker		T_{33}
34		Kanker		T_{34}
35	Janjgir	Janjgir		T_{35}
36	Durg	Durg	Anjora	T_{36}
37		Dhamda	Dhamda	T_{37}
38	Rajnandgaon	Rajnandgaon	Surgi	T_{38}
39			Mohba	T_{39}
40	Korba	Korba	KVK Farm	T_{40}

4.8.1.2: Sclerotial Parasitization

The sclerotia of *S. rolfsii* were smooth, small and round. After incubation of sclerotia under treatment with different isolates of *Trichoderma* in soil plating technique, it was observed that respective *Trichoderma* isolates grew over the sclerotia and green colored growth was observed above them. The colonized sclerotia, when punched with forceps, the number of parasitized sclerotia varied among different isolates. The maximum number of sclerotia was parasitized by a set of 8 isolates of *Trichoderma* namely T_5, T_{12}, T_{15}, T_{21}, T_{25}, T_{33}, T_{34} and T_{36}. Under treatment with these eight isolates of *Trichoderma*, 100 % sclerotia were colonized and parasitized. However, less than 50% of sclerotia were colonized by a set of 6 isolates namely T_7, T_{23}, T_2, T_{10}, T_{26} and T_{11}. The minimum number of average sclerotia (1.67) were parasitized by isolate T_7. Detailed data for average number of colonized sclerotia and % sclerotia colonized after 10 days of incubation period with each isolate of *Trichoderma* has been given in Table 14. Pictorial representation for all the isolates of *Trichoderma* in sclerotial parasitization (10 days of incubation period) along with control plates has been given in Figure 28. Graphical representation of per cent sclerotia colonization has been depicted in Figure 28(a,b).

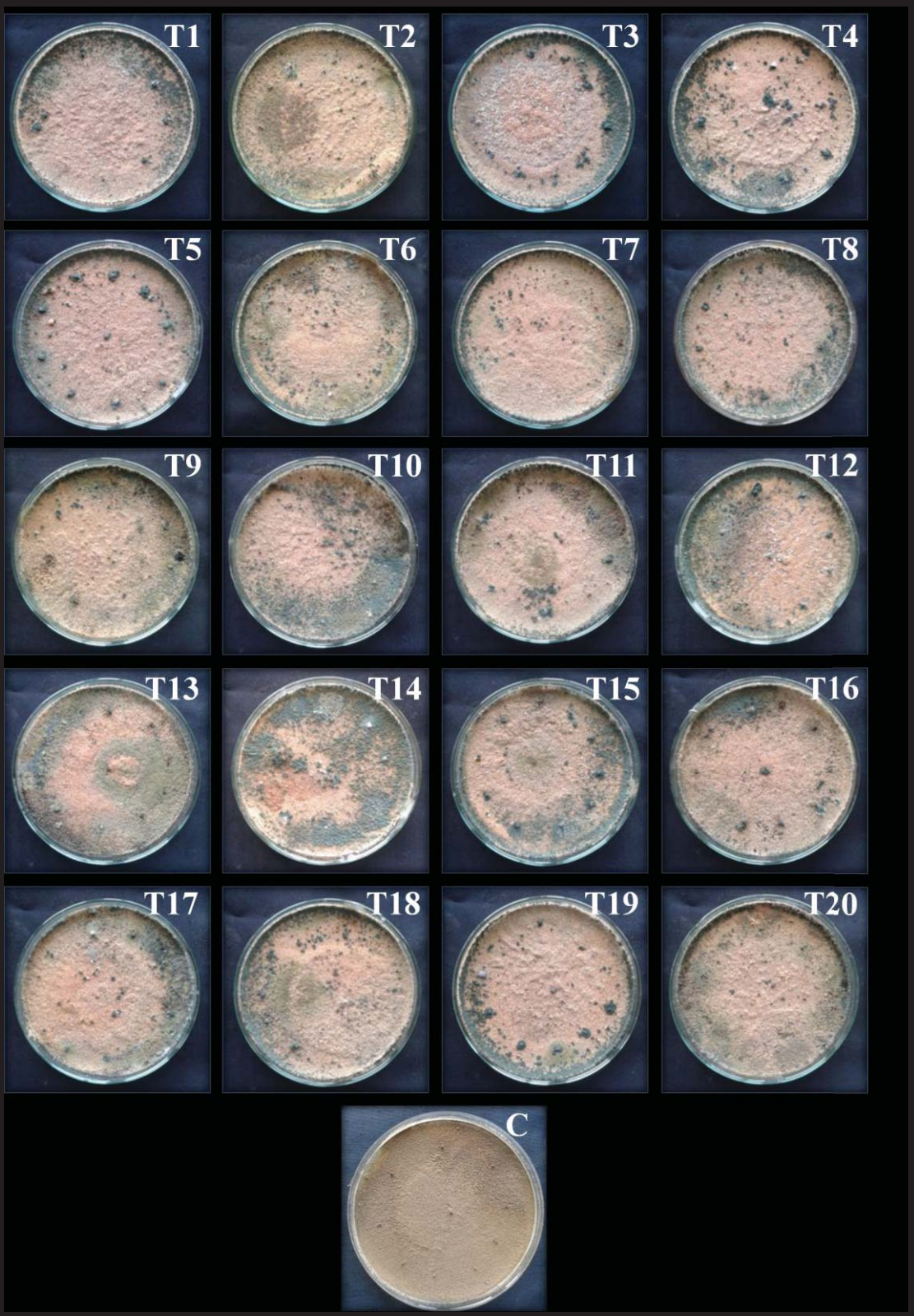


Fig 4.33(a) : Sclerotia parasitization of *S. rolfsii* by different isolates of *Trichoderma* using soil plating technique

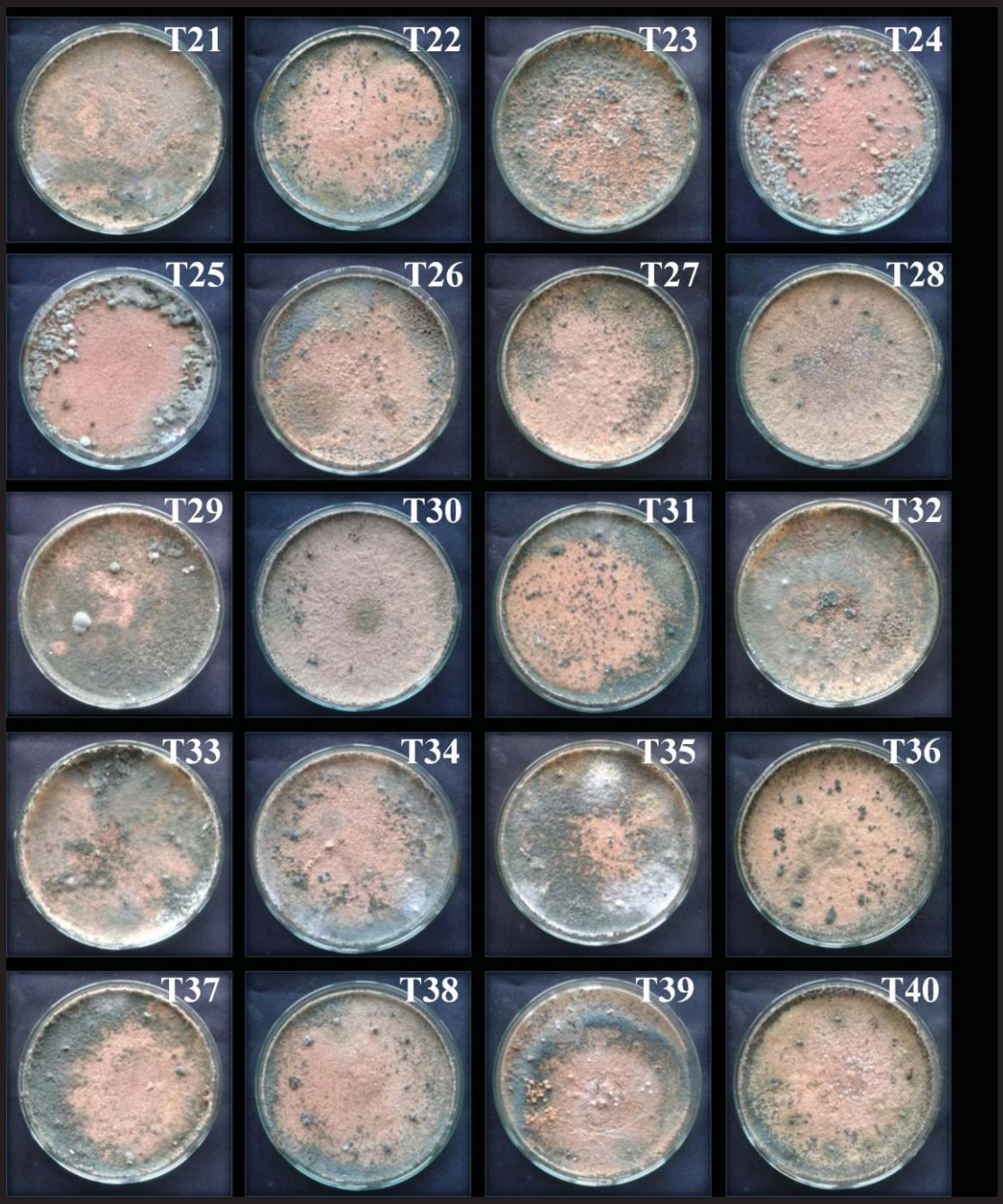


Fig 4.33(b) : Sclerotia parasitization of *S. rolfsii* by different isolates of *Trichoderma* using soil plating technique.

Table 4.29: Average sclerotia of *S. rolfsii* colonized by isolates of *Trichoderma* after 10 days incubation period

S. No.	Isolate code	Avg. number of colonized sclerotia	Per cent sclerotia colonized
1	T_1	6.67	66.67
2	T_2	2.67	26.67
3	T_3	8.67	86.67
4	T_4	9.00	90.00
5	T_5	10.00	100.00
6	T_6	6.67	66.67
7	T_7	1.67	16.67
8	T_8	6.67	66.67
9	T_9	8.00	80.00
10	T_{10}	3.00	30.00
11	T_{11}	4.67	46.67
12	T_{12}	10.00	100.00
13	T_{13}	9.33	93.33
14	T_{14}	8.67	86.67
15	T_{15}	10.00	100.00
16	T_{16}	9.00	90.00
17	T_{17}	8.00	80.00
18	T_{18}	8.00	80.00
19	T_{19}	7.33	73.33
20	T_{20}	7.00	70.00
21	T_{21}	10.00	100.00
22	T_{22}	8.33	83.33
23	T_{23}	2.00	20.00
24	T_{24}	9.33	93.33
25	T_{25}	10.00	100.00
26	T_{26}	3.00	30.00
27	T_{27}	8.33	83.33
28	T_{28}	6.33	63.33
29	T_{29}	8.00	80.00
30	T_{30}	7.00	70.00
31	T_{31}	5.33	53.33
32	T_{32}	9.33	93.33
33	T_{33}	10.00	100.00
34	T_{34}	10.00	100.00
35	T_{35}	7.00	70.00
36	T_{36}	10.00	100.00
37	T_{37}	8.33	83.33
38	T_{38}	8.00	80.00
39	T_{39}	9.00	90.00
40	T_{40}	6.00	60.00
	S.Em±	0.30	
	C.D. (5%)	0.84	

4.30 : Evaluation of antagonistic efficacy of *Trichoderma* spp by dual culture method

Among the 40 isolates of *Trichoderma*, most of the isolates inhibited the growth of *S. rolfsii*. However, maximum inhibition in growth of *S. rolfsii* was recorded in case of isolate T_{32} . The maximum inhibition percentage of 91.69 was recorded by isolate T_{32} while minimum inhibition of 50.26%was recorded by isolate $T_{16.}$ Maximum inhibition of T7(91.16%) and T3(90.88%) in growth of *S. rolfsii* was recorded while minimum inhibition of T16 (50.26) and T38 (50.27) was recorded by *Trichoderma.*

Table 4.30 Antagonistic efficiency of *Trichoderma* spp. isolates against *S. rolfsii* by dual culture technique

S. No.	Trichoderma spp. isolates	Per cent inhibition*
1	T1	88.28(69.99)
2	T2	67.21(55.05)
3	T3	90.88(72.80)
4	T4	72.14(58.13)
5	T5	52.15(46.22)
6	T6	75.20(60.12)
7	T7	91.16(72.74)
8	T8	88.29(71.87)
9	T9	86.22(68.21)
10	T10	66.29(54.50)
11	T11	87.26(69.10)
12	T12	60.12(50.83)
13	T13	83.27(65.86)
14	T14	76.18(60.78)
15	T15	86.26(68.25)
16	T16	50.26(45.13)
17	T17	73.46(58.98)
18	T18	55.66(48.23)
19	T19	74.26(59.50)
20	T20	90.12(71.72)
21	T21	60.76(51.20)
22	T22	63.20(52.64)
23	T23	81.39(64.44)
24	T24	65.24(53.86)
25	T25	76.46(60.97)
26	T26	90.36(71.95)
27	T27	77.43(61.63)
28	T28	68.20(55.66)
29	T29	71.22(57.54)
30	T30	65.26(53.87)
31	T31	66.24(54.46)
32	T32	91.69(72.80)
33	T33	77.25(61.51)
34	T34	69.16(56.26)
35	T35	72.27(58.21)
36	T36	66.37(54.54)
37	T37	76.35(60.89)
38	T38	50.27(45.14)
39	T39	68.34(55.74)
40	T40	70.26(56.94)
41	Control	0.00
	SE(m)	0.83
	C.D.	2.33

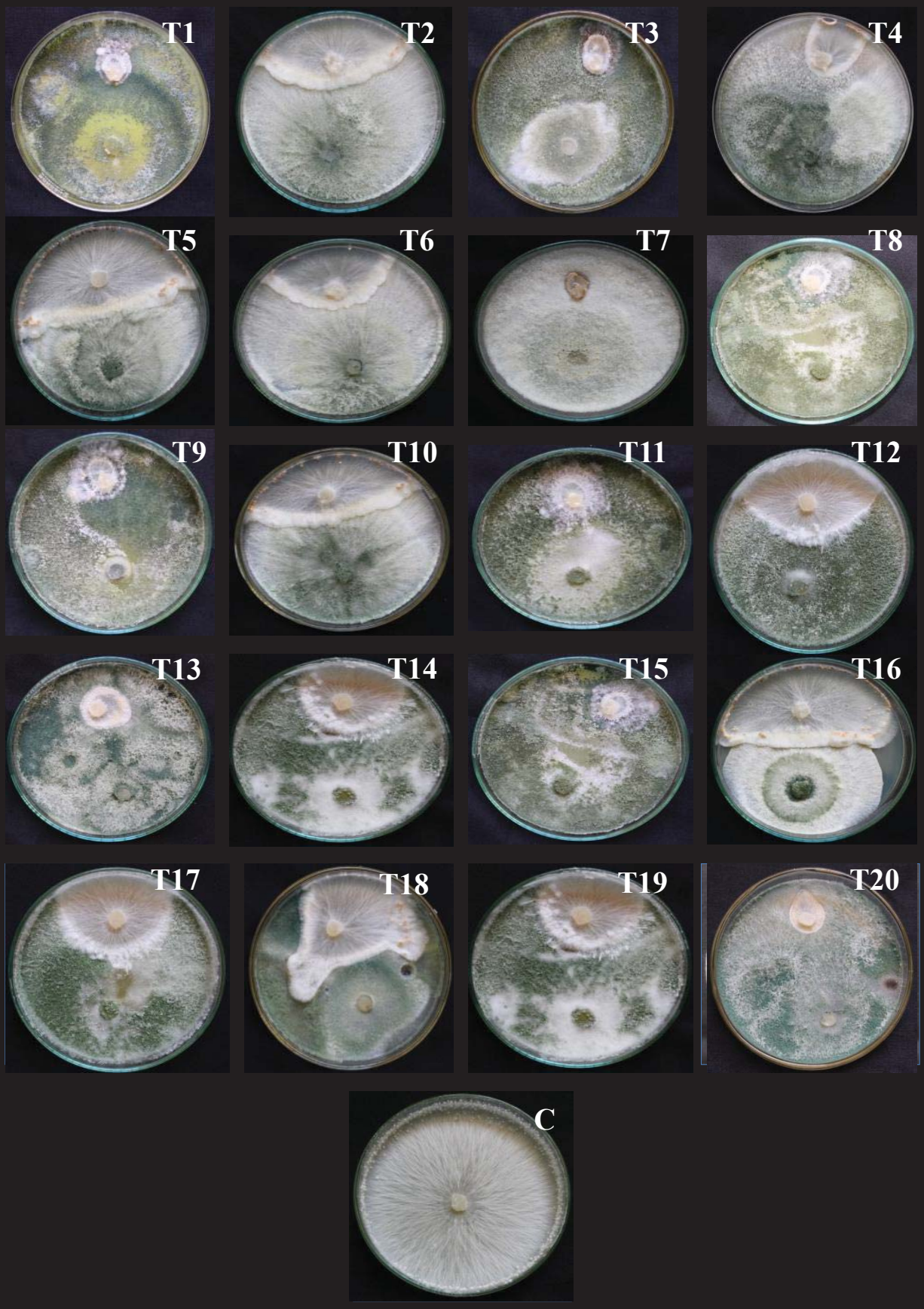


Fig 4.25 Antagonistic efficiency of *Trichoderma spp. 0* against *S. rolfsii by dual culture techanique*

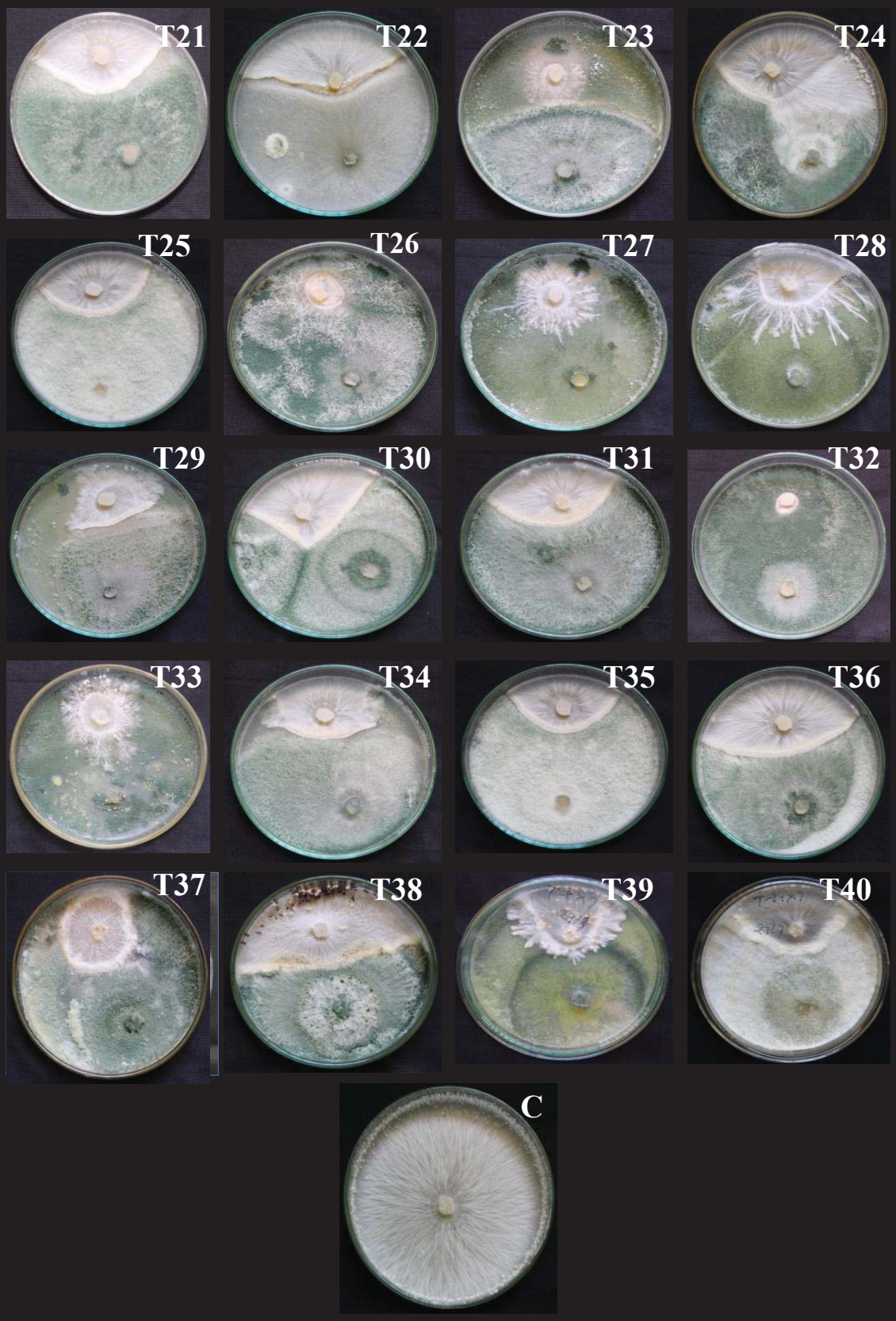


Fig 4.25 (b) Antagonistic efficiency of *Trichoderma* spp against *S. rolfsii*

By dual culture techanique

4.8.1.4 *In vivo* efficacy of *Trichoderma* spp under glass house condition

The *Trichoderma* isolates under this study also releaved varying degree of difference in mortality per cent, germination per cent, root length and shoot length against SR4 isolate under *in vivo* conditions as presented in Table 4.31

All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Isolate T23 and T40 were found to produce significantly least mortality 20.83 per cent among all, which was 75 per cent reduced over control, subsequently followed by T4 and T36 with mortality 25.00 per cent, 69.99 per cent reduced over control, and T22 exhibited 29.16 per cent mortality, 65.00 per cent reduced over control, these isolates were statistically at par with each other. These isolates showed significantly results, over other isolates and over control. Maximum mortality was observed in T7 that is 50.00 per cent whereas in control 83.33 per cent.

However, all the *Trichoderma* isolates in this study releaved variation in germination per cent, root length and shoot length and also among themselves over control, when challenge with SR4.

The influence of different *Trichoderma* spp. on germination per cent, root length and shoot length in lentil when challenge inoculated with *Sclerotium rolfsii* SR4, was studied and data are presented in Table 4.32

Highest germination per cent was observed in eight isolates like T12, T14, T19,T22, T29, T31a, T31b and T40 that is 90 per cent which was found significantly superior with other isolates and over control (86.66 per cent). Followed by T1, T20, T33 and T36 with 86.66 germination per cent which was at par with control.

All the *Trichoderma* isolates were significantly superior in increasing the root length over the control (9.33 cm), when challenged inoculated with *Sclerotium rolfsii* SR4 isolate. Maximum root length was recorded in T29 isolate (32.6 cm) (250.53 per cent increase over control) and T33 isolate 31.8 cm (241.93 per cent increase over control), which was at par and significantly superior over all the other treatments and control (9.33 cm). Followed by T40, 28.5 cm (206.45 per cent increase over control) and T23, 26.2 cm (181.72 per cent increase over control).

Least increased in root length was recorded T4, 11.4 cm (22.58per cent increase over control) and T1, 15.0 cm (61.29 per cent increase over control).

All the treatments in terms of shoot length, observed were at par with each other and with control. However, highest shoot length was exhibited by T7, T20 and T40 that is 17.2 cm, 15.3 cm and 15.0 cm (12.41, 1.29 and 1.96 per cent increase over control), respectively.

Table 4.31: Efficacy of *Trichoderma* spp. isolates against collar rot of lentil under glass house condition.

S.No.	Isolate	Per cent Mortality	Per cent decrease over control
1	T1	37.50 (37.57)	54.99
2	T4	25.00 (24.19)	69.99
3	T7	50.00 (44.98)	39.99
4	T12	41.66 (40.15)	50
5	T14	45.83 (42.57)	45
6	T19	33.33 (34.98)	60
7	T20	37.50 (37.57)	54.99
8	T22	29.16 (26.96)	65
9	T23	20.83 (22.19)	75
10	T29	37.50 (37.57)	54.99
11	T30	54.16 (44.98)	35
12	T31	41.66 (39.98)	50
13	T33	33.33 (34.98)	60
14	T36	25.00 (24.19)	69.99
15	T40	20.83 (22.19)	75
16	Control (untreated)	83.33 (66.17)	0
SEm±	4.45		
CD (5%)	12.89		

4.8.2: Isolation of fluorescent *Pseudomonas*

Fluorescent *Pseudomonas* were isolated from the soil samples by serial dilution method using Kings 'B' medium, thereafter, examined under UV light. Colonies with yellow - green and blue-white color pigmentation were marked and recorded. Twenty four fluorescent *Pseudomonas* isolates were isolated from collected soil sample from different location of Chhattisgarh and designated (Table 4.55).

Table 4.32: Effect of *Trichoderma* spp. isolates on germination and vigour index of lentil under sick pot condition.

S.No.	Isolate	Germination (%) *	Root Length(cm) *	Shoot length(cm) *
1	T1	86.66 (9.36)	15	13.6
2	T4	86.66 (9.36)	11.4	13
3	T7	83.33 (9.18)	15.9	17.2
4	T12	90.00 (9.53)	17.4	13.7
5	T14	90.00 (9.53)	20.2	14.5
6	T19	90.00 (9.53)	23.6	13.8
7	T20	86.66 (9.36)	24	15.3
8	T22	90.00 (9.53)	25.6	11.6
9	T23	86.66 (9.36)	26.2	14.2
10	T29	90.00 (9.53)	32.6	13.6
11	T30	90.00 (9.53)	22.4	13.2
12	T31	90.00 (9.53)	19.2	14.2
13	T33	86.66 (9.36)	31.8	11.8
14	T36	86.66 (9.36)	21.3	13.4
15	T40	90.00 (9.53)	28.5	15
16	Control (untreated)	83.33 (9.18)	9.33	15.5
		0.05	0.67	0.47
		0.15	1.69	2.13

4.8.2.1: Screening for antagonistic activity of fluorescent *Pseudomonas* against *S. rolfsii*

4.8.2.1.1: Evaluation of antagonistic efficacy of fluorescent *Pseudomonas* by dual culture method

Twenty fluorescent *Pseudomonas* isolates were isolated from collected soil samples from different locations of Chhattisgarh (Table 4.34). They were screened *in vitro* for their antagonistic activity against highly virulent isolate (SR4) of *S. rofsii* causing collar rot of lentil. Significant varying degree of antagonistic ability of fluorescent *Pseudomonas* isolates was found *in vitro* condition by dual culture technique and data are presented in Table 4.34 and Fig.4.27. Fluorescent *Pseudomonas* inhibited the mycelial growth by the antagonistic activity across *S. rofsii* ranged from 17.07 to 79.01 per cent over control.

Therty fluorescent *Pseudomonas* isolates were evaluated for their efficacy against *S. rolfsii.* Isolate P26 was found significantly efficient in reducing the mycelial growth of *S.rolfsii* by 79.01 % over control, followed by P7 (78.42 %), P13 (77.78 %), P22 (73.09 %), P3 (73.80 %), P16 (72.81 %), P29 (68.61%), P21

(67.98%), P12 (64.27%), P5 (61.90 %) which were statistically at par with each other. Minimum inhibition was recorded in P120 (15.53 %) followed by P28 (17.07 %), P2 (32.09 %) and P18 (34.99%) which were at par with each other.

Table 4.33: Isolates of fluorescent *Pseudomonas* collected from different locations of Chhattisgarh.

S.No.	Location	Block	District	Code of isolate
1	Raipur	Raipur	IGKV, Farm	P1
2		Raipur	Agronomy, Field	P2
3	Mungeli	Mungeli	Temari	P3
4		Mungeli	Navapara	P4
5		Lormi	Chilfi	P5
6		Lormi	Bhtha-Bhurka	P6
7	Bilaspur	Bilaspur	Takhatpur	P7
8	Dhamtari	Kurud	Gobra	P8
9	Baloda bazar	Simga	Surgi	P9
10		Bhatapara	Gogiya	P10
11		Bhatapara	Sandyi	P11
12	Bemetara	Bemetara	Bemetara	P12
13		Bemetara	Matia	P13
14		Bemetara	mohbhate	P14
15		Bemetara	Gadamod	P15
16	Kawardha	Kawardha	Newari farm	P16
17		Pandariya	Kapadah	P17
18		Pandariya	Ghuterkudi	P18
19	Griyaband	Gariyaband	Panduka	P19
20		Mainpur	Birighat	P20
21	Jagadalpur	Jagadalpur	Kumhravan	P21
22	Durg	Durg	Anjora	P22
23		Dhamda	Dhamda	P23
24	Rajnandgaon	Rajnandgaon	Surgi	P24
25		Rajnandgaon	Mohba	P25
26	Korba	Korba	KVK Farm	P26
27	Balod	Balod	Balod	P27
28	Kanker	Kanker	Kaanker	P28
29		Kanker	Narharpur	P29
30	Janjgir	Janjgir	Jangir	P30

In relation to other factor, fluorescent *Pseudomonas* isolate also inhibit sclerotial formation in *S. rolfsii.* Formation of sclerotia ranged from 0 to 147 after 12 DAI. In isolate, P14 and P29 sclerotia formation was scleotia formation was zero followed by isolate P27 (1), P10 (2), P11 (5), P (16), P17 (6), P26 (8), P12 (9), P19 (10), P9 (12), P3 (35), P21 (42), P22 (59) and P20 (67) which were

statistically at par with each other. Whereas, maximum sclerotia formation observed in isolate P6 (147) and P7 (144).

Present study is on conformity to the finding made by (Krishnamurthy and Gnanamanickam, 1998) they reported that antagonistic activity of *Pseudomonas* spp. against several fungus both *in vivo* and *in vitro* condition. Similar results were also obtained by Samanta *et al.* (2004) by *Pseudomonas fluorescens* against *Macrophomina phaseolina, R.solani, Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Kishore *et al*. (2005) reported that *P. aeruginosa* could inhibit mycelial growth of *S. rolfsii* up to 74 % using dual culture. Dewangan *et al*. (2014) reported that *Pseudomonas fluorescen* can efficiently inhibit the mycelial growth of all the fungal pathogens, *Sclerotium rolfsii* (63.15%), *Sclerotium rolfsai,* (61.85%) *Rhizoctonia bataticola* (55.56%) and *R. solani* (53.15 %) under in vitro condition by dual culture techniques. Chanutsa *et al.* (2014) reported 100 per cent inhibition in the growth of *S. rolfsii* with culture filtrate of *P. Florescence*.

Table 4.34: Antagonistic efficiency of fluorescent *Pseudomonas* isolates against *S.rolfsii* by dual culture technique.

4.8.2.1.2: *In vivo* efficacy of fluorescent *Pseudomonas* under glass house condition

The data on effect of antagonistic efficacy of fluorescent *Pseudomonas* on *S. rolfsii* under glass house condition are presented in Table 4.35

In vivo experiment was conducted to evaluate the efficacy of fluorescent *Pseudomonas* on the incidence of collar rot of lentil. The average mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Isolate P15 exhibited significant minimum mortality 15.24 per cent followed by P26 (15.57 per cent) and P22 (15.73 per cent) which were statistically at par with each other. These isolates showed significantly better results, over other isolates. Maximum mortality was observed in P8 (60.79 per cent) over control (86.26 per cent).

The influence of different fluorescent *Pseudomonas* isolate on germination per cent and vigour index in lentil when challenge inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.36.

Table 4.34: Antagonistic efficiency of *Psudomonas* spp. isolates against *S. rolfsii* by dual culture technique

S. No.	Isolates of *P.fluorescens*	**Percent inhibition*** %
1	P1	37.46(37.71)
2	P2	32.09(34.49)
3	P3	73.8(59.19)
4	P4	40.72(39.63)
5	P5	61.90(51.86)
6	P6	49.62(44.76)
7	P7	78.42(62.3)
8	P8	47.92(43.79)
9	P9	46.60(43.03)
10	P10	56.78(48.88)
11	P11	56.27(48.58)
12	P12	64.27(53.27)
13	P13	77.78(61.90)
14	P14	52.13(46.20)
15	P15	47.81(43.72)
16	P16	72.81(58.55)
17	P17	50.27(45.14)
18	P18	34.99(36.24)
19	P19	48.24(43.97)
20	P20	15.53(23.19)
21	P21	67.98(55.52)
22	P22	73.09(58.73)
23	P23	53.63(47.06)
24	P24	53.49(46.98)
25	P25	40.50(39.51)
26	P26	79.01(62.72)
27	P27	55.01(47.85)
28	P28	17.07(24.39)
29	P29	68.61(55.90)
30	P30	40.48(39.49)
31	Control	0(0)
	SE (m)	0.533
	C.D.	1.51

*Average of three replication

Figures in parentheses are angular transformation

Highest germination per cent was recorded in the isolates P26 (96.86 per cent) followed by P7 (94.62 per cent) and P13, P29 (93.42 and 91.54 per cent) which were at par with each other. The above isolates were found to be significantly superior with all the other isolates and over control (70.69 per cent).

All the isolates of with fluorescent *Pseudomonas* affect the vigour index of lentil plant. Isolate P17 was found best in increasing vigour index (4504.82) followed by P4 (4255.85) and P10 (4050.00) as compared to other isolates and control (2170.00).

Successful control of stem rot and root rot by *Pseudomonas* was demonstrated by Sharma (1994) and Sharma *et al.* (1999). In agreement to present finding Rakh *et al.* (2011) reported that *Pseudomonas monteilii* treated seeds showed decrease in incidence of disease up to 45.45 to 66.67% in comparison to untreated seeds in pot assay for control of *Sclerotium rolfsii*. Bhatia *et al.* (2005) reported that fluorescent *Pseudomonas* PS-I and PS–II coated seed sown in *S. rolfsii* infected soil significantly increased seed germination by 13.1 and 8.5 per cent respectively as compared to control. Seed bacterization with one of the endophytes (Endo PR8) of *Pseudomonas fluorescens* isolated from root and stem of health cotton seedlings, reducing damping off disease incidence caused by *Sclerotium rolfsii* by 76 per cent (Bowmik *et al.,* 2002). Hameeda *et al.* (2010) isolated bacteria from compost and macro fauna, seven of the 207 isolates showed antagonistic activity against *S. rolfsii* in pot culture. Two of the seven isolate were *Bacillus* sp. and rest belonged to *Pseudomonas* sp. Kaur and Sharma (2013) collected 35 isolates of rhizobacteria from 25 soil sample collected from healthy lentil rhizosphere. Out of thirty five isolates, ten isolates of rhizobacteria were characterized as *Pseudomonas* sp. on the basis of morphological and growth promotion activities.

Table 4.35: Efficacy of fluorescent *Pseudomonas* isolates against collar rot of lentil under glass house condition.

S. NO.	*Fluorescent Pseudomonas* Isolates	Per cent mortality*
1	**P1**	45.48(42.38)
2	**P2**	50.71(45.39)
3	**P3**	17.41(24.64)
4	**P4**	45.17(42.21)
5	**P5**	20.23(26.71)
6	**P6**	56.72(49.09)
7	**P7**	15.87(23.44)
8	**P8**	60.79(51.50)
9	**P9**	54.16(47.40)
10	**P10**	40.75(39.65)
11	**P11**	53.66(47.16)
12	**P12**	22.66(28.41)
13	**P13**	15.24(22.96)
14	**P14**	56.31(48.62)
15	**P15**	40.51(39.40)
16	**P16**	19.48(26.17)
17	**P17**	27.59(31.67)
18	**P18**	51.76(45.99)
19	**P19**	30.51(33.51)
20	**P20**	53.06(46.74)
21	**P21**	21.28(27.45)
22	**P22**	15.73(23.35)
23	**P23**	56.33(48.71)
24	**P24**	57.26(49.26)
25	**P25**	41.63(40.16)
26	**P26**	15.57(23.22)
27	**P27**	31.81(34.31)
28	**P28**	52.30(46.30)
29	**P29**	20.54(26.93)
30	**P30**	44.52(41.83)
31	**Control (untreated)**	86.26(68.23)
	SEm±	2.382
	CD (5%)	6.75

*Average of three replication

Figures in parentheses are angular transformation

Fig. 4.27 **Antagonistic efficiency of *Tricoderma* spp. isolates against *S. rolfsii* by dual culture technique**

Table 4.36: Effect of fluorescent *Pseudomonas* isolates on germination and vigour index of lentil under sick pot condition.

S. No.	Isolates of *P.fluorescens*	Germination (%)*	Root length (cm)**	Shootlength (cm)**	Vigour index
1	P1	72.21(8.55)	18.45	17.63	2605.34
2	P2	73.13(8.60)	17.46	18.60	2636.58
3	P3	90.70(9.57)	20.54	23.38	3983.39
4	P4	80.42(9.02)	17.87	18.62	2934.79
5	P5	83.15(9.17)	18.02	20.83	3230.78
6	P6	81.08(9.05)	19.22	17.17	2950.62
7	P7	94.62(9.77)	22.00	25.75	4518.11
8	P8	75.76(8.75)	19.11	18.05	2814.86
9	P9	78.64(8.92)	17.57	19.29	2899.06
10	P10	85.37(9.29)	17.52	17.62	3000.30
11	P11	81.72(9.09)	16.99	18.54	2903.90
12	P12	80.93(9.04)	18.86	18.96	3060.90
13	P13	93.42(9.71)	21.66	24.12	4277.07
14	P14	77.96(8.88)	18.18	18.32	2845.40
15	P15	80.3(9.01)	18.03	19.25	2993.32
16	P16	89.39(9.50)	20.19	22.40	3807.70
17	P17	77.31(8.84)	18.52	17.23	2764.09
18	P18	77.72(8.87)	16.95	16.29	2583.63
19	P19	81.25(9.06)	18.47	18.17	2977.24
20	P20	70.66(8.46)	15.56	16.41	2258.98
21	P21	85.77(9.31)	19.58	19.42	3345.00
22	P22	89.45(9.51)	20.41	21.76	3772.40
23	P23	79.87(8.98)	18.29	17.10	2826.72
24	P24	80.21(9.00)	18.59	16.79	2837.53
25	P25	74.35(8.67)	17.69	19.04	2730.63
26	P26	96.86(9.89)	21.87	24.48	4488.82
27	P27	77.96(8.88)	18.34	16.27	2698.69
28	P28	76.62(8.81)	16.24	16.83	2533.57
29	P29	91.54(9.62)	19.29	19.32	3535.23
30	P30	75.61(8.75)	18.99	17.70	2773.88
31	Control	70.69(8.46)	13.30	14.35	1954.58
	SE (m)±	0.12	1.12	0.86	
	C.D.	0.341	3.18	2.44	

*Average of three replication ** Average of five replication
Figures in parentheses are square root transformation

4.8.3.: Isolation of *Bacillus* spp.

Bacillus spp. were isolated from the soil samples by serial dilution method Using nutrient agar medium, thereafter, examined on the basis of morphological andcultural characteristic. Selected colonies inoculated in King's B broth and incubated in incubator shaker at 30^0C for 24 hours. Nine *Bacillus* spp. isolates

were isolated from the collected soil sample of different location of Chhattisgarh and designated (Table 4.59).

Table 4.37: Isolates of *Bacillus* spp. collected from different locations of Chhattisgarh.

S.No.	0.District	Block	Location	Isolte
1	Raipur	Raipur	IGKV, Farm	B1
2		Raipur	Agronomy, Field	B2
3	Mungeli	Mungeli	Temari	B3
4		Mungeli	Navapara	B4
5	Bemetara	Bemetara	Bemetara	B5
6		Bemetara	Matia	B6
7	Bilaspur	Bilaspur	takhatpur	B7
8	Bemetara	Bemetara	Bemetara	B8
9		Bemetara	Matia	B9
10		Bemetara	Gadamod	B10
11	Kawardha	Kawardha	Newari farm	B11
12		Pandariya	kapadah	B12
13		Pandariya	ghuterkudi	B13
14	Griyaband	Gariyaband	Panduka	B14
15	Baloda bazar	Simga	Surgi	B15
16		Bhatapara	Gogiya	B16
17	Dhamtari	Kurud	Gobra	B17
18	Kanker	Kanker	Kaanker	B18
19		Kanker	Narharpur	B19
20	Janjgir	Janjgir	Jangir	B20
21	Korba	Korba	KVK Farm	B21
22	Balod	Balod	Balod	B22

4.8.3.1: Screening of *Bacillus* spp. for antagonistic activity against *S. rolfsii*

4.8.3.1.1 Evaluation of antagonistic activity *Bacillus* spp. by dual culture method.

24 isolates *Bacillus* spp. were collected from different locations of Chhattisgarh and presented in Table 4.38. They were screened *in vitro* for their antagonistic activity against highly virulent isolate (SR4) of *S. rofsii* causing collar rot of lentil. Significant varying degree of antagonistic ability of *Bacillus* spp was found under *in vitro* by dual culture technique (Table 4.38 and Fig.4.28). *Bacillus* spp inhibited the mycelial growth by the antagonistic activity against *S. rofsii* and ranged from 18.73 to 72.33 per cent over control. Isolate B21 was found significantly efficient in reducing the mycelial growth of *S.rofsii* by 72.33 per cent

over control followed by B19 (70.39 per cent1) which was statistically at par with B21. Minimum inhibition was recorded in B14 (18.73 per cent).

The result revealed that isolates *Bacillus* spp. can efficiently inhibit the mycelial growth of *S. rolfsii* taken in this study.

In agreement to present finding, Shifa *et al.* (2015) observed that *B. subtilis* G-1, *B. amyloliquefaciens* B2 and *B. subtilis* EPCO 8 were found effective biocontrol agents in inhibiting the mycelial growth of *S. rolfsii in vitro* in dual culture assay.

Gholami *et al.* (2014) reported that *B. subtilis* sub sp. *subtilis* and *B. subtilis* subsp. *spizizenii*, which demonstrated the largest inhibition growth against *S. rolfsii* under *in vitro* dual culture. *Bacillus subtilis* has the potential to produce bioactive compound antagonistic against plant pathogenic fungi *Sclerotium rolfsii* (Gomashe *et al*., 2014). Suneeta *et al.* (2016) screened 26 isolates of *Bacillus* spp. against collar rot pathogen *in vitro*. Among them, five strains of *Bacillus* spp. showed highest antagonistic activity against *S. rolfsii*. Vishwanath *et al.* (2012) conducted experiment to screen potential biocontrol agents, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas* sp.-I, *Bacillus* sp.-I and *Bacillus* sp.-II for the management of *Sclerotium rofisii* the causal agent of collar rot of sunflower in dual culture test and they overgrew the pathogen upto 76.2, 88.8, 80.01, 35.9 and 77.8 per cent respectively *in vitro*.

Table 4.38: Antagonistic efficiency of *Bacillus* spp. isolates against *S. rolfsii* by dual culture technique.

S.No.	Bacillus spp. isolates	Percent inhibition* %
1	B1	41.42(40.03)
2	B2	56.34(48.63)
3	B3	58.31(49.77)
4	B4	41.71(40.21)
5	B5	41.86(40.29)
6	B6	29.85(33.07)
7	B7	28.79(32.42)
8	B8	31.96(34.39)
9	B9	24.94(29.92)
10	B10	64.26(53.27)
11	B11	24.31(29.52)
12	B12	26.24(30.78)
13	B13	66.86(54.83)
14	B14	18.73(27.01)
15	B15	26.8(31.15)
16	B16	58.87(50.09)
17	B17	56.91(48.95)
18	B18	65.19(53.83)
19	B19	70.39(57.01)
20	B20	58.36(49.79)
21	B21	72.32(58.23)
22	B22	64.61(53.49)
23	B23	61.45(51.59)
24	Control	0.00
	SEm ±	0.96
	CD (5%)	2.738

*Average of three replication

Figures in parentheses are angular transformation

4.8.3.1.2: *In vivo* efficacy of *Bacillus* spp. under glass house condition

The data on effect of antagonistic efficacy of *Bacillus* spp. on *S. rolfsii* under glass house condition are presented in Table 4.39

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Isolate B21 exhibited significant minimum mortality 17.35 per cent followed by B19 (mortality 22.64 per cent) and B22 (mortality 27.24 per cent). These isolates showed significantly better results, over other isolates. Maximum mortality was observed in B9 (69.54 per cent) as compared to control (81.95 per cent).

The influence of different *Bacillus* spp. isolate on germination per cent and vigour index in lentil when challenge inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.40

Highest germination per cent was observed in the isolates B21 (96.64 per cent) followed by B23 (92.34 per cent), B19 and B22 (91.32 and 90.50 per cent) which were at par with each other. The above *Bacillus* spp. isolates were found to be significantly superior with all the other isolates and over control (73.39 per cent).

All the isolates of *Bacillus* spp isolate affects the vigour index of lentil plant. Isolate B21 was found best in increasing vigour index (4400.66) followed by B19 (3964.51) and B23 (3728.38) as compared to other isolates and control (2180.42)

These greenhouse experiments using *Bacillus* spp. isolates showed agreement with results of Pleban *et al*. (1995) reported effectiveness of endophytic *Bacillus* spp. against *Rhizoctonia solani* and *Sclerotium rolfsii*. Also, Zaghloul *et al*. (2008) reported that *B. subtilis* I, II and III were capable of inhibiting damping-off in tomato seedlings by 80, 47 and 33%, respectively. Gholami *et al.* (2014) observed that reduction of root-rot of bean was the highest in the seeds pre-treated with the isolate *B. subtilis* subsp. *spizizenii*, *B. subtilis* subsp. *subtilis*, *B. atrophaeus*, *B. tequilensis* and *S. cyaneofuscatus*, which resulted in 50-58.5% reduction in severity of the disease root-Rot in glasshouse experiment. Shifa *et al.*

(2015) observed that groundnut seeds when treated with *B. subtilis* G-1 showed significant increases in per cent germination, root length, shoot length and seedling vigour and effectively reduced the incidence of stem rot of groundnut caused by *S. rolfsii.*

Table 4.39: Efficacy of *Bacillus* spp. isolates against collar rot of lentil under glass house condition.

S.No.	Bacillus spp. isolates	Per cent mortality* %
1	B1	45.47(40.06)
2	B2	48.94(48.64)
3	B3	54.98(49.79)
4	B4	53.09(40.23)
5	B5	49.14(40.32)
6	B6	48.82(33.12)
7	B7	47.42(32.45)
8	B8	51.5(34.43)
9	B9	69.54(29.96)
10	B10	51.05(53.29)
11	B11	65.7(29.54)
12	B12	69.46(30.82)
13	B13	25.63(54.86)
14	B14	67.08(25.64)
15	B15	68.66(31.18)
16	B16	32.01(50.11)
17	B17	45.75(48.97)
18	B18	30.21(53.85)
19	B19	22.64(57.03)
20	B20	38.7(49.81)
21	B21	17.35(58.26)
22	B22	27.24(53.50)
23	B23	37.61(51.62)
24	B24	81.95(64.86)
	SEm ±	2.291
	CD (5%)	6.533

*Average of three replication
Figures in parentheses are angular transformation

Table 4.40: Effect of *Bacillus* spp. isolates on germination and vigour index of Lentil under sick pot condition.

S.No	*Bacillus* spp. Isolates	Germination (%)	Root Length (cm)	Shoot Length (cm)	Vigour Index
1	B1	78.48(8.86)	18.08	17.65	2804.35
2	B2	80.17(8.95)	19.27	17.73	2966.56
3	B3	80.23(8.96)	18.90	18.07	2966.10
4	B4	80.02(8.95)	18.51	18.29	2944.74
5	B5	81.61(9.03)	18.08	18.62	2994.54
6	B6	77.85(8.82)	20.07	17.38	2914.96
7	B7	82.15(9.06)	19.56	17.30	3027.78
8	B8	81.58(9.03)	18.16	18.10	2957.55
9	B9	83.63(9.14)	19.53	17.89	3129.71
10	B10	84.66(9.20)	18.35	17.91	3069.77
11	B11	79.69(8.93)	18.28	17.34	2838.29
12	B12	81.89(9.05)	19.57	18.44	3112.64
13	B13	86.36(9.29)	21.86	21.50	3744.28
14	B14	78.87(8.88)	19.00	16.64	2811.19
15	B15	87.47(9.35)	19.22	18.22	3275.17
16	B16	83.77(9.15)	20.93	19.06	3349.40
17	B17	87.78(9.37)	19.78	20.35	3522.03
18	B18	84.98(9.22)	20.14	20.36	3441.69
19	B19	91.32(9.56)	22.92	20.49	3964.51
20	B20	86.75(9.31)	20.61	20.46	3562.82
21	B21	96.64(9.83)	23.37	22.17	4400.66
22	B22	90.5(9.51)	20.80	19.76	3670.38
23	B23	92.34(9.61)	20.06	20.31	3728.38
24	Control(untreate)	73.39(8.57)	14.84	14.87	2180.42
	SEm ±	1.29	0.94	0.90	
	CD (5%)	3.67	2.69	2.57	

* Average of three replication
**Average of five replication
Figures in parentheses are square root transformation **1.2**

4.8.4. : *In vitro* evaluation of botanicals

Ten botanicals *viz.*, Neem leaves, NSKE, *Agave*, *Clerodendron*, *Pongamia*, Henna, Garlic, Ginger, *Tridax* and *Eucalyptus*, were evaluated against *S. rolfsii* as described in ―Material and Methods‖. The data on per cent inhibition of radial growth of the fungus is presented in Table 18, Fig. 2 and Plate 7 .

Among the botanicals evaluated, *Agave* recorded maximum mycelial inhibition of 100 per cent at all the concentrations tested, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively with a mean of 65.25%. Neem leaves recorded 32.6, 41.1 and 41.3 per cent inhibition at 5, 10 and

15 per cent concentrations with a mean of 38.3%; NSKE 13.2, 17.4 and 22.4 per cent at 5, 10 and 15 per cent concentrations with a mean of 17.7%; *Clerodendron* 13.2, 18.6 and 28.9 per cent at 5, 10 and 15 per cent concentrations with a mean of 20.19%; *Eucalyptus* 10.4, 8.2 and 11.9 per cent at 5, 10 and 15 per cent concentrations with a mean of 10.1%; Ginger 5.2, 12.8 and 30.4 per cent at 5, 10 and 15 per cent concentrations with a mean of 16.1%; Garlic 2.6, 5.0 and 33.3 per cent at 5, 10 and 15 per cent concentrations with a mean of 13.6% respectively showed moderate inhibition.

Least inhibition was observed in *Tridax* with 0.0, 6.7 and 9.9 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 5.5% and *Pongamia* recorded 5.9, 5.4 and 9.9 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 7.1%.

Table 4.41. *In vitro* evaluation of botanicals against *Sclerotium rolfsii*

Sl. No.	Extract	Per cent mycelial inhibition			
		Conc. of botanicals			
		5%	10%	15%	Mean
1	Neem leaves	32.6(34.80)*	41.1(39.86)	41.3(39.97)	38.3(38.24)
2	NSKE	13.2(21.25)	17.4(24.65)	22.4(28.26)	17.7(24.85)
3	*Agave*	100.0(89.96)	100.0(89.96)	100.0(89.96)	100.0(89.96)
4	*Clerodendron*	13.2(21.25)	18.6(25.50)	28.9(32.49)	20.2(26.69)
5	*Pongamia*	5.9(14.09)	5.4(13.39)	9.9(18.31)	7.1(15.40)
6	Henna	34.4(35.92)	71.3(57.58)	90.0(71.54)	65.2(53.86)
7	Garlic	2.6(9.28)	5.0(12.92)	33.3(35.25)	13.6(21.67)
8	Ginger	5.2(13.16)	12.8(20.93)	30.4(33.47)	16.1(23.67)
9	*Tridax*	0	6.7(14.95)	9.9(18.31)	5.5(13.57)
10	*Eucalyptus*	10.4(18.78)	8.2(16.59)	11.9(20.13)	10.1(18.55)
11	Control	0	0	0	0
	Botanicals	**Conc.**	**Botanicals X Concentration**		
SEm+	**0.16**	**0.31**	**0.09**		
CD (P0.01)	**0.52**	**0.99**	**0.30**		

*Figures in parentheses are Arcsine transformed values

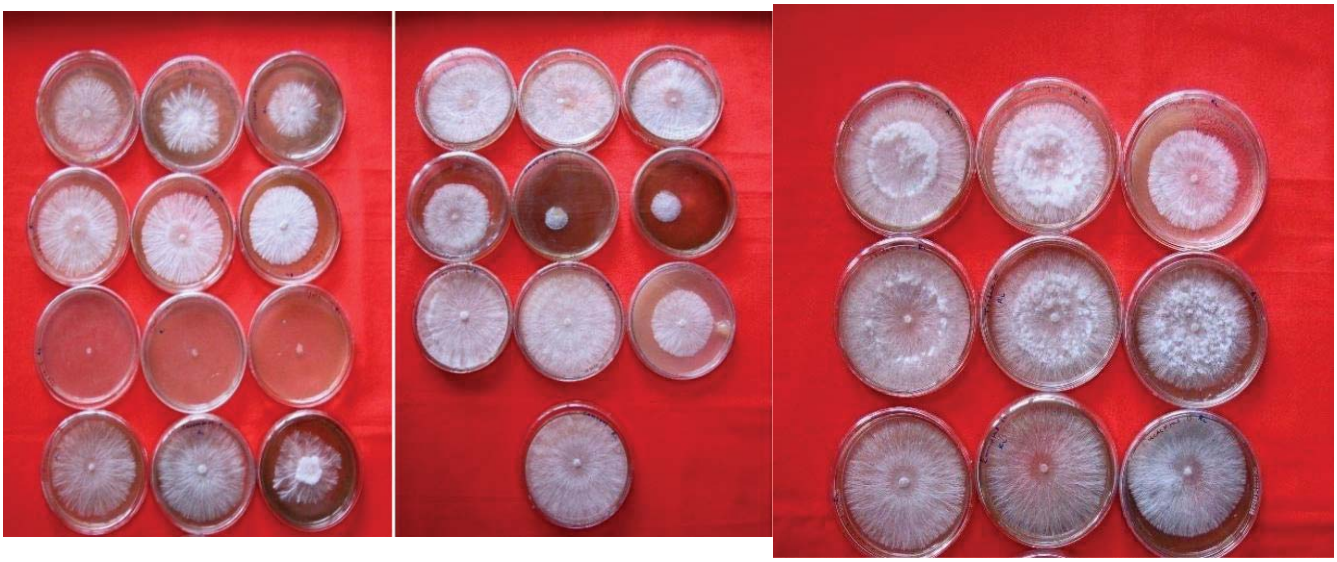


Fig : 4.29 Effect of different botanicals on *Sclerotium rolfsii*

T1: Neem leaves
T2: NSKE
T3: *Agave*
T4: *Clerodendron*
T5: *Pongamia*
T6: Henna/Mehndi
T7: Garlic
T8: Ginger
T9: *Tridax*
T10: *Eucalyptus*

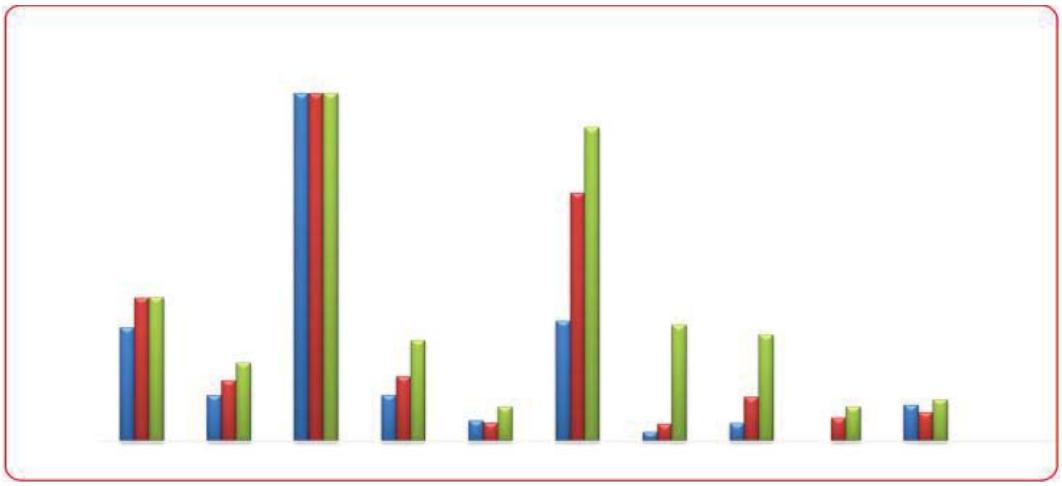


Fig. 4.38. *In vitro* evaluation of botanicals against *Sclerotium rolfsii*

T1: Neem leaves
T2: NSKE
T3: *Agave*
T4: *Clerodendron*
T6: Henna/Mehndi
T7: Garlic
T8: Ginger
T9: *Tridax*

4.8.5 . Seed treatment with biocontrol agent

4.8.6. Seed treatment with different doses

Evaluation of biocontrol agent *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp., seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) was carried out to find out effective seed treatment for control of collar rot of lentil under glass house condition.

In this experiment, talc based formulations (*Tricho*-9, *Pf*-26 and *Bs*-21) were used which were prepared after screening of potential isolate of respective biocontrol agent *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp., through *in vitro* and *in vivo* studies.

4.8.6.1 *Trichoderma* spp. seed treatment with different doses

The data on effect of *Trichoderma* spp. talc based formulation (*Tricho*-9) seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 4.42

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Seed treatment with T6 (dose 30 gm) exhibited significant minimum mortality 10.37 per cent (87.99 per cent decreased over control) followed by T5 (mortality 14.07 per cent), T4 (mortality 15.83 per cent) and T3 (mortality 20.37 per cent) with 83.70, 81.66, and 76.40 per cent decrease in mortality, respectively over control which were statistically at par with each other. Whereas maximum mortality was observed in T1 (38.42 per cent) followed by T2 (33.61 per cent) as compared to control (86.31 per cent).

Thus, the result revealed that *Trichoderma* spp. seed treatment with increased dose more than the recommended dose can efficiently control collar rot of lentil.

The data on effect of seed treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) on germination and vigour index of lentil under glass house condition are presented in Table 4.43

Highest germination per cent was observed in T6 and T3 (96.67 per cent) followed by T5 (93.33 per cent), T2 (90.00 per cent) and T1, T4 (86.67 per cent) which were at par with each other. The above treatments were found to be significantly superior over control (73.33 per cent).

Trichoderma spp. seed treatment with different doses affect the vigour index of lentil plant. Treatment T5 was found best in increasing vigour index (4293.18) followed by T6 (4060.14), T4 (3952.15) and T3 (3924.15) as compared to other doses and control (2258.56).

Table 4.42: Effect of seed treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) using different doses against collar rot of lentil.

S.No.	Treatment	Seed dose (g)	Per cent mortality*	Per cent decrease over Control
1	T1	5	38.42 (38.26)	55.47
2	T2	10	33.61 (35.39)	61.06
3	T3	15	20.37 (26.40)	76.40
4	T4	20	15.00 (23.03)	81.66
5	T5	25	14.07 (21.82)	83.70
6	T6	30	10.37 (18.77)	87.99
7	T7	Control (untreated)	86.30 (68.26)	0.00
		SEm ±	2.377	
		CD (5%)	7.28	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.43: Effect of seed treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) on vigour index of lentil.

S. No.	Treatment	Seed dose(g)	Germination (%)*	Root length (cm)**	Shoot length (cm)**	Vigour Index
1	T1	5	86.66 (9.3)	21.20	18.40	3432.13
2	T2	10	90.00 (9.5)	21.80	19.60	3726.00
3	T3	15	96.66 (9.8)	23.00	17.60	3924.80
4	T4	20	86.66 (9.3)	26.00	19.60	3952.15
5	T5	25	93.33 (9.7)	28.00	18.00	4293.18
6	T6	30	96.66 (9.8)	24.00	18.00	4060.14
7	T7	Control (untreated)	73.33 (8.6)	16.00	14.80	2258.56
		SEm ±	0.230	1.027	0.607	
		CD (5%)	0.704	2.990	1.768	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

4.8.6.2 Fluorescent *Pseudomonas* seed treatment with different doses

The data on effect of fluorescent *Pseudomonas* talc based formulation (*Pf*-26) seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 44

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Seed treatment with T5 (dose 30 gm) exhibited significant minimum mortality 10.00 per cent (88.41 per cent decreased over control) followed by T5 (mortality 10.83 per cent), T4 (mortality per cent 14.07) and T3 (mortality 18.52 per cent) with 87.45, 83.70 and 78.54 per cent mortality, respectively over control which were statistically at par with each other. Whereas maximum mortality was observed in T1 (31.94 per cent) as compared to control (86.31 per cent).

Thus, the result revealed that fluorescent *Pseudomonas* talc based formulation (*Pf*-26) seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed), when increased in dose more than the recommended can efficiently control collar rot of lentil.

The data on effect of seed treatment with potential antagonist fluorescent *Pseudomonas* talc based formulation (*Pf*-26) with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) on germination and vigour index of lentil under glass house condition are presented in Table 4.45

Highest germination per cent was observed in T6 (100 per cent) followed by T4 and T5 (93.33 per cent) and T3 (90.00 per cent) which were at par with each other. The above treatments were found to be significantly superior with all the other treatments and control (73.33 per cent).

Seed treatment of fluorescent *Pseudomonas* talc based formulation (*Pf*-26) with different doses affect germination and vigour index of lentil plant. Treatment T6 was found best in increasing vigour index (5000.00) followed by T4 (4946.49) and T5 (4666.50) as compared to other treatment and control (2258.56).

Table 4.44: Effect of seed treatment with potential antagonist fluorescent *Pseudomonas* formulation (*Pf*-26) using different doses against collar rot of lentil.

S. No.	Treatment	Seed dose (g)	Per cent mortality*	Per cent decrease over Control
1	T1	5	31.94 (34.32)	62.99
2	T2	10	27.50 (31.42)	68.14
3	T3	15	18.51 (24.72)	78.54
4	T4	20	14.07 (21.82)	83.70
5	T5	25	10.83 (19.18)	87.45
6	T6	30	10.00 (18.42)	88.41
7	T7	Control (untreated)	86.30 (68.26)	0.00
		SEm ±	2.68	
		CD (5%)	8.20	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.45: Effect of seed treatment with potential antagonist fluorescent *Pseudomonas* formulation (*Pf*-26) using different doses on vigour index of lentil.

S. No.	Treatment	Seed dose (g)	Germination (%)*		Root Length (cm)**	Shoot length (cm)**	Vigour Index
1	T1	5	83.33	(9.1)	25.80	18.20	3666.52
2	T2	10	86.66	(9.3)	26.40	19.00	3934.82
3	T3	15	90.00	(9.5)	26.80	19.20	4140.00
4	T4	20	93.33	(9.7)	32.40	20.60	4946.49
5	T5	25	93.33	(9.7)	28.40	21.60	4666.50
6	T6	30	100.0	(10.05)	28.00	22.00	5000.00
7	T7	Control (untreated)	73.33	(8.6)	16.00	14.80	2258.56
		SEm ±	0.221		1.066	0.499	
		CD (5%)	0.678		3.105	1.452	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

4.8.6.3 *Bacillus* spp. seed treatment with different doses

The data on effect of *Bacillus* spp. talc based formulation (*Bs*-21) seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 4.46

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Seed treatment with T6 (dose 30 gm) exhibited significant minimum mortality 21.48 per cent (75.11 per cent decreased over control) followed by T5 (mortality 23.14 per cent), T4 (mortality 25.00 per cent) with 73.18 and 71.03 per cent mortality, respectively over control which were statistically at par with each other. Whereas maximum mortality was observed in T1 that is 44.04 per cent whereas in control (86.30 per cent).

Thus, the result revealed that *Bacillus* spp. talc based formulation (*Bs*-6) seed treatment with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed), when increased in dose more than the recommended can efficiently control collar rot of lentil.

The data on effect of seed treatment with potential antagonist *Bacillus* spp. talc based formulation (*Bs*-6) with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) on germination and vigour index of lentil under glass house condition are presented in Table 4.47

Highest germination per cent was observed in T21 and T4 that is 93.33 per cent followed by T3 (90.00 per cent) and T2, T5 (86.66 per cent) which were at par with each other. The above treatments were found to be significantly superior with all the other treatment and control (73.33 per cent).

All the treatments of *Bacillus* spp. talc based formulation (*Bs*-21) seed treatment with different doses affect germination and vigour index of lentil plant. Treatment T6 found best in increasing vigour index (4386.51) followed by T5 (4229.50) and T4 (3994.52) as compared to other treatment and control (2258.56).

Table 4.46: Effect of seed treatment with potential antagonist *Bacillus* spp. formulation (*Bs-21*) using different doses against collar rot of lentil.

S. No.	Treatment	Seed dose (g)	Per cent mortality*	Per cent decrease over control
1	T1	5	44.04 (41.52)	48.96
2	T2	10	38.42 (38.26)	55.47
3	T3	15	37.77 (37.80)	56.23
4	T4	20	25.00 (29.91)	71.03
5	T5	25	23.14 (28.73)	73.18
6	T6	30	21.48 (27.59)	75.11
7	T7	Control (untreated)	86.30 (68.26)	0.00
		SEm ±	2.259	
		CD (5%)	6.919	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.47: Effect of seed treatment with potential antagonist *Bacillus* spp. formulation (*Bs*-21) on vigour index of lentil.

S. No.	Treatment	Seed dose (g)	Germination (%)*	Root Length (cm)**	Shoot length (cm)**	Vigour Index
1	T1	5	76.66 (8.8)	21.6	14.6	2775.45
2	T2	10	86.66 (9.3)	24.0	15.4	3414.80
3	T3	15	90.00 (9.5)	24.6	15.6	3618.00
4	T4	20	93.33 (9.7)	26.8	16.0	3994.52
5	T5	25	86.66 (9.3)	27.6	21.2	4229.50
6	T6	30	93.33 (9.7)	27.8	19.2	4386.51
7	T7	Control (untreated)	73.33 (8.6)	16	14.8	2258.56
		SEm ±	0.233	1.125	0.548	
		CD (5%)	0.715	3.276	1.595	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

4.8.7. Seed treatment and soil application with biocontrol agent.

The experiment was conducted to find out an effective antagonistic treatment (*Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp.) which may enhance germination, shoot length, root length and minimize mortality of seedling by collar rot of lentil.

In this experiment, talc based formulations (*Tricho*-9, *Pf*-26 and *Bs*-21) were prepared after screening of potential isolate of respective biocontrol agent

Trichoderma spp., fluorescent *Pseudomonas* and *Bacillus* spp., through *in vitro* and *in vivo* studies.

4.8.7.1 Seed treatment and soil application with *Trichoderma* spp.

The data on effect of seed and soil treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 4.48

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Combined application of seed treatment + soil application (T4) of potential antagonist *Trichoderma* spp. talc based formulation (*Tricho*-9) exhibited significant minimum mortality 18.14 per cent (79.11 per cent decreased over control) followed by seed priming + soil treatment (T5) (mortality 22.22 per cent) (74.43 per cent decrease over control) as compared to only seed treatment (T1) (mortality 32.22 per cent), soil treatment (T3) (mortality 33.33 per cent) and seed priming treatment (T2) (mortality 36.11) with 62.92, 61.64 and 58.44 per cent mortality, respectively over control.

The treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-12) use in different forms also affect germination and vigour index of lentil under glass house condition. The data presented in Table 4.70 revealed that the seed and soil treatment with *Trichoderma* spp. (T4) was found best in increasing vigour index 4722.50 as well as germination (96.66 per cent) as compared to the other treatment and untreated control.

In agreement to above findings, Vanitha and Suresh (2002) observed that application of *T. harzianum* as seed treatment and soil application of adathoda leaf powder and FYM resulted, the lowest collar rot of brinjal (9.44%) caused by *Sclerotium rolfsii.* Saralamma and Vithal Reddy (2003) reported that seed treatment @ 10 conidia ml-1 and soil application @ 5g kg-1 soil with *T. harzianum* (H) were found to be optimum in increasing percent seedling emergence to an extent of 80 and 84, reducing disease incidence to 26.6 and 13.0% with an increased yield of 1373 and 1413 kg /ha. Biswas and Sen (2000) reported that *Trichoderma harzianum* isolates (T8 and T10) reduced stem rot incidence significantly when delivered as seed dressing or soil application in pot trials. Per

cent disease reduction through seed dressing was 33% to 50% over control and through direct soil application it was up to 72% to 83%.

Table 4.48: Effect of seed and soil treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) against collar rot of lentil.

S.No.	Tretment	Type of Treatment	Doses	Per cent Mortility*	Per cent Disease over Control
1	T1	Seed Treatment	10g/kg Seed	32.22(34.56)	62.22
2	T2	Seed Priming	10g/kg Seed soaked for 2 hr	36.33(36.91)	58.44
3	T3	Soil Treatment	50g/kg Soil	33.33(35.24)	61.64
4	T4	Seed + Soil Treatment	10g/kg Seed + 50g/kg Soil	18.14(24.88)	79.11
5	T5	Seed Priming+ soil Treatment	10g/kgSeed Soaked for 2hr + 50g/kg Soil	22.22(27.60)	74.43
6	T6	Control(Untreated)		86.90(72.57)	0
		SEm ±		4.34	
		CD (5%)		13.54	

*Average of three replication
Figures in parentheses are angular transformation

4.8.7.2 Seed treatment and soil application with fluorescent *Pseudomonas*

The data on effect of seed and soil treatment with potential antagonist fluorescent *Pseudomonas* formulation (*Pf*-17) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 4.50

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Combined application of seed priming + soil application (T5) of potential antagonist fluorescent *Pseudomonas* talc based formulation (*Pf*-17) exhibited significant minimum mortality 10.00 per cent (87.18 per cent decreased over control) followed by Seed priming (T2) (mortality 13.70 per cent), Seed + soil treatment (T4) (mortality 14.44 per cent) with 82.43 and 81.48 per cent mortality over control respectively as compared to soil treatment (T2) (mortality 19.07 per cent) and Seed treatment (T1) (mortality 25.93 per cent) with 75.55 and 66.75 per cent mortality, respectively over control.

The treatment with potential antagonist fluorescent *Pseudomonas* formulation (*Pf*-26) use in different forms also affect germination and vigour index of lentil under glass house condition.

Table 4.49: Effect of seed and soil treatment with potential antagonist *Trichoderma* spp. formulation (*Tricho*-9) on vigour index of lentil.

S. No.	Treatment	Types of treatment	Germination (%)*	Root Length(cm)**	Shoot length(cm)**	Vigour index
1	T1	Seed treatment	93.33 (9.7)	21.00	19.60	3789.20
2	T2	Seed Priming	83.33 (9.1)	20.20	25.60	3816.51
3	T3	Soil treatment	90.00 (9.5)	24.00	27.00	4590.00
4	T4	Seed + soil treatment	96.66 (9.8)	23.60	22.00	4722.50
5	T5	Seed Priming+ soil treatment	86.66 (9.3)	23.00	22.00	4050.00
6	T6	Control (Untreated)	76.66 (8.8)	15.40	16.40	2331.89
	SEm ±		0.163	1.288	1.186	
	CD (5%)		0.507	3.783	3.696	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

The data presented in Table 4.51revealed that all the treatment enhanced germination per cent but highest was observed in the seed priming + soil treatment (T5) that is 100 per cent followed by seed priming (T2) (96.67 per cent), seed + soil treatment (T4) (93.33 per cent) and seed, soil treatment (90 per cent) The above treatments were found to be significantly superior over control (76.67 per cent).The treatment seed priming (T4) was found best in increasing vigour index 4930.17 followed by seed priming + soil (T5) that is 4540.00.

In agreement to above findings, Usharani *et al*. (2009) conducted pot experiment to evaluate different delivery system of *P. fluorescens* for the management of Fusarial wilt of tomato and revealed that FYM enriched with *P. fluorescens* as seed and soil application was very effective in minimizing wilt incidence. Rakh *et al.* (2011) conducted experiment in pot assay for control of *Sclerotium rolfsii*. *Pseudomonas cf. monteilii 9* treated seeds showed decrease in incidence of disease up to 45.45 to 66.67% in comparison to untreated seeds.

Table 4.50: Effect of seed and soil treatment with potential antagonist Fluorescent *Pseudomonas* formulation (*Pf*-26) against collar rot of

S.No.	Treatment	Type of treatment	Doses	Per cent Mortility	Per cenr Decrease Over Control
1	T1	Seed Treatment	10g/kg Seed	25.92(30.49)	66.75
2	T2	Seed Priming	10g/kg Seed soaked for 2 hr	13.70(21.48)	82.43
3	T3	Soil Treatment	50g/kg Soil	19.07(25.5)	75.55
4	T4	Seed + Soil Treatment	10g/kg Seed + 50g/kg Soil	14.44(22.00)	81.48
5	T5	Seed Priming+ soil Treatment	10g/kgSeed Soaked for 2hr+ 50g/kg Soil	10.00(18.42)	87.18
6	T6	Control(Untreated)		77.97(62.30)	0
		SEm ±		2.799	
		CD (5%)		8.72	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.51: Effect of seed and soil treatment with potential antagonist Fluorescent *Pseudomonas* formulation (*Pf*-26) on vigour index of lentil.

S.No.	Treatment	Types of Treatment	Germination %	Root Length (cm)**	Shoot Lengh(cm)**	Vigour
1	T1	Seed treatment	90.00(9.5)	25.4	22.4	4302
2	T2	Seed Priming	96.66(9.8)	30.2	20.8	4930.17
3	T3	Soil treatment	90.00(9.5)	24.4	24.4	4410
4	T4	Seed + soil treatment	93.33(9.7)	22	23.4	4237.18
5	T5	Seed Priming +soil treatment	100.00(10.05)	25.8	19.6	4540
6	T6	Control (Untreated)	76.66(8.8)	16.2	15.2	2407.44
	SEm ±		0.176	0.987	0.594	
	CD (5%)		0.549	2.897	1.745	

* Average of three replication, **Average of five replication Figures in parentheses are square root transformation

4.8.7.3. Seed treatment and soil application with *Bacillus* spp.

The data on effect of seed and soil treatment with potential antagonist *Bacillus* spp. formulation (*Bs*-6) against collar rot of lentil caused by *S. rolfsii* under glass house condition are presented in Table 4.52

The per cent mortality of lentil in sick pots after 45 days of challenge inoculation with *S. rolfsii* (SR4) was recorded. All the treatments were significantly superior, in decreasing the incidence of collar rot disease in lentil, over control. Combined application of seed priming + soil application (T5) of potential antagonist *Bacillus* spp. talc formulation (*Bs*-6) exhibited significant minimum mortality 21.48 per cent (72.24 per cent decreased over control) followed by seed+ soil treatment (T4) (mortality 25.19 per cent), seed priming treatment (T2) (mortality 26.85 per cent) with 67.45 and 65.30 per cent mortality, respectively over control as compared to soil treatment (T3) (mortality 33.33 per cent) and seed treatment (T1) (mortality 36.11 per cent), alone in comparision to control (77.38 per cent).

The treatment with potential antagonist *Bacillus* spp. formulation (*Bs*-21) used in different forms also affect germination and vigour index of lentil under glass house condition.

The data presented in Table 4.53 revealed that the seed priming and soil treatment (T5) is found best in increasing vigour index (4237.18) as well as germination (93.33 per cent) as compared to the other treatments and untreated control.

Shifa *et al.* (2015) reported that seed treatment with the powder formulation of *B. subtilis* G-1 alone was effective in controlling stem rot disease as compared to control but combined seed treatment and soil application significantly increased the plant height and reduced the incidence of stem rot up to 93% under greenhouse conditions. Vishwanath *et al.* (2012) observed that *B. subtilis* as seed treatment was more effective in disease control by producing minimum collar rot incidence (10-17 %) in sunflower caused by *S. rolfsii* and significantly increase in seedling emergence, plant stand and biomass.

Table 4.52: Effect of seed and soil treatment with potential antagonist *Bacillus* spp. formulation (*Bs*-21) against collar rot of lentil.

S.No.	Treatment	Type of treatment	Doses	Per cent Mortility*	Per cenr Decrease Over Control
1	T1	Seed treatment	10g/kg Seed	36.11(36.91)	53.33
2	T2	Seed Priming	10g/kg Seed soaked for 2 hr	26.85(31.11)	65.3
3	T3	Soil treatment	50g/kg Soil	33.33(35.16)	56.93
4	T4	Seed + soil treatment	10g/kg Seed + 50g/kg Soil	25.19(29.97)	67.45
5	T5	Seed Priming +soil treatment	10g/kgSeed Soaked for 2hr+ 50g/kg Soil	21.48(27.59)	72.24
6	T6	Control (Untreated)		77.38(61.80)	0
	SEm ±			2.351	
	CD (5%)			7.324	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.53: Effect of seed and soil treatment with potential antagonist *Bacillus* spp. formulation (*Bs*-21) on vigour index of lentil.

S.No.	Treatment	Type of treatment	Germination %	Root Length (cm)**	Shoot Lengh(cm)**	Vigour Index
1	T1	Seed treatment	83.33 (9.1)	23.4	21.2	3716.52
2	T2	Seed Priming	86.67 (9.3)	19.4	22	3588.14
3	T3	Soil treatment	80.00 (9.0)	19.2	21.2	3232
4	T4	Seed + soil treatment	93.33 (9.7)	19.6	20.6	3751.87
5	T5	Seed Priming +soil treatment	93.33 (9.7)	24.4	21	4237.18
6	T6	Control (Untreated)	73.33 (8.6)	14.4	14.8	2141.24
	SEm ±		0.163	0.539	0.772	
	CD (5%)		0.507	1.581	2.268	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

4.9. Evaluation of fungicides against *S. rolfsii*

4.9.1. *In vitro* evaluation of fungicides against *S. rolfsii* by poison food technique

The efficacy of seven systemic fungicides(Carbendazim 50% WP, Tricyclozole 75% WP, Hexaconazole 5% EC, Propiconazole 25% EC, Azoxystrobin 35% EC, Benomyl 50% WP and Thiophanate Methyl 70% WP), four non-systemic fungicides (Mancozeb 75%WP, Thiram 75% WS, Copper oxychloride, and Propineb 70% WP) and six combo fungicides (Metalaxyl 8% +Mancozeb 64% , Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol, Carboxin 37.5% + Thiram 37.5% and Carbendazim 12%+ Mancozeb 63% WP) were evaluated *in vitro* at different concentrations of 20, 50, 100,200 and 500 ppm against *Sclerotium rolfsii* on potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 1982).

The data presented in Table 4.75 revealed that among the seven systemic fungicides tested, Hexaconazole 5% EC and Propiconazole 25% EC were found highly effective at all concentrations with 100 per cent inhibited mycelial growth of *S. rolfsii.* Azoxystrobin 35% EC also inhibited the mycelial growth 60.83 per cent at 20 ppm, 78.46 per cent at 50 ppm and 100 per cent at 100, 200 and 500 ppm while, Tricyclozole 75% WP inhibited mycelial growth 68.88 per cent at 50 ppm, 70.27 per cent at 100 ppm and 100 per cent at 200 and 500 ppm. The other fungicides namely Carbendazim 50% WP (66.24 per cent) and Benomyl 50% WP (72.21 per cent) were found to inhibited mycelial growth at 500 ppm. Thiophanate Methyl 70% WP was found to be least effective in inhibiting mycelial growth of pathogen (37.49 per cent) at 500 ppm concentration.

Table 4.54: Effect of systemic fungicides on per cent inhibition of radial growth of *S. rolfsii* at different concentrations (ppm).

Fungicide/	Per cent inhibition of mycelial growth of *S. rolfsii* *					Mean
Treatments	20	50	100	200	500	
T1:Carbendazim 50%WP	0.00 (2.97)	0.00 (2.97)	9.72 (17.25)	16.24 (23.73)	66.24 (54.48)	18.44
T2:Tricyclazole 75%WP	11.11 (19.46)	68.88 (56.08)	70.27 (56.95)	100.00 (86.93)	100.00 (86.93)	70.00
T3:Hexaconazole 5%EC	100.00 (86.93)	100.00 (86.93)	100.00 (86.93)	100.00 (86.93)	100.00 (86.93)	100.00
T4:Propiconazole 25%EC	100.00 (86.93)	100.00 (86.93)	100.00 (86.93)	100.0 (86.93)	100.0 (86.93)	100.00
T5: Azoxystrobin 35% EC	60.83 (51.27)	78.46 (62.37)	100.00 (86.93)	100.00 (86.93)	100.00 (86.93)	87.85
T6: Benomyl 50% WP	5.82 (13.95)	7.08 (15.34)	27.91 (31.79)	33.33 (35.24)	72.21 (58.45)	29.27
T7:Thiophanate methyl 70%WP	3.88 (10.59)	8.05 (16.34)	17.77 (24.74)	28.05 (31.96)	37.49 (37.73)	19.05
T8: Control	0.00	0.00	0.00	0.00	0.00	
Mean	40.23	51.74	60.69	68.23	82.28	
		Fungicide	Concentration	F × C		
SEm±		0.527	0.445	1.178		
C.D at 5%		1.480	1.251	3.309		

*Average of four replication

Figures in parentheses are angular transformation

These results were supported by several previous workers namely Chowdhury *et al.* (1998); Virupaksha Prabhu and Hiremath (2003) and Arunasri *et al*. (2011); who reported that the Triazoles (Hexaconazole, Propiconazole, Difenconazole) were highly effective to inhibit the growth of *S. rolfsii*. Whereas, Johnson and Subramanyam (2000) found carbendazim least effective against *S. rolfsii*. Manu *et. al.* (2012) reported that Hexaconazole, Tebuconazole & Propiconazole were found to be having strong inhibitory effect on the growth of *S. rolfsii* isolated from finger millet at lower concentration. Das *et al.* (2014) reported that Hexaconazole & Tebuconazole were highly effective at all the concentrations against *S. rolfsii* followed by Propiconazole and mycobutnil. Least inhibition was observed in Thiophanate methyl and Bavistin.

The data presented in Table 4.76 revealed that among the four non-systemic fungicides Thiram 75%WS was highly effective at higher concentration as it inhibited the *S. rolfsii* by 55.55 per cent at 20 ppm, 86.80 per cent at 50 ppm

and 100 per cent at 100, 200 and 500 ppm concentrations. Mancozeb75%WP also inhibited the mycelial growth of pathogen (100 per cent) at 100, 200 and 500 ppm, while Propineb70%WP inhibited (75.41 per cent) at 100 ppm and 100 per cent at 200 as well as 500 ppm concentrations. Copper oxychloride 50 WP was found to be least effective in inhibiting the growth of *S. rolfsii* as it inhibited only 5.83 per cent at 500 ppm concentration.

These results were in accordance with the results of Sujatha (1991) and Johnson and Subramanyam (2000). Das *et al.* (2014) reported that Mancozeb75%WP and Captan showed higher inhibitory effect as compared to Copper oxychloride. Dutta and Das (2002) studied the efficacy of thiram and mancozeb at 0.1 per cent concentration against tomato isolate of *S. rolfsii in vitro* and reported that thiram inhibited 70.3 per cent mycelial growth and 96.5 per cent sclerotial production of *S. rolfsii*. Khan and Javaid (2015) reported that Mancozeb fungicide significantly declined the *S. rolfsii* growth at various concentrations by 99-100% over control.

Fig 4.30: ***In vitro*** **evaluation of systemic fungicide in different concentration against** ***S. rolfsii***

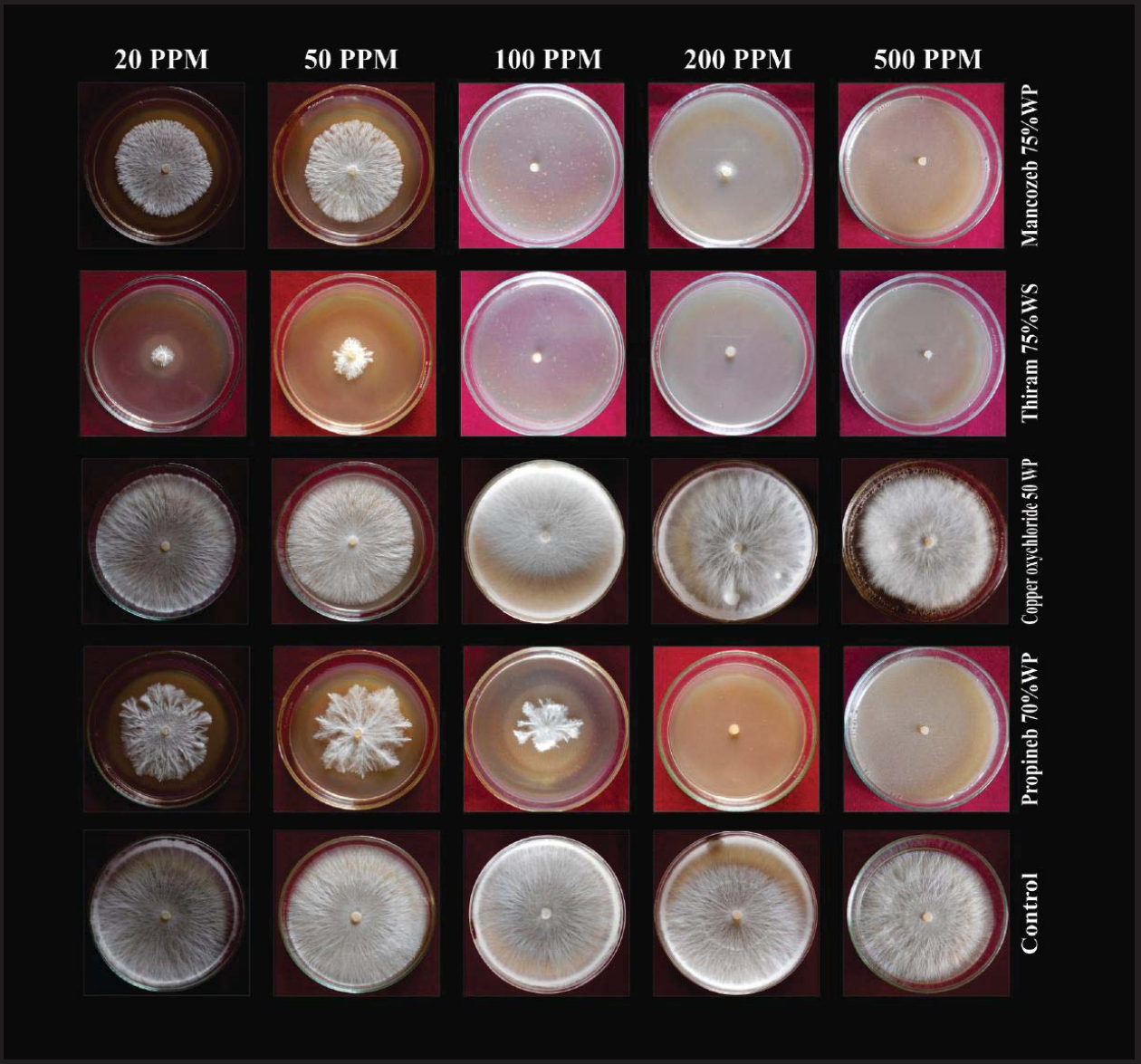


Fig. 4.31 ***In vitro*** **evaluation of non systemic fungicidfes in different concentration against** ***S. rolfsii***

Fig. 4.32 .*In vitro* evaluation of combined fungicide in different concentration against *S.rolfsii*

Table 4.55: Effect of Non-systemic fungicide on per cent inhibition of radial growth of *S. rolfsii* at different concentrations (ppm).

Fungicide/	Per cent inhibition of mycelial growth of *S. rolfsii* *					Mean
Treatments	20	50	100	200	500	
T1: Mancozeb 75%WP	33.32 (35.16)	39.85 (39.09)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	74.63
T2: Thiram 75%WS	55.55 (48.59)	86.80 (73.00)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	88.47
T3: Copper oxychloride 50 WP	0.00 (2.97)	0.00 (2.97)	0.00 (2.97)	0.00 (2.97)	5.83 (13.95)	1.16
T4: Propineb 70%WP	20.82 (26.80)	34.44 (35.87)	75.41 (60.26)	100.0 (86.93)	100.00 (86.93)	66.13
T5:Control	0.00	0.00	0.00	0.00	0.00	
Mean	27.42	40.27	68.85	75.00	76.45	
		Fungicide	Concentration	F × C		
SEm±		1.104	1.235	2.470		
C.D at 5%		3.132	3.502	7.004		

*Average of four replication
Figures in parentheses are angular transformation

The data presented in Table 4.56 revealed that out of the six combo fungicides, four *viz*., Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% were found to be highly effective at all the concentrations with 100 per cent inhibition in mycelial growth of *S. rolfsii*. Metalaxyl 8%+ Mancozeb 64% also inhibited the mycelial growth of pathogen 48.88 per cent at 20 ppm, 53.86 per cent at 50 ppm and 100 per cent at 100, 200, 500 ppm concentrations. Carbendazim12%+ Mancozeb 63%was found to be least effective in lower concentrations but at higher concentration (500 ppm), showed 100 per cent inhibition of mycelial growth of *S. rolfsii*. These results are in agreement with the findings of Virupaksha Prabhu and Hiremath (2003) and Arunasri *et al*. (2011) who reported that the combo products containing triazoles *viz.*, Avatar, Merger and Nativo were highly inhibitive to the growth of *Sclerotium rolfsii*. Vyas and Joshi (1977), Sujatha (1991) and Manu *et al.* (2012) reported that carboxin was highly effective against *Sclerotiumrolfsii*. Das *et al.* (2014) also reported that Carboxin 37.5% + Thiram 37.5% is found to be highly inhibitory on the growth of *S. rolfsii*.

Thus, it is concluded that Systemic fungicides like, Hexaconazole 5% EC, Propiconazole 25% EC and combi products Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% showed complete inhibition of the pathogen at all the concentrations. Whereas, the non systemic fungicides namely Mancozeb 75%WP, Thiram 75% WS and Propineb 70% WP were found inhibitive only at higher concentrations against *S. rolfsii* under *in vitro* condition.

Table 4.56: Effect of combo fungicides on per cent inhibition of radial growth of *S. rolfsii* at different concentrations (ppm).

Fungicide/ Treatments	Per cent inhibition of mycelial growth of *S. rolfsii* *					Mean
	20	50	100	200	500	
T1: Metalaxyl 8%+ Mancozeb 64% WP	48.88 (44.35)	53.86 (47.21)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	80.55
T2:Tebuconzole 50%+ Trifloxystrobin 25% WG	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
T3: Captan 70% + Hexaconazole 5%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
T4:Propiconazole 13.9% +Difenoconazole13.9%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
T5: Carboxin 37.5% +Thiram 37.5%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
T6: Carbendazim 12% + Mancozeb 63%	15.69 (23.27)	31.10 (33.87)	39.16 (38.72)	52.77 (46.57)	100.0 (86.93)	47.74
T7:Control	0.00	0.00	0.00	0.00	0.00	
Mean	77.42	80.82	89.86	92.12	100.0	
		Fungicide	Concentration	F × C		
SEm±		0.303	0.277	0.678		
C.D at 5%		0.853	0.779	1.908		

*Average of four replication

Figures in parentheses are angular transformation

4.9.2 *In vivo* efficacy of fungicides as seed treatment against collar rot of lentil under glass house condition

The experiment was conducted to find out an effective fungicidal seed treatment in which seven systemic fungicides (Carbendazim 50% WP, Tricyclozole 75% WP, Hexaconazole 5% EC Propiconazole 25% EC, Azoxystrobin 35% EC, Benomyl 50% WP and Thiophanate Methyl 70% WP); four non-systemic fungicides (Mancozeb 75%WP, Thiram 75% WS, Copper

oxychloride, and Propineb 70% WP) and six combo fungicides (Metalaxyl 8% +Mancozeb 64% , Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol, Carboxin 37.5% + Thiram 37.5% and Carbendazim 12% + Mancozeb 63% WP) were tested against collar rot disease of lentil and their effect on germination, shoot length, root length and vigour index of plant under sick pot with challenge inoculation of *S. rolfsii* (SR4) under glass house condition.

The data on effect of seed treatment with systemic fungicides against collar rot of lentil under glass house condition are presented in Table 4.57

All the treatments were significantly superior in decreasing the incidence of collar rot in lentil over control. Seed treatment with Hexaconazole 5% EC (T3), Propiconazole 25% EC (T4) and Azoxystrobin 35% EC (T5) exhibited zero mortality and 100 per cent decrease in disease incidence over control. Whereas, the seed treatment with Benomyl 50% WP (mortality 20.37 per cent) and Thiophanate Methyl 70% WP (mortality 36.94 per cent) showed 75.20 and 55.03 per cent decrease in disease incidence, respectively over control.

Maximum mortality was observed in treatment with Carbendazim 50% WP (T1) that is 69.84 per cent followed by Tricyclazole 75%WP (57.97 per cent) as compared to control (82.14 per cent).

The influence of seed treatment with systemic fungicides on germination per cent and vigour index in lentil inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.58.

Highest germination per cent was observed in seed treatment with fungicide Propiconazole 25% EC (T4) and Azoxystrobin 35% EC (T5) that is 100 per cent followed by Benomyl (T6) (96.66 per cent). Seed treatment with Hexaconazole 5% EC (T3) was found best in increasing vigour index (3330) followed by Benomyl (3286.78) and Propiconazole 25% EC (3220).

Table 4.57: Effect of seed treatment with systemic fungicides against collar rot of lentil.

S. No.	Treatment	Percent mortality*	Per cent decrease over control
1	T1: Carbendazim 50% WP	69.84 (57.18)	14.97
2	T2: Tricyclazole 75% WP	57.97 (49.70)	29.41
3	T3: Hexaconazole 5% EC	0.00 (0.906)	100.00
4	T4: Propiconazole 25% EC	0.00 (0.906)	100.00
5	T5: Azoxystrobin 35% EC	0.00 (0.906)	100.00
6	T6: Benomyl 50% WP	20.37 (7.091)	75.20
7	T7: Thiophanate methyl 70%WP	36.94 (13.34)	55.03
8	T8: Control (Untreated)	82.14 (65.16)	0.00
	SEm±	4.882	
	C.D at 5%	14.76	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.58: Effect of seed treatment with systemic fungicides on vigour index of lentil.

S. No.	Treatment	Germination (%)*		Root Length (cm)**	Shoot length (cm)**	Vigour index
1	T1: Carbendazim 50% WP	63.33	(8.0)	16.20	17.66	2144.35
2	T2: Tricyclazole 75% WP	83.33	(9.1)	17.90	17.16	2921.55
3	T3: Hexaconazole 5% EC	100.0	(10.0)	15.00	18.30	3330.0
4	T4: Propiconazole 25% EC	100.0	(10.0)	16.10	16.10	3220.0
5	T5: Azoxystrobin 35% EC	100.0	(10.0)	14.60	15.70	3030.0
6	T6: Benomyl 50%WP	96.66	(9.8)	15.00	19.00	3286.78
7	T7:Thiophanate methyl 70%WP	90.00	(9.5)	16.20	16.40	2934.0
8	T8: Control (Untreated)	73.33	(8.6)	14.00	16.00	2199.9
	SEm±	0.231		0.579	0.497	
	C.D at 5%	0.697		1.679	1.438	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

The data on effect of seed treatment with four non-systemic fungicides against collar rot of lentil under glass house condition are presented in Table 4.80 All the treatments were significantly superior, in decreasing the incidence of collar rot in lentil, over control. Seed treatment with fungicide Propineb 70%WP (T4) exhibited significant minimum mortality 10.37 per cent (87.99 per cent decreased over control) followed by seed treatment with Mancozeb 75%WP (mortality 16.66

per cent) and Thiram 75%WS (mortaliy 20 per cent) with 79.71 and 75.65 per cent mortality, respectively over control. Whereas maximum mortality was observed in seed treatment with Copper oxychloride (T3) that is 73.21 per cent as compared to control (82.14 per cent).

The influence of seed treatment with non-systemic fungicides on germination per cent and vigour index in lentil when challenge inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.59

Highest germination per cent was observed in seed treatment with Propineb 70%WP (T4) and Mancozeb 75%WP (T1) that is 96.66 per cent. The seed treatment with Propineb 70%WP (T4) found best in increasing vigour index (3712.1) followed by Mancozeb 75%WP (3586.53).

*Average of three replication
Figures in parentheses are angular transformation

Table 4.59: Effect of seed treatment with non-systemic fungicides on vigour index of lentil.

S. No.	Treatment	Germination (%)*	Root Length (cm)**	Shoot length (cm)**	Vigour index
1	T1: Mancozeb 75%WP	96.66 (9.8)	17.10	20.40	3586.5
2	T2: Thiram 75%WP	86.66 (9.3)	14.40	18.80	2877.4
3	T3:Copper oxychloride 50%WP	73.33 (8.6)	19.60	19.60	2874.5
4	T4: Propineb 70%WP	96.66 (9.8)	18.20	20.20	3712.1
5	Control (Untreated)	73.33 (8.6)	14.00	16.00	2199.9
	SEm±	0.225	0.651	0.590	
	C.D at 5%	0.719	1.934	1.753	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

The data on effect of seed treatment with six combo fungicides against collar rot of lentil under glass house condition are presented in Table 4.61

All the treatments were significantly superior in decreasing the incidence of collar rot in lentil over control. Seed treatment with Tubaconzole 50%+Trifloxystrobin 25% WG (T2), Captan 70%+ Hexaconazole 5% WP (T3), Propiconazole 13% + Difenoconazol (T4) and Carboxin 37.5% + Thiram 37.5%

(T5) exhibited zero mortality that is 100 per cent decrease over control. Whereas, the seed treatment with Metalaxyl 8% +Mancozeb 64% (T1) (mortality 29.81 per cent) and Carbendazim 12% + Mancozeb 63% WP (T6) (mortality 38.42 per cent) showed 63.71 and 53.21 per cent decrease, respectively over control.

The influence of seed treatment with combo fungicides on germination per cent and vigour index in lentil inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.61

Highest germination per cent was observed in seed treatment with Tubaconzole 50%+Trifloxystrobin 25% WG (T2), Captan 70%+ Hexaconazole 5% WP (T3), and Carboxin 37.5% + Thiram 37.5% (T5) that is 100 per cent followed by Propiconazole 13% + Difenoconazol (T4) (96.66 per cent). Seed treatment with Carboxin 37.5% + Thiram 37.5% (T5) and Tubaconzole 50%+Trifloxystrobin 25% WG (T2) found best in increasing vigour index that is 3710 and 3700 respectively.

Table 4.60: Effect of seed treatment with combo fungicides against collar rot of lentil.

S. No.	Treatment	Percent mortality*	Per cent decrease over control
1	T1: Metalaxyl 8%+ Mancozeb 64%	29.81 (32.77)	63.71
2	T2:Tebuconzole 50%+Trifloxystrobin 25%	0.025 (0.906)	100.0
3	T3: Captan 70%+ Hexaconazole 5%	0.025 (0.906)	100.0
4	T4: Propiconazole 13.9% + Difenoconazole 13.9%	0.025 (0.906)	100.0
5	T5: Carboxin 37.5%+Thiram 37.5%	0.025 (0.906)	100.0
6	T6: Carbendazim 12%+ Mancozeb 63%	38.42 (38.26)	53.21
7	T7: Control (Untreated)	82.14 (65.16)	
	SEm±	2.127	
	C.D at 5%	6.513	

*Average of three replication
Figures in parentheses are angular transformation

Table 4.61 : Effect of seed treatment with combo fungicides on vigour index of lentil.

S. No.	Treatment	Germination (%)*	Root Length (cm)**	Shoot length (cm)**	Vigour index
1	T1: Metalaxyl 8%+ Mancozeb 64%	90.0 (9.5)	18.00	22.20	3618.0
2	T2:Tebuconzole 50%+ Trifloxystrobin25%	100.0 (10.0)	18.20	18.80	3700.0
3	T3: Captan 70%+ Hexaconazole5%	100.0 (10.0)	15.40	18.60	3400.0
4	T4:Propiconazole 13.9% + Difenoconazole 13.9%	96.67 (9.8)	16.20	18.80	3383.5
5	T5: Carboxin37.5 %+Thiram37.5%	100.0 (10.0)	17.30	19.80	3710.0
6	T6: Carbendazim 12%+ Mancozeb 63%	86.67 (9.3)	16.40	18.40	3016.1
7	T7: Control (Untreated)	73.33 (8.6)	14.00	16.00	2199.9
	SEm±	0.165	0.366	0.619	
	C.D at 5%	0.504	1.067	1.802	

* Average of three replication

** Average of five replication

Figures in parentheses are square root transformation

Charde *et al.* (2002) reported that seed treatment with propiconazole and hexaconazole were superior in checking stem rot of groundnut caused by *S. rolfsii* and increasing the shoot and root length. Seedling root dip in mancozeb (0.1%) and thiram (0.1%) effectively reduced the collar rot of tomato caused by *S. rolfsii* (Dutta and Das, 2002). Seed treatment of lentil with hexaconazole and propiconazole inhibited *S. rolfsii*. These fungicides were found to be absorbed by roots and translocated to shoot and leaf length. (Tajane *et al.*, 2002).

4.9.3 *In vitro* determination of compatibility of biocontrol agents *Trichoderma* spp., fluorescent *Pseudomonas*, *Bacillus* spp., with fungicides.

4.9.3.1: *In vitro* determination of compatibility of *Trichoderma* spp. (*Tricho*-9) with fungicides.

The experiment was conducted *in vitro* determination of compatibility of *Trichoderma* spp. (Tricho-19) with selected fungicides (Hexaconazole 5% EC, Propiconazole 25% EC, Mancozeb 75% WP, Thiram 75% WS, Propineb 70% WP, Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% + Thiram 37.5%) at different concentrations (100, 200 and 500 ppm) on potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 1982).

The data presented in Table 4.84 revealed that among the seven fungicides tested for compatability with *Trichoderma* spp. (Tricho-9) *in vitro* at different concentrations (100, 200 and 500 ppm), only Propineb 70% WP was found to be highly compatible with *Trichoderma* spp. (*Tricho*-9) at all concentrations (100, 200 and 500 ppm). While, Mancozeb 75% WP (T3), Thiram 75% WS (T4), Metalaxyl 8% +Mancozeb 64% (T6) and Carboxin 37.5% + Thiram 37.5% (T7) were found to be least compatible at high concentration (500 ppm) showing 60.37, 66.67,67.41 and 70.00 per cent inhibition of radial growth respectively. Hexaconazole 5% EC (T1) and Propiconazole 25% EC (T2) was incompatible with *Trichoderma* spp. showing 100 per cent inhibition of radial growth at all concentrations.

Thus, it is concluded that fungicide like Propineb 70% WP were found to be compatible and comparatively safer with *Trichoderma* spp. (*Tricho*-9) as compared to other fungicides.

Sarkar *et al*. (2010) reported that among the systemic fungicides, hexaconazole was the most toxic, followed by propiconazole and triflumizole with *T. harzianum*. Toxicity of the non systemic fungicides was lower than that of the systemic fungicides, among which copper oxychloride and copper hydroxide were highly compatible with no inhibition at lower concentrations. Bagwan (2010) studied that compatability tests under *in vitro* condition to find out safer fungicides, pesticides, different cakes and botanicals against *Trichoderma.* Results indicate that among the fungicides, thiram (0.2%), copper oxychloride (0.2%) and

mancozeb (0.2%) were found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride* as compared to other fungicides. *Trichoderma* was most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil .

Table 4.62: Effect of fungicide on the mycelial growth of *Trichoderma* spp. (*Tricho*-9) at different concentrations.

S.No.	Fungicide	Per cent inhibition of mycelial growth of *Trichoderma* spp. (*Tricho* -9)		
		100	**200**	**500**
1	T1:Hexaconazole 5%EC	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
2	T2:Propiconazole 25%EC	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
3	T3:Mancozeb 75%EC	45.18 (42.21)	51.11(45.61)	60.37 (50.96)
4	T4:Thiram 75% ES	0.00 (0.906)	15.55(23.19)	66.67 (54.71)
5	T5:Propineb 70%WP	0.00 (0.906)	0.00 (0.906)	0.00 (0.906)
6	T6:Metalaxy 8%+ Mancozeb 64%	34.81 (36.14)	45.92(42.64)	67.41(55.167)
7	T7:Carboxin 37.5%+ Thiram 37.5%	0.00 (0.906)	49.63(42.77)	70.00 (56.78)
	SEm±	0.234	0.452	0.441
	C.D at 5%	0.717	1.385	1.351

*Average of three replication
Figures in parentheses are angular transformation

4.9.3.2: *In vitro* determination of compatibility of fluorescent *Pseudomonas* and *Bacillus* spp., with fungicides.

The experiment was conducted *in vitro* determination of compatibility of bacterial bioagents fluorescent *Pseudomonas*, *Bacillus* spp. with fungicides (Hexaconazole 5% EC, Propiconazole 25% EC, Mancozeb 75% WP, Thiram 75% WS, Propineb 70% WP, Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% + Thiram 37.5%) at different concentrations (100, 200 and 500 ppm) on King's B medium (KBM) using poisoned food technique (Nene and Thapliyal, 1982).

The data presented in Table 4.63 revealed that among the seven fungicides tested for compatability with fluorescent *Pseudomonas* (*Pf* -26) *in vitro* at different concentrations (100, 200 and 500 ppm) Hexaconazole 5% EC, Propiconazole 25% EC and Propineb 70% WP were found to be highly compatible with fluorescent *Pseudomonas* (*Pf* -17) and observed good growth (+++) at all concentrations (100, 200 and 500 ppm). While, Mancozeb 75% WP (T3), Thiram 75% WS (T4), Metalaxyl 8% +Mancozeb 64% (T6) and Carboxin 37.5% + Thiram 37.5% (T7)

were found to be compatible at low concentration (100 ppm) but least compatible at high concentration (500 ppm).

Similar results were also obtained by Louis *et al*. (2016). They reported that seven fungicides viz., propiconazole, hexaconazole, tebuconazole, difenconazole, azoxystrobin, carbendazim and famoxadone + cymoxanil were compatible at all the concentrations. Kresoxim methyl was less compatible and mancozeb, copper oxy chloride and copper hydroxide were not compatible with *P. fluorescens*.

Table 4.63: Compatibility of fluorescent *Pseudomonas* (*Pf* -26) with selected fungicides at different concentrations.

S. No.	Fungicide	Compatibility of fluorescent *Pseudomonas*(*Pf* -26) *		
		100	**200**	**500**
1	T1: Hexaconazole 5%EC	+++	+++	+++
2	T2: Propiconazole 25%EC	+++	+++	+++
3	T3: Mancozeb 75% WP	+++	++	+
4	T4: Thiram 75%WS	+++	+++	++
5	T5: Propineb 70% WP	+++	+++	+++
6	T6: Metalaxyl 8%+ Mancozeb 64%	+++	++	+
7	T7: Carboxin 37.5%+Thiram 37.5%	+++	+++	++

(-): no growth, (+): poor growth, (++): moderate growth, (+++): good growth
*Average of three replication

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The data presented in Table 4.64 revealed that among the seven fungicides tested for compatability with *Bacillus* spp. (*Bs*-21) *in vitro* at different concentrations (100, 200 and 500 ppm), only Propineb 70% WP (T5) was found to be compatible at low concentration (100 ppm) but least compatible at high concentration (500 ppm). Whereas, Hexaconazole 5% EC (T1), Propiconazole 25% EC (T2), Mancozeb 75% WP (T3), Thiram 75% WS (T4,) Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% +Thiram 37.5% were incompatible with *Bacillus* spp. (*Bs*-6) showing 100 per cent inhibition of growth at all concentration.

Basamma and Kulkarni (2017) observed that Mancozeb, Metalaxyl MZ, Tebuconazole were less compatible with *B. subtilis.* Whereas, Carbendazim, Hexaconazole and Difenconazole showed maximum tolerance limit upto their

recommended concentration for disease management and showed high compatibility with *B. subtilis*.

The present study clearly demonstrated that, the biocontrol agents *Trichoderma* spp. (*Tricho*-9), fluorescent *Pseudomonas* (*Pf* -126), and *Bacillus* spp., (*Bs*-21) are compatible with Propineb 70% WP fungicides under *in vitro* condition. Therefore, Propineb 70% WP fungicide can be used for combined application with biocontrol agents (*Trichoderma* spp. (*Tricho*-9), fluorescent *Pseudomonas* (*Pf* -26), and *Bacillus* spp., (*Bs*-21)) for integrated management of collar rot in lentil caused by *S. rolfsii*.

Table 4.65: Compatibility of *Bacillus* spp. (*Bs*-21) with selected fungicides at different concentrations.

S.No.	Fungicide	100	200	500
1	T1: Hexaconazole 5%EC	-	-	-
2	T2: Propiconazole 25% EC	-	-	-
3	T3: Mancozeb 75%WP	-	-	-
4	T4: Thiram 75%WS	-	-	-
5	T5: Propineb70% WP	+++	+++	++
6	T6: Metalaxyl 8%+Mancozeb 64% WP	-	-	-
7	T7:Carboxin37.5%+Thiram - 37.5%WP		-	-

(-): no growth, (+): poor growth, (++): moderate growth, (+++): good growth.
*Average of three replication

4.10. Integration of effective *Trichoderma* spp., fluorescent *Pseudomonas*, *Bacillus* spp., and fungicide seed treatment *in vivo* pot experiment

The experiment was carried out to assess the efficacy of an integrated management strategy for collar rot of lentil that combined the use of biocontrol agents (*Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp.) and fungicide under glass house condition.

The seed dressing talc powder formulation developed from highly effective isolate of *Trichoderma* spp. (*Tricho*-9), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*Bs*-21) was selected for evaluation along with fungicide Propineb 70% WP. These compatible treatments were evaluated alone and in combinations as seed treatment against the pathogen *S. rolfsii*. The data on effect of various seed treatment on collar rot incidence in lentil under glass house condition are presented in Table 4.66

All the treatments were significantly superior in decreasing the incidence of collar rot in lentil over control. Seed treatment with *Pf*- 26 + *Bs*-21 + Propineb 70%WP (T14) exhibited significant minimum incidence 13.70 per cent (83.08 per cent decreased over control) followed by treatment T15 (*Tricho*-9 + *Pf*- 26 + *Bs*-21 + Propineb 70%WP) (14.07 per cent), T 13(*Tricho*-9 + *Bs*-21 +Propineb 70%WP) (14.53 per cent), T12 (*Tricho*-9 + *Pf*- 26 + Propineb 70%WP)(15.83 per cent), T9 (*Pf*- 26 + Propineb 70%WP)(17.77 per cent), T7 (*Tricho*-9 + Propineb)(19.90 per cent) and T4 (Propineb 70%WP) (20.74 per cent) with 82.62, 82.04, 80.44, 78.04, 75.40 and 74.68 per cent decrease, respectively over control which were statistically at par with each other. These seed treatments showed significantly better results, over other seed treatments. Maximum collar rot incidence was observed in seed treatment T3 (*Bs*-6) (39.28 per cent) followed by T1 (*Tricho*-9) (34.25 per cent) and T6 (*Tricho*-19 + *Bs*-21) (32.50 per cent) against 80.95 % in control.

Table 4.65: Effect of various seed treatment on collar rot incidence in lentil.

S. No.	Treatment	Collar rot Incidence (%)*	Per cent Decrease over control
1	T1: *Tricho*-9	34.25 (35.69)	57.68
2	T2: *Pf*- 26	28.51 (32.19)	64.77
3	T3: *Bs*-21	39.28 (38.80)	51.46
4	T4: Propineb 70%WP	20.74 (27.08)	74.38
5	T5: *Tricho*-9 + *Pf*- 26	25.18 (29.98)	68.88
6	T6: *Tricho*-9 + *Bs*-21	32.50 (34.72)	59.85
7	T7: *Tricho*-9 + Propineb 70%WP	19.90 (26.27)	75.40
8	T8: *Pf*- 26 + *Bs*-21	27.38 (31.54)	66.18
9	T9: *Pf*- 26 + Propineb 70%WP	17.77 (24.72)	78.04
10	T10: *Bs*-9 + Propineb 70%WP	26.85 (31.12)	66.83
11	T11: *Tricho*-9 + *Pf*- 26 + *Bs*-21	24.07 (29.30)	70.27
12	T12: *Tricho*-9 + *Pf*- 26 + Propineb 70%WP	15.83 (23.30)	80.44
13	T13: *Tricho*-9 + *Bs*-21 +Propineb 70%WP	14.53 (22.24)	82.04
14	T14: *Pf*- 9 + *Bs*-21 + Propineb 70%WP	13.70 (21.49)	83.08
15	T15: *Tricho*-9 + *Pf*- 26 + *Bs*-21 + Propineb 70% WP	14.07 (21.83)	82.62
16	T16: Control (untreated seed)	80.95 (64.42)	0.00
	SEm ±	2.398	
	CD5%	6.911	

*Average of three replication
Figures in parentheses are angular transformation

Best isolate of bioagents

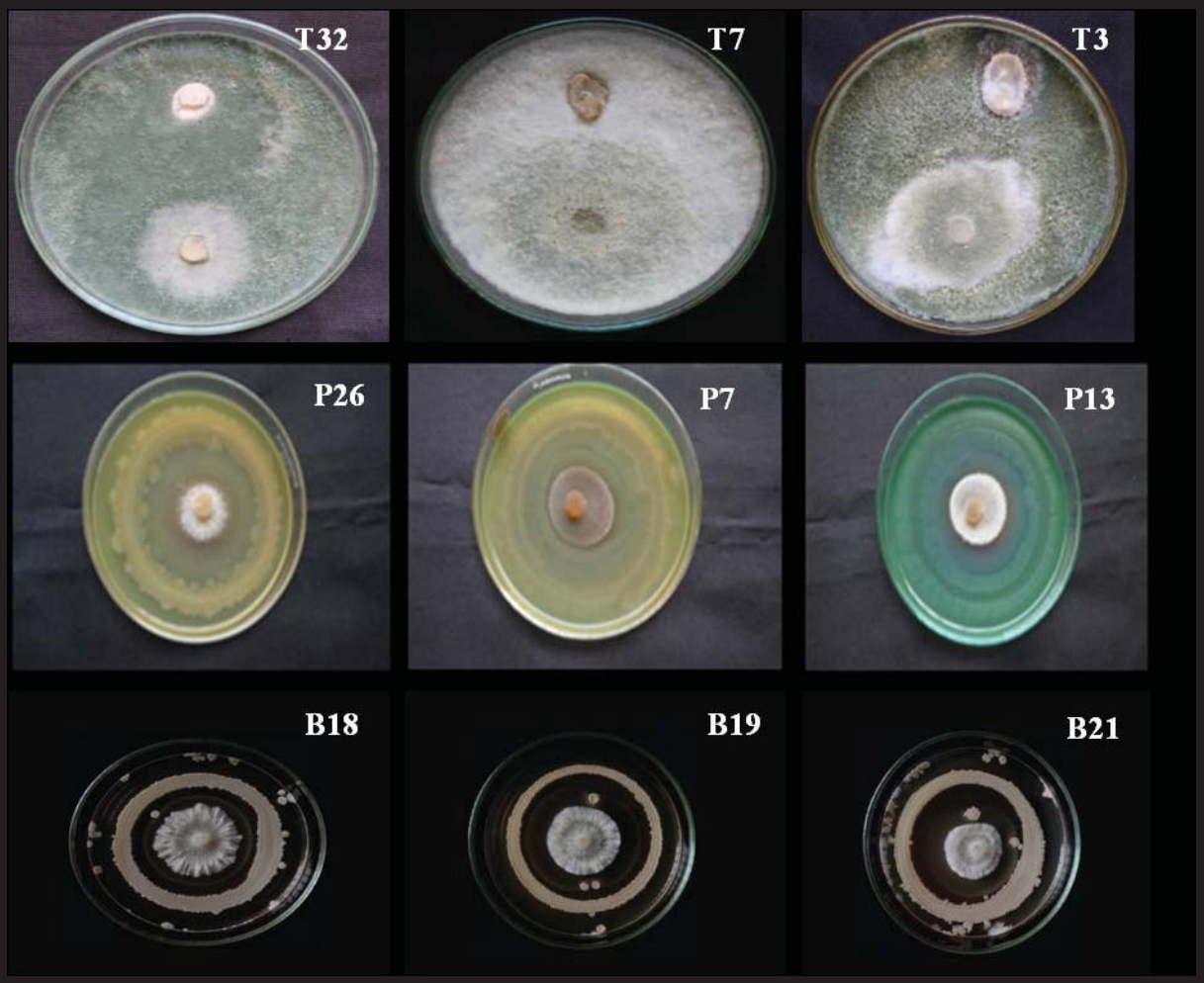

Table 4.66: Effect of various seed treatment on collar rot incidence in lentil

The influence of different seed treatment on germination per cent and vigour index in lentil when challenge inoculated with *S. rolfsii* (SR4) was studied and data are presented in Table 4.88. Highest germination per cent was observed in the treatment T14 (*Pf*- 26 + *Bs*-21 +Propineb), T11 (*Tricho*-9 + *Pf*- 26 + *Bs*-21) and T4 (Propineb 70%WP) that is 96 per cent followed by T2 (*Pf*- 26), T5 (*Tricho*-9 + *Pf*- 26),T6 (*Tricho*-9 + *Bs*-21), T9 (*Pf*- 26 + Propineb 70% WP) and T15 (*Tricho*-6 + *Pf*- 26 + *Bs*-9 + Propineb 70% WP) that is 93.33 per cent. The above seed treatments were found to be significantly superior with all the other treatment and control (70.00 per cent).

All the seed treatments affect the vigour index of lentil plant. The seed treatment, T14 (*Pf*- 26 + *Bs*-21 + Propineb 70% WP) was found best in increasing vigour index (4864.90) followed by T5 (*Tricho*-9 + *Pf*- 26) and T11 (*Tricho*-9 + *Pf*-26 + *Bs*-21) (4606.90) as compared to other treatment and control (2053.10).

However, all the seed treatments in this study revealed variation in germination per cent and vigour index, when challenge inoculated with *S. rolfsii* (SR4). These results were supported by the finding of previous workers. Rama yalla reddy (2002) found that integrated use of *T. viride*, *P. fluorescens*, neem cake and thiram as seed treatment of groundnut improved seed yield and controlled soil microflora *viz*., *A. niger, Alternaria spp, Curvularria sp, Sclerotium rolfsaispp, Penicillium sp, R. stolonifer, R. solani, S. rolfsii and Verticillium sp.*. Arunasri (2003) reported that seedling root dip in thiram @ 0.1 per cent + *Trichoderma* suspension + *Pseudomonas* spp. reduced the *S. rolfsii* incidence in Crossandra (about 6.66 per cent) compared to control (73.66%). The use of either *P. fluorescens* or *Trichoderma virens* or Thiram alone or in combination with each other significantly reduced the stem rot caused by *S. rolfsii* in groundnut plant compared to control under greenhouse conditions (Manjula *et al*. 2004). Abeysinghe (2009a) suggested that a combination of *Bacillus subtilis* with *Pseudomonas* strains can lead to greater plant protection against *R. solani* and *S. rolfsii* than the biocontrol strains were used individually. Seed treated with *T. harzianum* + carboxin provided maximum protection to the crop against collar rot of lentil and give maximum seedling emergence, final plant stand and grain yield. (Ravinder Kumar *et al.* 2008). Singh *et al.* (2013) selected *Trichoderma* spp. and

Pseudomonas spp. for seed and seedling treatment in tomato, to assess the synergistic effect of compatible isolates for plant growth promotion and management of *S. rolfsii*. He concluded that the application of a consortium of compatible bioagents enhanced the plant growth and biological control of the pathogen in contrast to treatment with single bioagent. Rajendra prasad *et al.* (2017) observed that the combination of potential *Trichoderma harzianum* -1 and *Pseudomonas fluorescence* bacterial biocontrol agents proved effective in increasing germination, reduce pre and post emergence collar rot and increasing the shoot and root weight and fresh and dry weight of tomato plants in the pots when inoculated with *Sclerotium rolfsii*.

Thus, the result showed that, the combination of seed dressing formulation *Trichoderma* spp. (*Tricho*-12), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*Bs*-21) and Propineb 70%WP provide maximum protection to emerging seedlings. The seed treated with *Pf*- 26 + *Bs*-21 + Propineb 70%WP provided the highest germination, effectively help to increase plant growth and minimum per cent disease incidence of collar rot of lentil.

Table 4.66: Effect of various seed treatment on seedling parameters of lentil under glass house condition.

S. No.	Treatment	Germination (%)*	Root Length (cm)**	Shoot length (cm)**	Vigour index
1	T1: *Tricho*-9	86.66 (9.3)	24.00	18.67	3697.78
2	T2: *Pf*- 26	93.33 (9.7)	29.00	19.67	4542.37
3	T3: *Bs*-21	76.66 (8.8)	22.67	16.67	3015.80
4	T4: Propineb	96.66 (9.8)	16.33	18.33	3350.24
5	T5: *Tricho*-9 + *Pf*- 26	93.33 (9.7)	29.67	19.67	4604.90
6	T6: *Tricho*-9 + *Bs*-21	93.33 (9.7)	26.00	19.33	4230.65
7	T7: *Tricho*-9 + Propineb	83.33 (9.1)	24.67	19.67	3694.85
8	T8: *Pf*- 26 + *Bs*-21	73.33 (8.6)	26.67	18.67	3324.78
9	T9: *Pf*- 26 + Propineb	93.33 (9.7)	27.33	20.33	4448.11
10	T10: *Bs*-21 + Propineb	86.66 (9.3)	26.33	18.33	3870.24
11	T11: *Tricho*-9 + *Pf*- 26 + *Bs*-9	96.66 (9.8)	28.33	19.33	4606.82
12	T12: *Tricho*-9 + *Pf*- 26 + Propineb	86.33 (9.3)	25.67	19.67	3914.20
13	T13: *Tricho*-9 + *Bs*-21 +Propineb	90.00 (9.5)	24.67	19.33	3960.00
14	T14: *Pf*- 26 + *Bs*-21 + Propineb	96.66 (9.8)	29.33	21.00	4864.90
15	T15: *Tricho*-9 + *Pf*- 26 + *Bs*-9 + Propineb	93.33 (9.7)	28.00	20.67	4542.37
16	T16: Control (untreated seed)	70.00 (8.4)	14.33	15.00	2053.10
	SEm ±	0.221	0.500	0.410	
	CD5%	0.619	1.442	1.159	

* Average of three replication

** Average of five replication

Figures in parentheses are square root transformation

4.11. Evaluation of new strain of *Trichoderma* along with combination of fungicide for the management of collar rot of lentil

1. T-1—*Trichoderma herzianum* T-6 (@10g/kg seed)
2. T-2—*Trichoderma herzianum* T-28 (@10g/kg seed)
3. T-3—*Trichoderma viride* T-18 (@10g/kg seed)
4. T-4—*Trichoderma* mutant (@10g/kg seed)
5. T-5—Propineb(Antracol) @3g/kg seed
6. T-6—Hexaconazol+Zineb (Avtar)@3g/kg seed
7. T-7—*Trichoderma herzianum* + Propineb (@10g+1.5g/kg seed
8. Control

Field experiments were conducted during *Rabi*, 2018 and 2019 in RBD with three replications and eight treatments. Seeds of lentil cultivar-Bragg were dipped in bioagents/ fungicidal solution for 10 minutes dried in shad and planted 4×3 m sized plot with 30 x 10 cm spacing. Control plots were maintained without seed treatment, without soil application/ drenching of bioagent/fungicides. Per cent disease incidence was recorded in all treatment by counting the number of infected plants.

4.67: Evaluation of new strain of *Trichoderma* along with combination of fungicide for the management of collar rot of lentil

Treatment	Treatment Details	Plant hight	No. of Branches			No. of leaflets	No. of flawer	No. of pods	Length of leaflets	Shoot Lengh	Root length	Nodule	100 seed weight
			1	2	3								
T1	*Trichoderma herzianum* T-6	53.47	2.86	3.48	3.63	79.28	52.53	40.38	4.86	45.03	18	4.06	3.3
T2	*Trichoderma herzianum* T-28	54.08	2.58	4	5.2	75.61	53.46	43.19	5.59	42.35	16.33	3.38	3.38
T3	*Trichoderma viride* T-18	53.43	2.43	3.13	3.32	79.02	50.33	38.83	5.05	44.38	15.18	3	3.12
T4	*Trichoderma* mutant	57.05	2.47	4.53	2.82	84.4	52.32	43.23	5.9	46.82	20.03	3.23	3.52
T5	Propineb(Antracol)	53.62	2.5	4.3	3.66	73.27	49.37	43.2	5.82	44.98	16.47	3.85	3.22
T6	Hexaconazol+Zineb (Avtar)	54.33	3.37	5.03	3.48	75.25	51.07	43.22	4.55	43.85	18.97	3.83	3.18
T7	*Trichoderma herzianum* + Propineb	58.28	2.53	4.68	3.17	66.62	51.47	44.44	5.33	45.43	20.97	3.6	3.75
	Control	53.57	2.25	4.85	3.28	65.76	43.12	34.95	4.52	41.99	15.8	3.77	2.53
	C.D.at 5%	**1.53**	**0.18**	**0.25**	**1.35**	**6.76**	**4.84**	**4.19**	**7.64**	**1.50**	**1.14**	**0.34**	**0.22**
	C.V.	**4.80**	**0.58**	**0.78**	**4.23**	**21.22**	**15.21**	**13.15**	**23.99**	**4.72**	**3.58**	**1.06**	**0.69**

Untreated Seed Treated Seed

Fig 4.36 Untreated and treated seed

Observations on various plant growth parameters like No. of leaflets, No. of flawer, No. of pods, Length of leaflets, Shoot length, Root length, NO. of Nodule, yield, 100 seed weight, plant emergence and Disease Incidence were recorded 45 days after sowing. Most of the strain of Trichoderma along with combination of fungicide showed positive response for plant growth promoting activity. Seeds treated with Trichoderma isolates, showed significant increase in plant hight (53.62cm in T5 to 58.28cm in T7; 53.57cm in control treatment), No. of tiller (2.43 in T3 to 4.68 in T6; 2.25 in control treatment), Number of leaflets (66.62 cm in T7 to 84.40 cm in T4; 65.76 in control treatment), Number of flawer (49.37 in T5 to 53.46 in T7; 40.63 in control treatment), Number of pods (38.83 in T3 to 44.44 in T7; 34.95 in control treatment), Length of leaflets (4.55 cm in T6 to 5.90 cm in T4; 4.52cm in control treatment), Shoot Length (42.35 cm in T2 to 46.82 cm in T4; 41.99 cm in control treatment), Root lengh (16.33 cm in T2 to 20.97 cm in T7; 15.80 cm in control treatment), No. of root nodule3.12 in T3 to 3.75 in T7; 2.53 in control treatment), plant emergence (342.33 in T3 to 471.67 in T7; 307 in control treatment), Disease incidence (28.91 in T3 to 12.59 in T7; 307 in control treatment), Yield / hac. (1696 in T5 to 1762.27 in T7; 1631.87 in control treatment), (Table- 4.67). Among these, seven treatment *viz.*, T1, T2, T3, T4, T5, T6, and T7 showed maximum increase in shoot length ranging from 45.43 cm (T7) to 46.82 cm (T4), root length ranging from 20.03 cm (T4) to 20.97 cm (T7) and yield 1728.33 (T4) to 1762.27 (T7) (Table- 4.67).

Table 4.68: Evaluation of new stains of *Trichoderma* along with fungicides for the management of collar rot of lentil – Seedling emergence

Treatment	Treatment Details	Doses	Plant Emergence 2019	Plant Emergence 2020	Pooled Data
T1	*Trichoderma herzianum T*-6	(@10g/kg seed)	416.67	428	422.33
T2	*Trichoderma herzianum* T-28	(@10g/kg seed)	423.33	460.67	442
T3	*Trichoderma viride* T-18	(@10g/kg seed)	333.33	351.33	342.33
T4	*Trichoderma* mutant	(@10g/kg seed)	428	468.33	448.17
T5	Propineb(Antracol)	(@3g/kg seed)	408.67	396.67	402.67
T6	Hexaconazol+Zineb (Avtar)	(@3g/kg seed)	412.67	414	413.33
T7	*Trichoderma herzianum* + Propineb	(@10g+1.5g/kg seed)	458	485.33	471.67
	Control		292.33	323	307.67
	C.D.at 5		**4.04**	**4.47**	
	C.V.		**12.68**	**14.04**	

Fig. 4.37(a)*Trichoderma* along with fungicides for the management of collar rot of lentil – Seedling emergence

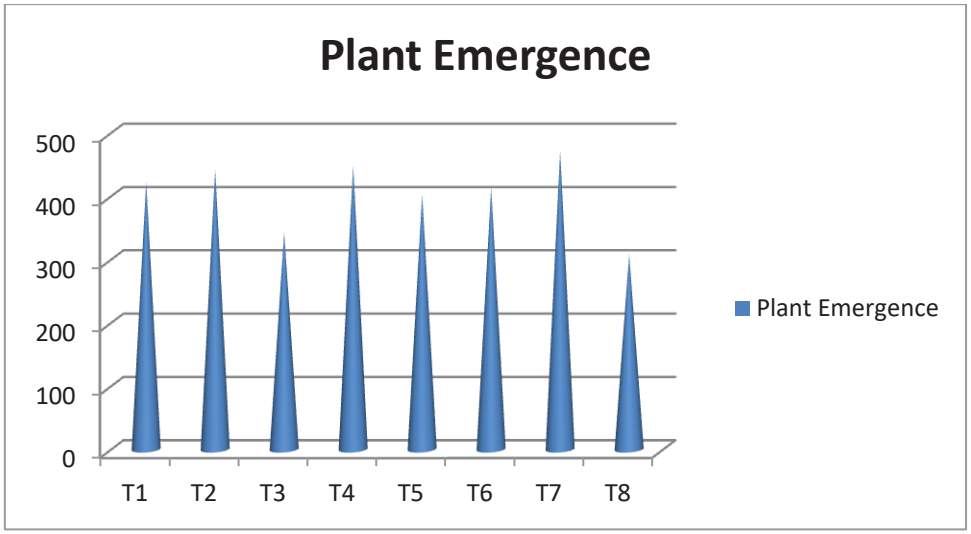

*Fig.*4.37(b) Seedling emergence of treated and untreated lentil seed of *Trichoderma* along with fungicides

Table 4.69 | Evaluation of new stains of *Trichoderma* along with fungicides for the management of collar rot of lentil – Disease incidence (%)

Treatment	Treatment Details	Doses	Disease Incidence		Pooled Data
			2019	2020	
T1	*Trichoderma herzianum* T-6	(@10g/kg seed)	23.65	21.86	22.76
T2	*Trichoderma herzianum* T-28	(@10g/kg seed)	24.19	18.86	21.52
T3	*Trichoderma viride* T-18	(@10g/kg seed)	32.75	25.08	28.91
T4	*Trichoderma* mutant	(@10g/kg seed)	19.73	14.06	16.9
T5	Propineb(Antracol)	(@3g/kg seed)	23.48	19.48	21.48
T6	Hexaconazol+Zineb (Avtar)	(@3g/kg seed)	20.26	21.93	21.09
T7	*Trichoderma herzianum* + Propineb	(@10g+1.5g/kg seed)	13.26	11.93	12.59
T8	Control		37.83	30.83	34.33
	C.D.at 5		**4.48**	**2.04**	
	C.V.		**14.08**	**6.42**	

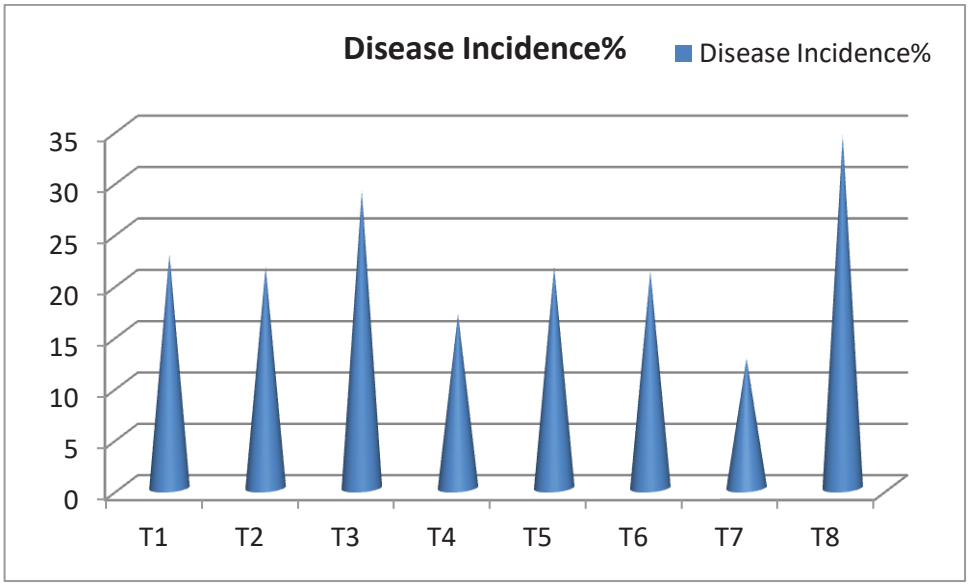

***Fig4.38* Disease incidence of treated and untreated lentil seed of *Trichoderma* along with fungicides**

Table 4.70 Evaluation of new stains of *Trichoderma* along with fungicides for the management of collar rot of lentil – Yield (kg/ha)

Treatment	Treatment Details	Doses	Yield /hac.		Pooled Data
			2018	2019	
T1	*Trichoderma herzianum* T-6	(@10g/kg seed)	1702.67	1711.33	1707
T2	*Trichoderma herzianum* T-28	(@10g/kg seed)	1706.33	1729	1717.67
T3	*Trichoderma viride* T-18	(@10g/kg seed)	1698.33	1660	1679.17
T4	*Trichoderma* mutant	(@10g/kg seed)	1711	1745.67	1728.33
T5	Propineb(Antracol)	(@3g/kg seed)	1689.33	1702.67	1696
T6	Hexaconazol+Zineb (Avtar)	(@3g/kg seed)	1665.33	1707	1686.17
T7	*Trichoderma herzianum* + Propineb	(@10g+1.5g/kg seed)	1721.09	1803.45	1762.27
	Control		1608.41	1655.32	1631.87
	C.D.at 5		**4.4**	**3.99**	
	C.V.		**13.8**	**12.54**	

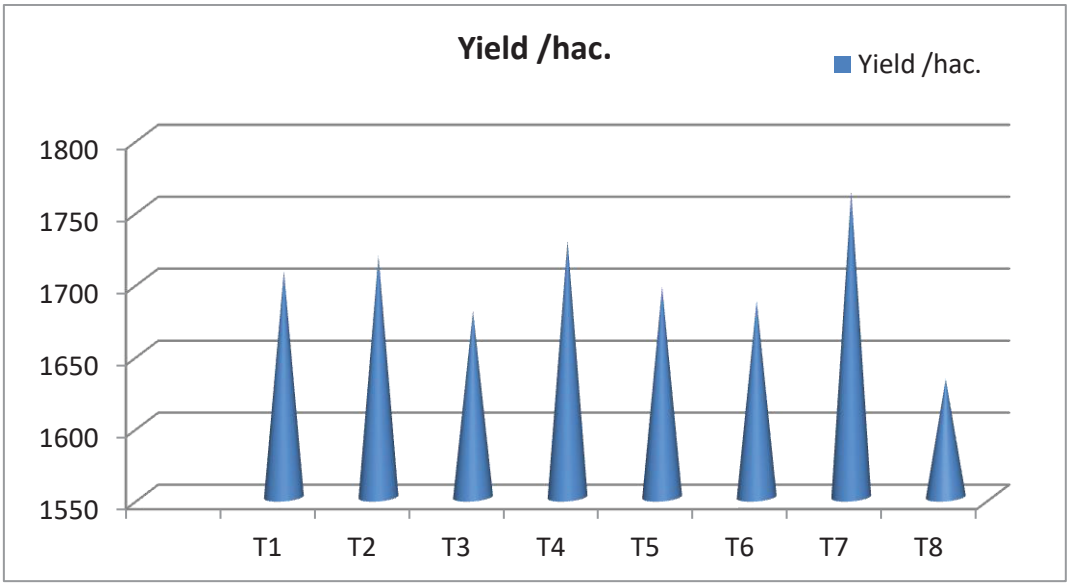

Fig.4.39: Yield of treated and untreated lentil seed of *Trichoderma* along with fungicides

Trials for control of lentil collar rot was conducted in the 2018–2019 and 2019–2020 crop seasons at Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. In Raipur in 2015–2016, experiment was taken in a plot of 4 m × 3 m. lentil seeds treated with *Trichoderma* along with combination of fungicide was sown and observation on seed yield and plant charecters was recorded

Seeds were treated *Trichoderma* along with combination of fungicide seeds of k-34 variety, and observations on disease incidence and grain yield were recorded, and compared with non-treated plots.

The Formulation is Effective in Reducing disease incidence and Improving Yield of lentil Under Field Conditions *Trichoderma herzianum*+Propineb and *Trichoderma* mutant formulation significantly reduced lentil plant mortality (12.59% wilted plants, as against 33.34% in control plot) and was superior to other *Trichoderma* based formulations (Table4.9).

In trials on collar-rot control, *Trichoderma herzianum*+Propineb and *Trichoderma* mutant was evaluated and compared with a few other *Trichoderma* strains, fungicides, and combination treatment, at form of CoA, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh.over 2 years. *Trichoderma herzianum*+Propineb and *Trichoderma* mutant treatment of seeds significantly

improved seedling emergence, reduced disease incidence, and improved yield (Tables4.39)

Trichoderma along with combination of fungicide Seed Treatment Improved Lentil Yield In 2018 and 2019, the maximum yield recorded in the *Trichoderma herzianum*+Propineb and *Trichoderma* mutant –treated plot being 1728.33 and 1762.27 kg/ha and the minimum being 1686.17 kg/ha, compared to 1631.87 kg/ha in control fields, and the yield gain was statistically significant.

4.12. Screening of lentil entries

4.12.1 Field screening

To find out the sources of resistance, lentil entries were evaluated for their reaction against *Sclerotium rolfsii* under natural field condition as per standard evaluation system. The reactions of the entries are depicted in Table 4.71 to 4.94.

During first *rabi* season 2018- 2019, total 139 lentil entries were screened against collar rot pathogen under field conditions. All the entries tested under Lentil LS AVT-1(7), Lentil LS AVT-2(6), Lentil EE IVT(21), Lentil EE IVT(10), Lentil MMLT(13), Lentil Germplasm(52), Lentil Released Variety(30) were susceptible to highly susceptible to collar rot**.** None entry was resistant or moderately resistant to collar rot.

While during second *rabi* season 2019-20, total 132 lentil entries were screened against collar rot pathogen under field conditions.

All the entries tested under lentil AVT 2 Large seed CV(5), Lentil IVT Rice Fallow NEPZ(13), Lentil IVT Large Seed(22), ,lentil germplasm(34) , lentil germplasm(52) 132 lentil germplasm accessions were screened against collar rot pathogen under natural conditions. Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as resistant. Fourteen germplasm lines were found tolerant while 02 were moderately susceptible and 05 were highly susceptible to the disease. These resistant sources can further be exploited in breeding program for the development of disease resistant commercial cultivars.

Gaurkhede *et al.* (2015) reported that in a field screening of 284 lentil germplasm accessions against collar rot, 9 were found free from disease and 29

exhibited < 10 per cent mortality due to collar rot. Gupta and Mishra, (2009) screened among 120 lines of lentil in disease sick fields for 3 consecutive years and 32 entries performed consistent resistant reaction to collar rot. Twelve accessions were found free from collar rot during the testing years under high disease pressure. Hussain *et al*., (2005), screened 57 cultivars and found only one genotype highly resistant. Sugha *et al*., (1991) evaluated 210 lentil lines/cultivars from different sources. None of these were resistant or even moderately resistant.

Fig.4.40: Field screening of lentil

Table 4.71: Screening of lentil entries available lentil germplasm against collar rot of lentil during 2018-19.

S. No.	Entries	Per cent emergence	Per cent mortality
1	DPL-62	30.00	34.45
2	VL-1	61.44	69.00
3	VL-4	54.35	71.49
4	DPL-15	60.00	75.85
5	ASHA	52.00	79.46
6	NDL-1	51.33	82.14
7	PL-5	48.33	76.15
8	Ranjan	38.33	63.58
9	PL-406	70.25	55.54
10	PL-234	51.00	80.64
11	VL-103	40.33	75.94
12	Kirsey fokar	51.67	72.94
13	Dehati Masoor	48.33	78.64
14	JL-1	35.00	78.54
15	JL-3	18.21	28.45
16	K-75	48.33	82.64
17	PL-4	46.67	62.75
18	Masoor Dal	19.65	29.15
19	Deshi Lal	60.00	62.15
20	Chhoti Sunhari	63.43	78.65
21	Chhoti Masoor	46.57	74.54
22	Adlika	55.00	78.51
23	Chootki Masoor K	42.34	80.15
24	Local	60.33 63.00	72.16
25	Desi Safed	43.00	73.15
26	Deshi Masoor Gol	54.32	72.64
27	Deshi Masoor-1	52.33	75.54
28	Masoor-1	49.25	65.48
29	Masoor- 2	41.25	31.57
30	Moti Masoor	56.78	70.84
31	Baban Masoor	64.85	55.14
32	Subrata	72.94	31.84
33	Masuri	58.65	67.54
34	Kashor Masur	62.48	72.64
35	JL3(Susceptible check)		82.64

Table–4.72: Sceening of available lentil germplasm against *Sclerotium rolfsii 2018-2019*

S.No.	Grading	Mortility	Total no. of germplasm line	Name of germplasm line
1	High Resistance	Less then 1	3	DPL-62, VL-1, VL-4,
2	Resistance	1-10%	10	DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103, Kirsey fokar, Dehati Masoor
3	Tolerance	11-20%	14	JL-1, K-75,PL-4, Masoor Dal, Deshi Lal, Chhoti Sunhari, Chhoti Masoor, Adlika, Chootki Masoor K, Local, Desi Safed,
4	Moderate suscetible	21-50%	2	Deshi Masoor Gol, Deshi Deshi Masoor-1, Masoor-1 Masoor- 2
5	High susceptible	More than50%	5	Moti Masoor, Baban Masoor, Subrata, Masuri, Kashor Masur
		Total	34	

Table 4.73: Screening of lentil entries under AVT-1 (LARGE SEED) against collar rot of lentil during 2018-19.

S. No.	Entries	Per cent emergence	Per cent mortality
1	LLS 18- 82	72.75	42.65
2	LLS 18- 83	82.33	38.53
3	LLS 18- 84	80.45	49.54
4	LLS 18- 85	64.14	39.14
5	LLS 18- 86	46.25	68.24
6	JL3 (Susceptible check)	76.54	71.84

Table 4.74: Reaction of lentil entries under AVT-1 (LARGE SEED) against collar rot of lentil during 2018-19.

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	4	LLS 18- 83 , LLS 18- 85,
5	More than 40	5 (HS)	0	LLS 18- 82, LLS 18- 84, LLS 18- 86.
	Total entries		4	
	LSI		2.25	

Table 4.75: Screening of lentil entries under AVT-2 (LARGE SEED) against collar rot of lentil during 2018-19.

S. No.	Entries	Per cent emergence	Per cent mortality
1	LLS-18-68	81.64	37.15
2	LLS-18-69	83.54	39.53
3	LLS-18-70	66.00	35.94
4	LLS-18-71	81.44	39.92
5	LLS-18-72	65.25	40.15
6	JL3 (Susceptible check)	77.95	66.84

Table 4.76: Reaction of lentil entries under AVT-2 (LARGE SEED) against collar rot of lentil during 2018-19.

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	4	LLS 18- 68, LLS 18- 69, LLS 18- 70, LLS 18- 71, LLS 18- 72.
5	More than 40	5 (HS)	0	Nil
	Total entries		4	
	LSI		2.25	

Table 4.77: Screening of lentil entries under IVT (LARGE SEED) against collar rot of lentil during 2018-19

S. No.	Entries	Per cent emergence	Per cent mortality
1	LLS 18- 129	53.14	47.25
2	LLS 18- 130	77.25	41.25
3	LLS 18- 131	66.67	35.42
4	LLS 18- 132	61.67	38.62
5	LLS 18- 133	40.00	39.40
6	LLS 18- 134	53.33	38.56
7	LLS 18- 135	45.00	36.14
8	LLS 18- 136	20.00	40.29
9	LLS 18- 137	83.33	38.06
10	LLS 18- 138	65.00	37.69
11	LLS 18- 139	28.33	31.53
12	LLS 18- 140	28.33	40.59
13	LLS 18- 141	75.00	37.01
14	LLS 18- 142	60.00	38.42
15	LLS 18- 143	50.00	38.56
16	LLS 18- 144	71.67	36.26
17	LLS 18- 145	51.67	34.27
18	LLS 18- 146	25.00	38.02
19	LLS 18- 147	46.67	39.00
20	LLS 18- 148	65.00	37.25
21	LLS 18- 149	55.25	42.68
22	JL3(Susceptible check)	41.67	70.99

Table 4.78: Reaction of lentil entries under IVT (LARGE SEED) against collar rot during 2018-19.

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	23	LLS 18- 131, LLS 18- 132, LLS 18- 133, LLS 18- 134, LLS 18- 135, LLS 18- 137, LLS 18- 138, LLS 18- 139, LLS 18- 141, LLS 18- 142, LLS 18- 143, LLS 18- 144, LLS 18- 145, LLS 18- 146, LLS 18- 147, LLS 18- 148.
5	More than 40	5 (HS)	0	LLS 18- 129, LLS 18- 130, LLS 18- 136, LLS 18- 140, LLS 18- 149
	Total entries		23	
	LSI		5	

Table 4.79: Screening of lentil entries under AVT (EXTR LARGE) against collar rot of lentil during 2018-19.

S. No.	Entries	Per cent emergence	Per cent mortality
1	LEE18-165	70.25	77.25
2	LEE18-166	66.67	65.25
3	LEE18-167	51.67	79.76
4	LEE18-168	43.33	83.77
5	LEE18-169	59.36	73.33
6	LEE18-170	51.67	77.57
7	LEE18-171	31.67	75.00
8	LEE18-172	48.33	68.25
9	LEE18-173	48.33	70.69
10	JL3(Susceptible check)	90.00	89.86

Table 4.80: Reaction of lentil entries under AVT (EXTR LRGE) against collar rot of lentil during 2018-19.

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	0	Nil
5	More than 40	5 (HS)	9	LEE18-165, LEE18-166, LEE18-167, LEE18-168, LEE18-169, LEE18-170, LEE18-171, LEE18-172, LEE18-173.
	Total entries		10	
	LSI		5	

5 Table 4.81: Screening of lentil entries under MLT against collar rot of lentil during 2018-19

S. No.	Entries	Per cent emergence	Per cent mortality
1	RL-6-1	64.25	68.63
2	RL-8	72.32	66.84
3	RL9	65.14	72.35
4	RL10	63.33	59.10
5	RL-11	65.00	71.35
6	RL-12	55.00	68.25
7	JL-3	43.33	72.36
8	RVL-13-5	51.67	76.26
9	L- 4796	63.33	81.65
10	RVL-6071-1	38.33	70.55
11	IPL-316	55.00	80.33
12	RVL-14-4	76.25	65.94
13	RL-3-5-1	72.68	68.24
14	JL3(Susceptible check)	80.00	93.65

Table 4.82: Reaction of lentil entries under MLT against collar rot during 2018-19.

S. No.	Per cent mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	0	Nil
5	More than 40	5 (HS)	14	RL-6-1, RL-8, RL9, RL10, RL-11, RL-12, JL-3, RVL-13-5, L- 4796, RVL-6071-1, IPL-316, RVL-14-4, RL-3-5-1
	Total entries		14	
	LSI		5	

Table 4.83: Screening of lentil entries under lentil germplsm against collar rot of lentil during 2018-19

	Germplasm	Germination Per cent	Mortility Percentge
1	RL-4	66.44	65.25
2	RL-8	55.69	75.98
3	DPL-62	63.33	59.18
4	IPL-532	69.54	81.36
5	IPL-522	55.75	72.36
6	L-4676	43.33	80.95
7	JL-3	51.63	76.26
8	RL-3	63.33	75.36
9	IC-29983	46.85	81.36
10	SKY/AC-1420	55.25	78.36
11	IC-496773-1	78.33	65.94
12	EC-299645	78.33	83.06
13	IC-208353	68.33	72.46
14	IC-408019	82.36	68.24
15	IC-201689	53.33	68.63
16	PRCOI-EE-758	63.33	84.16
17	EC-78451-A	65	85.97
18	IC-558821	55	81.36
19	JL-29	95	75.85
20	IC-201714	16.67	71.36
21	IC-271999	36.67	88.36
22	IC-201101	56.67	81.36
23	IC-2220168	65	77.36
24	IC-267114	16.67	86.45
25	IC-4197	56.67	81.35
26	IC-2016IC-299647	61.67	75.36
27	IC-299647	50.48	82.36
28	EC-28514	68.33	82.36

29	RI-5	78.33	75.36
30	RL-9	85.87	70.85
31	RL-10	63.33	69.25
32	PL-10	78.33	70.58
33	IPL-521	76.67	77.36
34	L-4049	43.85	81.75
35	RL-6	83.94	74.54
36	RL-7	61.67	80.36
37	VKG-15/275	68.33	72.84
38	IC-21268	48.33	83.45
39	IC-211609	88.33	80.76
40	IC-268271	84.36	83.64
41	IC-267121	90.74	78.15
42	IC-201714	66.67	68.15
43	IC-212276	85.78	74.56
44	IC-267657	88.33	83.14
45	IC-277444	48.33	83.05
46	IC-271999	60.84	70.65
47	EC-299644	85.36	88.13
48	IC-201665	83.33	58.35
49	VKG-15/367	89.52	66.58
50	VKG-15/151	85.14	84.48
51	IC-271332	86.67	78.36
52	JL3(Susceptible check)	63.33	81.36

Table 4.84: Reaction of lentil entries under LENTIL GERMPLASM against collar rot during 2018-19

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries,
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	3	Nil
4	31-40	4 (S)	2	Nil
5	More than 40	5 (HS)	22	RL-4, RL-8, DPL-62, IPL-532, IPL-522, L-4676, JL-3, RL-3, IC-29983, SKY/AC-1420, IC-496773-1, EC-299645, IC-208353, IC-408019, IC-201689, PRCOI-EE-758, EC-78451-A, EC-78451-A, IC-558821, JL-29, IC-201714, IC-271999, IC-201101, IC-2220168, IC-267114, IC-4197, IC-2016IC-299647, IC-299647, EC-28514, RI-5, RL-9, RL-10, PL-10, IPL-521, L-4049, RL-6, RL-7, VKG-15/275, IC-21268, IC-211609, IC-268271, IC-267121, IC-201714, IC-

		212276, IC-267657, IC-277444, IC-271999, EC-299644, IC-201665, VKG-15/367, VKG-15/151, IC-271332, IC-271995.
	Total entries	27
	LSI	4.70

Table 4.85: Screening of lentil entries under AVT 2 (Large Seed) against collar rot of lentil during 2019-20.

S. No.	Entries	Per cent emergence	Per cent mortality
1	LLS 19-73	68.00	59.00
2	LLS 19- 74	83.67	68.00
3	LLS 19-75	65.33	70.00
4	LLS 19- 76	79.67	60.00
5	JL3 (Susceptible check)	39.00	89.00

Table 4.86: Reaction of lentil entries under AVT 2 (Large Seed) against collar rot of lentil during 2019-20

S. No.	Per cent Mortality	Score/ Reaction	No. of entries	Name of entries
1	Less than 10	1	0	Nil
2	10-20	2	0	Nil
3	21-30	3	0	Nil
4	31-40	4	0	Nil
5	More than 40	5	4	LLS 19-73, LLS 19- 74, LLS 19-75, LLS 19- 76, LLS 19-77
Total entries			4	
LSI			5	

Table 4.87: Screening of lentil entries under IVT Rice FALLOW against collar rot of lentil during 2019-20

S. No.	Entries	Per cent emergence	Per cent mortality
1	LRF19-148	69.54	68.45
2	LRF19-149	70.15	70.96
3	LRF19-150	48.25	80.43
4	LRF19-151	58.94	62.84
5	LRF19-152	36.48	81.45
6	LRF19-153	52.14	71.00
7	LRF19-154	55.44	70.00
8	LRF19-155	52.14	52.00
9	LRF19-156	70.57	68.00
10	LRF19-157	83.15	78.00
11	LRF19-158	36.85	65.00
12	LRF19-159	79.15	65.48
13	LRF19-160	22.58	79.00
14	JR3 (Susceptible check)	27.67	90.00

Table 4.88: Reaction of lentil entries under IVT Rice FALLOW against against collar rot during 2019-20

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	0	Nil
5	More than 40	5 (HS)	0	LRF19-148, LRF19-149, LRF19-150, LRF19-151, LRF19-152, LRF19-153, LRF19-154, LRF19-155, LRF19-156, LRF19-157, LRF19-158, LRF19-159, LRF19-160,
	Total entries		13	
	LSI		5	

Table 4.89: Screening of lentil entries under IVT (LARGE SEED) against collar rot of lentil during 2019-20

S. No.	Entries	Per cent emergence	Per cent mortality
1	LLS 19-126	68.67	84.00
2	LLS 19-127	51.33	80.00
3	LLS19- 128	71.67	65.00
4	LLS 19-129	71.67	59.00
5	LLS19-130	60.00	67.00
6	LLS19-131	43.00	82.00
7	LLS 19-132	28.33	75.00
8	LLS19-133	65.00	84.00
9	LLS19-134	53.33	82.00
10	LLS19-135	26.67	73.00
11	LLS19-136	41.67	63.00
12	LLS19-137	35.00	60.00
13	LLS19-138	76.67	59.00
14	LLS19-139	75.00	74.00
15	LLS19-140	68.33	79.00
16	LLS19-141	73.33	69.00
17	LLS19-142	41.67	60.00
18	LLS19-143	55.54	76.00
19	LLS19-144	71.67	64.00
20	LLS19-145	69.33	71.00
21	LLS19-146	75.67	71.00
22	LLS19-47	78.00	69.00
23	JL3 (Susceptible check)	33.33	90.00

Table 4.90: Reaction of lentil entries under entries under IVT (LARGE SEED) against collar rot of lentil during 2019-20

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries S	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	0	Nil
5	More than 40	5 (HS)	22	LLS 19-126, LLS 19-127, LLS 19-128, LLS 19-129, LLS 19-130, LLS 19-131, LLS 19-132, LLS 19-133, LLS 19-134, LLS 19-135, LLS 19-136, LLS 19-137, LLS 19-138, LLS 19-139, LLS 19-140, LLS 19-141, LLS 19-142, LLS 19-143, LLS 19-144, LLS 19-145, LLS 19-146, LLS 19-147.
	Total entries		22	
	LSI		5	

Table 4.91: Screening of lentil entries under Released varieties (2019) against collar rot of lentil during 2019-20

S. No.	Entries	Per cent emergence	Per cent mortality
1	RLV11-6	90.00	68.59
2	RLV13-5	98.33	86.49
3	RLV-31	95.00	85.50
4	RLV13-7	96.67	83.40
5	JL-3	96.67	75.71
6	HUL-57	70.00	83.33
7	Kota Mashoor-1	100.0	77.25
8	KotaMashoor-2	98.33	85.21
9	RLG-5	98.33	86.49
10	L-4727	95.00	76.62
11	L-4717	38.33	85.28
12	L-4147	71.67	81.65
13	L-4076	96.67	77.65
14	LH-89-48	5.00	76.97
15	LH-84-8	80.00	79.62
16	LH-82-6	76.67	87.65
17	LL-699	86.66	86.51
18	LL-1373	80.00	75.36
19	LL-931	75.00	86.02
20	DPL-15	95.00	85.30
21	DPL-62	55.00	73.21

22	IPL-81	6.67	79.52
23	IPL-406	68.33	87.92
24	IPL-316	60.00	74.77
25	IPL-220	61.67	89.73
26	WBL-77	78.33	80.64
27	Pant L-7	78.33	86.75
28	Pant L -8	88.33	87.29
	Narendra Mashoor-1		
	Narendr Mashoor 2		
31	JL3(Susceptible check)	85.00	98.44

Table 4.92: Reaction of lentil entries under Released varieties (2019) against collar rot during 2019-20

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	0	Nil
4	31-40	4 (S)	0	Nil
5	More than 40	5 (HS)	28	RLV11-6, RLV13-5, RLV-31, RLV-31, RLV-31, RLV13-7, JL-3, HUL-57, Kota Mashoor-1, KotaMashoor-2, RLG-5, L-4727, L-4717, L-4147, L-4076, LH-89-48, LH-84-8, LH-82-6, LL-699, LL-1373, LL-931, DPL-15, DPL-62, IPL-81, IPL-406, IPL-316, IPL-220, WBL-77, Pant L-7, Pant L -8, Narendra Mashoor-1 , Narendr Mashoor 2

Total entries	28
LSI	5

Table: 4.93: lentil entries under lentil germplsm against collar rot of lentil during 2019-20.

S. No.	Entries	Per cent emergence	Per cent mortality
1	IC-267363	79.12	86.47
2	IC-261720	89.65	72.34
3	IC-267198	90	80.14
4	IC-421795	75.45	78.51
5	IC-429195	72.19	89.68
6	IC-201697	90	83.45
7	IC-201694	68.33	85.78
8	IC-208343	96.67	81.3
9	SKY-IC-1420-2	38.33	85.85
10	IC-207029	48.33	88.33
11	IC-4967732	48.33	72.5
12	IC-267113	38.33	57.25
13	VKG-15/362	73.33	82.46
14	IC-20168	55	79.09
15	VKG-15/319	38.33	81.25
16	IC-371632	51.67	81.58
17	VKG-15/227	48.33	85.42
18	VKG-15/336	35	84.29
19	VK-15/362	15	23.03
20	EC-303712	48.33	80.53
21	IC-201740	46.67	60.87
22	IC-201688	16.67	23.04
23	IC-55944	70	79.42
24	IC-559776	53.33	73.29
25	IC-560127	33.33	68.74
26	IC-560212	45	75.85
27	IC-560299	56.67	71.96
28	C-S-CL-4595	61.67	84.11
29	EC-78415	60	85.52
30	EC-78540	60	83.48
31	EC-78461	51.67	79.76
32	CE-267554	43.33	83.77
33	EC-267604	36.67	73.33
34	EC-267627	51.67	77.57
35	EC-267638	31.67	75.12
36	EC-223222	48.33	80.63
37	EC-267537	48.33	81.44
38	EC-267538	61.67	73.95
39	EC-267601	85	69.52
40	EC-267626	80	87.76
41	EC-267628-B	85	98.43

42	IC-396758	51.67	79.76
43	VKG-LE-1	43.33	83.77
44	IGL-1014-5	36.67	73.33
45	RVL-13-7	51.67	77.57
46	L-4727	31.67	75.85
47	RVL-13-5	48.33	81.8
48	L-4735	48.33	75.54
49	L-4769	92.14	66.85
50	RVL-14-4	65	76.35
51	L-4762	71.15	78.95
52	RVL-14-5	72	42.14.
53	JL3(Susceptible check)	77	84.52

Table 4.94: Reaction of lentil entries under LENTIL GERMPLASM against collar rot during 2019-20

S. No.	Per cent Mortality	Score/ Reaction	No. of Entries	Name of entries
1	Less than 10	1 (R)	0	Nil
2	10-20	2 (MR)	0	Nil
3	21-30	3 (MR)	3	Nil
4	31-40	4 (S)	2	Nil
5	More than 40	5 (HS)	22	IC-267363, IC-261720, IC-267198, IC-421795, IC-429195, IC-201697, IC-201694, IC-208343, SKY-IC-1420-2, IC-207029, IC-4967732, IC-267113, VKG-15/362, IC-20168, VKG-15/319, IC-371632, VKG-15/227, VKG-15/336, VK-15/362, EC-303712, IC-201740, IC-201688, IC-55944, IC-559776, IC-560127, IC-560212, IC-560299, C-S-CL-4595, EC-78415, EC-78540, EC-78461, CE-267554, EC-267604, EC-267627, EC-267638, EC-223222, EC-267537, EC-267538, EC-267601, EC-267626, EC-267628-B, IC-396758, VKG-LE-1, IGL-1014-5, RVL-13-7, L-4727, RVL-13-5, L-4735, L-4769, RVL-14-4, L-4762, RVL-14-5
	Total entries		27	
	LSI		4.70	

CHAPTER-V
SUMMARY AND CONCLUSION

The present investigation on **"Studies on collar rot *(Sclerotium rolfsii* sacc.) of lentil and it's management."** was carried out in the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The investigation mainly consists of four objectives- 1.) Survey and Collection of *Sclerotium rolfsii* Sacc. of lentil from different growing areas of Chhattisgarh.2.) Isolation, purification, characterization and pathogenicity of different isolates of *Sclerotium rolfsii* Sacc. of lentil. 3.) Study of soil factors affecting development of *Sclerotium rolfsii* Sacc. and collar rot disease in lentil.4.) Collection and characterization of native isolates of *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp. 5.) Management of collar rot of lentil. The summary and conclusion of findings of the present investigations are given below:

The survey for the occurrence and severity of lentil collar rot were undertaken at seedling and vegetative stage of the crop from fourteen locations in seven lentil growing districts of Chhattisgarh state (Raipur, Mungeli, Bilaspur, Dhamrari, Balodabajar, Kawardha, and Bemetara,) during 2018 and 19. It is apparent that lentil collar rot disese incidence was more sever in Raipur, Mungeli, Bemetara, Raipur, Bilaspur districts of Chhattisgarh than other surveyed districts of Chhattisgarh, "during different cropping seasons. The average" collar rot incidence of lentil (*Sclerotium rolfsii*.) in different districts varied from 12.57 to 39.97 per cent. In Mungeli district, it was maximum (39.63%) followed by Bemetara (37.63%), Kawardha (32.00%), Raipur (23.73%), Balodabazar (16.89) Bilaspur (16.84%), Dhamtari (12.57%), Least wilt incidence was observed in Dhamtri (12.57%) district of Chhattisgarh.

Symptoms of collar rot disease was observed in lentil under natural field conditions. The characteristic symptoms found were drying plants whose foliage turns slightly yellow scattered throughout the field was observed. Affected young seedling turned yellow and collapsed but the older seedlings dried without collapsing. No clear cut drooping of leaves was seen. Uprooted seedling showed

rotting at the collar region and downwards. The collar portion was covered with whitish mycelia strands. White mycelia growth was also observed on the tap root of a completely dried seedling and rapseed-like sclerotia were observed attached to mycelia growth around the collar region.

Isolation of the test fungus was made from infected plants of lentil, collected from forty lentil growing areas of Chhattisgarh state ((Raipur IGKV farm- *SR*1, Agronomy, Field-*SR*2, Khurha-*SR*3, Mungeli, Temari *SR*4, Chilfi-*SR*5, Bhtha-Bhurka *SR*6, Bilaspur, takhatpur*SR*7, Dhamtari Kurud, Gobra *SR*8, Baloda bazaar, Simga Surgi-S*R*9, Bhatapara, Gogiya-*SR*10, Kawardha, Pandariya-kapadah-*SR*11, ghuterkudi-*SR*12, Bemetara Gadamod- *SR*13, Matia-*SR*14)). Forty isolates of *S. rolfsii* were isolated from collected samples and the cultures were purified by single hyphal tip method and were maintained on PDA at 25 ± 2^{0}C. Based on mycological characters the pathogen identified as *Sclerotium rolfsii* Sacc.

Forty isolates were characterized for cultural and morphological characteristics on PDA medium. All the isolates shared characteristics of *S. rolfsii.* The isolates varied in mycelial growth and classified into 3 groups: fast growing (diameter >80-90mm), moderately fast growing (70-79 mm) and slow growing (< 70). First group comprised of 09 isolates *SR*1, *SR*3, *SR*4, *SR*6, SR7, *SR*9, *SR*10 , *R*11, *SR*13 were fast growing (diameter >80-90 mm). Second group include moderately fast growing (70-79 mm) 03 isolates, *SR*2, *SR*5, *SR*14, as mycelial growth. The third group consist 01 isolates *SR*8 were slow growing (< 70mm).

On the basis of variation in hyphal width among isolates, the pathogen was categorized in three groups . First group comprised of 02 isolate SR9, and SR10, with hyphal width ranging between 7-9 µm. Second group include 9-11µm in 09 isolates *SR*1 (IGKV Farm), *SR*2, *SR*3, *SR*5, *SR*6, *SR*7, *SR*11, *SR*13, *SR*14" as hyphal width and third group consist of 03 isolates SR4, SR8, SR12, with hyphal width of more than 11 µm. However, maximum and minimum hyphal width observed in *SR*12 (12.00 µm) and *SR*10 (7.2 µm) respectively.

All isolates varied in distance between septa and categorized in four groups. First group comprised of 01 isolate SR10, distance between septa with ranging between 13-18 µm. Second group include 18-23 µm in 04 isolates *SR*7,

*SR*8, S*R*13, *SR*14 as distance between septa and third group Include 24-26 μm consist of 06 isolates *SR*1, *SR*2, *SR*4, S*R*5, *SR*6, *SR*9. Fourth group include 27-31 μm in 2 isolates, *SR*3, S*R*11 , as septa distance in hypha of pathogen.

The isolates showed variation in pattern of mycelial growth. Based on mycleial growth pattern on PDA after five days of incubation, the forty isolates were placed in two groups. The first group includes *SR*8, *SR*9, *SR*13, *SR*14 isolates having fluffy mycelia growth. Whereas *SR*1, *SR*2, *SR*3, *SR*4, *SR*5, *SR*6, *SR*7, *SR*10 , *SR*11and *SR*12 isolates comprised of second group with compact mycelial growth pattern. .

The variations were observed in sclerotia production among the *S. rolfsii* isolates. Highest number of sclerotia production per plate was noted in *SR*9 (258.33) followed by *SR*7 (203) which were at par with each other. Least sclerotia production per plate was found in *SR*4 (67.66) followed by *SR*11, *SR*12.

The isolates were also varied in sclerotial weight taken after 30 days of incubation. Higher weight of the the maximum weight of the 100 sclerotia was found in isolate *SR*10 (0.242mg) followed by *SR*3 (0.241 mg) and *SR*9 (0.172 mg) isolate. Lowest weight of 100 sclerotia was found in isolate *SR*12 (0.06 mg) followed by isolate *SR*4 (0.10 mg).

The average size of 10 sclerotia in the fortin isolates varied from 0.4 to 1.6 in diameter. Maximum sclerotial size was found in isolate *SR*5 that is 1.6 mm followed by *SR*10 (1.5 mm) and *SR*4, *SR*6, *SR*27, *SR*40 (1.5). Whereas least sclerotia size observed in isolate *SR*12, *SR*4, *SR*7, *SR*9, *SR*8, and *SR*6 (0.8 mm).

The isolates also varied in sclerotial shape, Spherical shape sclerotia recorded in 11 isolates *SR*1, *SR*2, *SR*4, *SR*5, S*R*6, *SR*7, *SR*8, *SR*10, *SR*11, *SR*12, *SR*13 ", whereas, shape Ellipsoidal sclerotia were found in 3 isolates *SR*3, *SR*9 and *SR*14 Based on pigmentation of the sclerotia, isolates were assigned to three different colour groups , light brown, reddish brown and dark brown. Isolate *SR*4, *SR*11, *SR*12, were observed to have light brown colour, and isolate *SR*1, *SR*3, *SR*8, *SR*9, *SR*13, and *SR*14 had reddish brown colour, whereas the sclerotia of isolates S*R*2, *SR*5, *SR*6 , *SR*7, and *SR*10 were dark brown in colour.

The pattern of sclerotial production was also studied and based on their arrangement on the culture medium, the isolates were divided into two main categories. It was noted that the isolates had a particular pattern of formation of sclerotia in culture. In the first category ,9 isolate *SR*1, *SR*2, *SR*5, *SR*6, *SR*8, *SR*9, *SR*10, *SR*11, *SR*12 produced sclerotia in irregular manner scattered all over the culture and rim of the plate. In the second category, 5 isolate *SR*3, *SR*4, *SR*7, *SR*13 and *SR*14 produced sclerotia in group.

Pathogenicity of the fungus was tested, all the isolates were found to pathogenic under artificially inoculated condition and expressed typical symptoms of collar rot. *In vivo* sick pot experiment revealed that the isolates were variable in their aggressiveness ; all isolates varied in average morality per cent of 66.66 to 100 %. Highest mortality was observed in *SR* 4 which was significantly superior than other isolates, followed by *SR*14 (96.29 %), *SR*5 (89.68 %), *SR*3 (85.71%), *SR*8 (85.18 %), *SR*11 (83.33 %) and *SR*12 (81.48%). However, lowest mortality 66.66 % was observed in isolate followed by *SR*13 (69.29%), S*R*6 (69.64%) and *SR*9 (73.54%). All isolates also affected the germination, root and shoot length of lentil. The isolate SR4 (Temari) was most virulent, caused 100 per cent mortality. Therefore, it can be used as inoculum (Mass multiplication on wheat grain medium) to make sick soil for further screening studies.

Among four different soil types found in Chhattisgarh region, mortality per cent (19.17 %) was recorded in Matasi. However, highest mortality was observed in Kanhar 57.41 %.

Highest germination was observed in Kanhar (92.67 per cent) followed by Dorsa and Bhata 88.65 per cent, which were found to be at par with each other. While, the lowest germination per cent was observed in Matasi that is 83.94 per cent. Maximum root length was observed in Kanhar (36.19 cm) and Bhatha (35.16 cm) followed by Dorsa (33.78 cm) which were at par with each other. Minimum root length was recorded in Dorsa that is 33.78 cm. Maximum shoot length was observed in Khanar (26.85) followed by Bhata (24.70 cm) which were at par with each other . Minimum shoot length was recorded in Matasi (23.39 cm) and Dorsa (23.45 cm).

Soil type also affects the vigour index of plant. The vigour index is multiplication of germination percentages and seedling length of the final day count . Maximum vigour index was found in Kanhar that is 5841.91 where as minimum vigour index was recorded in Matasi (4709.03)

In case of soil texture, least mortality per cent (23.14%) was recorded in sandy loam texture followed by sandy texture with mortality per cent of 26.82 % which were at par with each other. However, highest mortality was observed in clay texture soil 62.96 %, followed by Clay loam 46.59 %. Highest germination was found in sandy textured soil (100.0 per cent) followed by Sandy loam (96.22%) and Silt loam (95.55%) which were significantly superior with other treatments. Loam , Silt clay and Clay loam soil supported lentil seed to germinate by 93.11, 93.33 and 86.55 per cent, respectively. While, lowest germination per cent was observed in Clay (85.11%).

Maximum root length was observed in Sandy soil (22.27 cm) which significantly superior than other treatments. Minimum root length was recorded in Clay soil (14.07 cm). The root length Silt loam (19.47 cm), Silt clay (19.00 cm), Sandy loam (18.60 cm) and Loam (20.47) were statistically at par among themselves. Maximum shoot length was recorded in Clay soil (19.27 cm) followed by LSandy loam (15.67 cm), Clay loam (16.60 cm) Silt clay and Silt loam (17.20 cm) which were at par with each other. Minimum shoot length was observed in Clay soil (19.27 cm). Soil type also affects the vigour index of plant. Maximum vigour index was found in Sandy soil (4054) where as minimum vigour index was in Clay loam (2837.56).

An experiment was conducted to know which soil pH is favorable or unfavorable for collar rot disease development caused by *S. rolfsii* in lentil. Significantly least mortality per cent (20.97%) was recorded at pH 8.5 followed by pH 8 (24.02 %), pH 7.5 (39.81 %), pH 7 (43.33 %), pH 5 (44.21 %) and pH 5.5 (52.99 %). However, significantly highest mortality was observed at Ph 6.0 that is, 81.25 % followed by pH 6.5 (65.56). Highest germination was observed in pH 7.0 that is 90.00 per cent, followed by pH 7.5 with 86.22 per cent. Whereas, Whereas lowest germination was observed in pH 5 that 65.44 per cent followed by pH 5.5 (68.22 per cent) which were at par with each other. Maximum root

length was observed in pH 7.0 (20.20 cm) followed by pH 8.0, pH 8.5 (19.07 cm) and pH 7 (18.60 cm) which were at par, but significantly better than other treatments. Minimum root length was recorded in pH 5 that is 14.87 and pH 5.0 (15.53 cm). Maximum shoot length was observed in pH 7.0 (21.27 cm) followed by pH 8.5. (20.33 cm), pH 8.0 (20.27 cm),pH 7.5(20.20 cm) and pH 6.5 (19.80 cm) which were at par with each other. Whereas , minimum shoot length was recorded in pH 5.0 (17.20 cm). Highest vigour index was found in pH 7 that is 3771.00 whereas least was in pH 5 that is 2141.85.

Fifteen *Trichoderma* spp. were isolated from the soil samples collected from different location of Chhattisgarh and were characterized for cultural and morphological characteristics on PDA medium. All the isolates shared characteristics of *Trichoderma*.

Trichoderma isolates varied in growth pattern, that is, effused (*T*4, *T*12, *T*22 and *T*23) and subdued (*T*1, *T*7, *T*14, *T*19, *T*20, *T*29, *T*31, *T*32, *T*33, *T*36 and *T*40). Difference was observed in colony appearance like, uniform ringed velvety (T1), velvety at periphery (*T*4, *T*14 and *T*20), velvety ringed (*T*7, *T*19, *T*29, *T*31, *T*32, *T*33 and *T*40) and scattered (*T*12, *T*22, *T*23 and *T*36).

Trichoderma varied in the ability to form pustules. No pustules was found in isolate *T*1, *T*7, *T*19, *T*20, *T*29, *T*31, *T*32, *T*33 and *T*40. Scattered pustules were observed in isolate *T*4, compact pattern pustules in *T*12 and *T*23 whereas, minute pustules were observed in *T*22 and *T*36

Pigmentation of *Trichoderma* isolates were studied on PDA plates. The production of diffusible yellowish orange pigments were found in *T*1, *T*7, *T*12, *T*19, *T*22, *T*29 and *T*40 isolates, whereas, in *T*4, *T*14, *T*20, *T*23, *T*31, *T*32, T33 and T36 isolates no pigmentation were found. However, no odour was found to produce from *Trichoderma* isolates.

The cultural characteristics of the isolates were significantly variable from each other. All the forty isolates showed fast growth covering the full petriplate growth (85mm) with in 3rd day of incubation at 25°C. Conidiation was predominantly effuse and conidial/colony colour change was observed of different shades like light green, dark green-white, light green-white, yellow green and white with aerial, sub-aerial and submerged-aerial type of growth pattern.

Among 40 isolates of *Trichoderma*, one isolate of *Trichoderma* T_{23} was producing completely yellow colour in petriplate growth which was observed not only from back side of petriplate but also from the front side growth in petriplate. This shows its high capacity to produce antibiotics.

In Gelatin hydrolysis test, twenty two isolates were categorized in two group based on liquification/hydrolysis of gelatine media nineteen isolates releaved positive whereas, three isolates were negative to gelatin hydrolysis test (Table 4.65). Positive result means liquification of media, *P*2, *P*3, *P*8, *P*14, *P*19, *P*20, *P*22, *P*23, *P*25, *P*27, and *P*29 isolates may be considered as *Pseudomonas fluorescens* or *Pseudomonas aeruginosa.* Negative result means solidification of media and may considered as *Pseudomonas putida* (*P*4, *P*7 and *P29).*

Nitrate utilization nineteen isolates were assign positive (+) (*P*2, *P*3, *P*4, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*23, *P*25, *P*27, *P*29 ,) and may considered as *P. fluorescens* whereas three showed negative (-) response (*P*7, and *P*29) unable to utilized nitrate and regarded as *P. putida* .

In starch hydrolysis test isolates were grouped in two categories, negative (-) giving blue colour and positive (+) with no colour change. This was grouped on the basis of blue colour formation when iodine indicator was added to the Petri plates. Nineteen isolates (*P*2, *P*4, *P*7, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*23", and *P*29,) exhibited negative (-) response which confirms the presence of *P. fluorescens* whereas three isolates (*P*3, *P*25 and *P*27) showed positive result.

In casein hydrolysis test seventeen isolates (*P*2, *P*3, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*25 and *P*29) exhibited positive (+) response and they may be considered as *P. fluorescens* or *P. aeruginosa* whereas, five isolates (*P*4, *P*7, P23 *P*27 and *P*29) showed negative result may assign as *P. putida*

In lypolytic test four isolates produce positive (+) to test (*P*2, *P*3, *P*7 and *P*40). These isolates may be considered as *P. fluorescens* or *P. aeruginosa* whereas, rest of the eighteen isolates (*P*4, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*23, P25, *P*27 and P29) exhibited negative (-) response

Isolates when inoculated in King's B medium and incubated at 4°C for 48 hours. Nine isolates (*P*2, *P*3, *P*4, *P*7, *P*8, *P*14 , and *P*29) showed growth therefore

they may be considered as *P. fluorescens* whereas, rest of the isolates (*P*18 and *P*27) may be *P. aeruginosa*

Antibiotic sensitivity test was used to distinguish different species of fluorescent *Pseudomonas.* Isolates (*P*2, *P*4, *P*3, *P*7, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*23, *P*25, *P*27 and *P*29) resistance to kanamycin may be considered as *P. aeruginosa*, except P41 which exhibited sensitivity towards kanamycin can assign as fluorescent *Pseudomonas* (Plate 29). On other hand in carbenicillin test isolates showed resistance response may be considered as fluorescent *Pseudomonas* or, *P. putida* (*P*2, *P*3, *P*4, *P*18, *P*19, *P*20, *P*22, *P*25, and *P*29) while isolates sensitive to carbenicillin may be *P. aeruginosa* (*P*7, *P*8, *P*14, *P*23, *P*27 and *P*40).

In triple sugar iron agar test Nineteen isolates (*P*4, *P*3, *P*7, *P*8, *P*14, *P*18, *P*19, *P*20, *P*22, *P*25, *P*27 and *P*29) produced red colour after on slant and butt after incubation whereas isolate (*P*2 and *P*23) produced yellow colour on slant

In hydrogen sulphide test the black colour is due to the production of H_2S from sodium thiosulphate present in the medium that then combines with ferrous ammonium sulphate resulting in the formation of black insoluble ferrous sulphide. This was found in ten isolates (*P*3, *P*4, *P*8, *P*14, *P*18 and *P*19).

Biochemical Characterization of *Bacillus* spp. all the thirty four isolates showed positive results to Gram reaction, Growth at 45^{o}C temperature, utilization of citrate, starch hydrolysis test and acid production. Out of 34 isolates of *Bacillus* spp., 25 isolates showed positive results (i.e. bacterial growth) in 7% NaCl concentration while remaining 12 isolates (*BC*-13, *BC*-14, *BC*-45, *BC*-52, *BC*-53, *BC*-56, *BC*-57, B*C*-64, *BC*-65, *BC*-118, *BC*-131 and *BC*-133) did not show any bacterial growth when inoculated in liquid nutrient broth plus 7% NaCl. All the isolates of *Bacillus* spp. except two (*BC*-14 and *BC*-118) exhibited turbid growth when grown in liquid casein medium (with 0.5% glucose) at pH 5.7. Out of thirty four isolates tested, twelve isolates (*BC*-17, *BC*-28, *BC*-34, *BC*-51, *BC*-52, *BC*-57, *BC*-65, *BC*-108, *BC*-123, *BC*-125, *BC*-131 and *BC*-133) showed negative reaction and remaining isolates showed positive reaction for VP test. All thirty four isolates of *Bacillus* spp. exhibited negative result as none of the isolates could grow under anaerobic condition. Based on the results of different biochemical tests, 15 (out of

34 isolates) could be identified as *Bacillus subtilis.* The remaining isolates could not be grouped into a particular species, as the biochemical character.

Effect of plant age on collar rot disease development was significantly highest per cent mortality of 90 per cent was recorded in 10 days old plant. This was followed by stage 20 days old plant with 80 per cent mortality. Plant age of 30 days and 45 days recorded 60 per cent and 40 per cent mortality respectively.

Effect of different date of sowing on Collar rot disease development was shows that incidence of wilt was recorded higher in early sown crop that is 20th Oct. in both the year of testing. Maximum mean disease incidence 62.90 per cent was recorded in 20th Oct sown crop followed by 30th Oct sown crop (54.67%). The minimum collar disease incidence was recorded 23.25 per cent in the late sown crop 30November in both years.

Effect of Tillage practices for disease control was in shows that incidence of collar rot was recorded higher in zero tillage in both the year of testing. Maximum mean disease incidence 37.12 per cent was recorded in lentil crop followed by Conventional tillage (31.60%). The minimum collar rot disease incidence was recorded 16.73 per cent in the minimum tillage in both years.

Effect of seed priming for disease control was shows that incidence of collar rot was recorded higher in no seed priming in both the year of testing. Maximum mean disease incidence 14.40 per cent was recorded in lentil crop. The minimum collar rot disease incidence was recorded 12.68 per cent in the seed priming in both years.

Fortin *Trichoderma* spp. isolates were isolated from the soil samples collected from different infected areas of Chhattisgarh and were characterized for cultural and morphological characteristics on PDA medium. All the isolates shared characteristics of *Trichoderma* spp.

Among 40 isolates of *Trichoderma*, one isolate of *Trichoderma* T_{23} was producing completely yellow colour in petriplate growth which was observed not only from back side of petriplate but also from the front side growth in petriplate. This shows its high capacity to produce antibiotics.

It was observed that *Trichoderma* isolate T_{32} (91.69) showed its maximum inhibition capacity against plant pathogenic fungi namely *S. rolfsii*. Apart from the

direct inhibition of plant pathogens by mycoparasitism on hyphae of the different plant pathogenic fungi, *Trichoderma* spp. also have capacity to parasitize and kill the hard resting structure, sclerotia perpetuating in soil with their differential activity. In present investigation, it was interesting to note that a set of eight isolates namely T_5, T_{12}, T_{15}, T_{21}, T_{25}, T_{33}, T_{34} and T_{36} effectively parasitized the 100 % sclerotia of *S. rolfsii*.

Among the 40 isolates of *Trichoderma*, most of the isolates inhibited the growth of *S. rolfsii*. However, maximum inhibition in growth of *S. rolfsii* was recorded in case of isolate T_{32} . The maximum inhibition percentage of 91.69 was recorded by isolate T_{32} while minimum inhibition of 50.26%was recorded by isolate $T_{16.}$ Maximum inhibition of T7(91.16%) and T3(90.88%) in growth of *S. rolfsii* was recorded while minimum inhibition of T16(50.26) and T38(50.27) was recorded by *Trichoderma*

In vivo pot experiment, among the fifteen *Trichoderma* spp. isolates tested for their efficacy in overcoming collar rot disease of lentil by seed treatment, *T*23 and *T*40 were found to produce least mortality 20.83 % among all, subsequently followed by *T*4 and *T*36 with mortality 25.00%. Maximum mortality was observed in *T*7 that is 50.00% whereas in control 83.33%. Highest germination per cent was observed in eight isolates like *T*12, *T*14, *T*19, *T*22, *T*29, *T*31, *T*32 and *T*40 that is 90 per cent All the *Trichoderma* spp. isolates were significantly superior in increasing the root length over the control (9.33 cm), when challenged inoculated with *S. rolfsii* SR4 isolate. Maximum root length was recorded in *T*29 isolate (32.6 cm) and *T*33 isolate 31.8 cm. However, all the treatments in terms of shoot length, observed were at par with each other and with control. However, highest shoot length was exhibited by *T*7, *T*20 and T40 that is 17.2 cm, 15.3 cm and 15.0 cm respectively.

Therty fluorescent *Pseudomonas* isolates were evaluated for their efficacy against *S. rolfsii.* Isolate *P*26 was found significantly efficient in reducing the mycelial growth of *S.rolfsii* by 79.01 % over control, followed by *P*7 (78.42 %), *P*13 (77.78 %), *P*22 (73.09 %), *P*3 (73.80 %), *P*16 (72.81 %), *P*29 (68.61%), *P*21 (67.98%), *P*12 (64.27%), *P*5 (61.90 %) which were statistically at par with each

other. Minimum inhibition was recorded in *P*120 (15.53 %) followed by *P*28 (17.07 %), *P*2 (32.09 %) and *P*18 (34.99%) which were at par with each other.

In vivo pot experiment, among thirteen fluorescent *Pseudomonas* isolates tested for their efficacy in overcoming collar rot disease of lentil by seed treatment, isolate P15 exhibited significant minimum mortality 15.24 per cent followed by *P*26 (15.57 per cent) and *P*22 (15.73 per cent) which were statistically at par with each other. These isolates showed significantly better results, over other isolates. Maximum mortality was observed in *P*8 (60.79 per cent) over control (86.26 per cent).

Highest germination per cent was recorded in the isolates *P*26 (96.86 per cent) followed by *P*7 (94.62 per cent) and *P*13, *P*29 (93.42 and 91.54 per cent) which were at par with each other. The above isolates were found to be significantly superior with all the other isolates and over control (70.69 per cent).

All the isolates of with fluorescent *Pseudomonas* affect the vigour index of lentil plant. Isolate *P*17 was found best in increasing vigour index (4504.82) followed by *P*4 (4255.85) and *P*10 (4050.00) as compared to other isolates and control (2170.00).

Twenty four *Bacillus* spp. isolates were isolated from collected soil sample from different location of Chhattisgarh and were evaluated for their efficacy against *S. rolfsii in vitro* and *in vivo* condition.

Bacillus spp. inhibited the mycelial growth by the antagonistic activity against *S. rofsii* and ranged from 18.73 to 72.33 per cent over control. Isolate *B*21 was found significantly efficient in reducing the mycelial growth of *S. rofsii* by 72.33 per cent over control followed by *B*19 (70.39 per cent1) which was statistically at par with *B*21. Minimum inhibition was recorded in *B*14 (18.73 per cent).

In vivo pot experiment, among *twenty isolates Bacillus* spp. isolates tested for their efficacy against collar rot disease of lentil by seed treatment. Isolate *B*21 exhibited significant minimum mortality 17.35 per cent followed by *B*19 (mortality 22.64 per cent) and *B*22 (mortality 27.24 per cent). These isolates

showed significantly better results, over other isolates. Maximum mortality was observed in *B*9 (69.54 per cent) as compared to control (81.95 per cent).

Highest germination per cent was observed in the isolates *B*21 (96.64 per cent) followed by *B*23 (92.34 per cent), *B*19 and *B*22 (91.32 and 90.50 per cent) which were at par with each other. The above *Bacillus* spp. isolates were found to be significantly superior with all the other isolates and over control (73.39 per cent).

All the isolates of *Bacillus* spp. isolate affects the vigour index of lentil plant. Isolate B21 was found best in increasing vigour index (4400.66) followed by *B*19 (3964.51) and *B*23 (3728.38) as compared to other isolates and control (2180.42)

After screening of potential biocontrol agent *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp., through *in vitro* and *in vivo* studies. Talc based formulations were prepared from respective isolate of biocontrol agent *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas* (*P-26*) and *Bacillus* spp. (*B-21*) and used for seed treatment and soil application.

Among the botanicals evaluated, *Agave* recorded maximum mycelial inhibition of 100 per cent at all the concentrations tested, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively with a mean of 65.25%. Neem leaves recorded 32.6, 41.1 and 41.3 per cent inhibition at 5, 10 and 15 per cent concentrations with a mean of 38.3%; NSKE 13.2, 17.4 and 22.4 per cent

Biocontrol agents, *Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp., seed treatment were evaluated with different doses (5g, 10g, 15g, 20g, 25g and 30g per kg seed) to find out effective dose for the control of collar rot of lentil under glass house condition.

In case of *Trichoderma* spp. (*T32*), all the treatments were significantly superior in decreasing the incidence of collar rot disease in lentil, over control. Seed treatment with *Trichoderma* spp. *23* (30 g/kg seed) exhibited least mortality (10.37 per cent) followed by *T*5 (25 g/kg seed) (14.07 per cent)*T*4 (20 g/kg seed) (15.83per cent) and *T*3 (15g/kg seed) (20.37 per cent) which were statistically at par with each other. Whereas maximum mortality was observed in T1 (5g/kg seed)

that is 38.42 per cent and *T*2 (10g/kg seed) (33.61 per cent) AS compared to control (86.31 per cent). Highest germination per cent was observed in seed treatment with *Trichoderma* spp. *T*6 (30 g/kg) and *T*3 (15g/kg seed) (96.67 per cent) followed by *T*5 (25g/kg seed) (93.33 per cent), *T*2 (10g/kg seed) (90.00 per cent) and *T*1 (5g/kg), *T*4 (20g/kg seed) (86.67 per cent) which were at par with each other. Treatment *T*4 (20g/kg seed) found best in increasing vigour index (4293.18) followed by *T*5 (25 g/kg) (4060.14), *T*3 (15g/kg) (3952.15) and *T*4 (20g/kg) (3924.15) as compared to other treatment and control (2258.56).

The seed treatment with fluorescent *Pseudomonas* (*Pf*-17), T23 (30 g/kg seed) exhibited significant minimum mortality (10.00 per cent) followed by *T*5 (25g/kg seed) (10.83 per cent), *T*4 (20g/kg seed) (14.07 per cent) and *T*3 (15g/kg seed) (mortality 18.52 per cent) which were statistically at par with each other. Whereas maximum mortality observed in *T*1 (5g/kg seed) that is 31.94 per cent as compared to control (86.31 per cent). Highest germination per cent was observed in *T*32 (30 g/kg seed) that is 100 per cent followed by *T*4 (20 g/kg seed) and *T*5 (25g/kg seed) (93.33 per cent) and *T*3 (15g/kg seed) (90.00 per cent) which were at par with each other. The above treatments were found to be significantly superior over control (73.33 per cent). Treatment *T*32 (30g/kg seed) was found best in increasing vigour index (5000.00) followed by *T*4 (20 g/kg seed) (4946.49) and *T*5 (25 g/kg seed) (4666.50) as compared to other treatment and control (2258.56).

Seed treatment with *Bacillus* spp. (*B-21*), *T*32 (30g/kg seed) exhibited least mortality (21.48 per cent) followed by *T*5 (25g/kg seed) (23.15 per cent), *T*4 (20g/kg seed) (25.00 per cent) which were statistically at par with each other. Whereas maximum mortality observed in *T*1 (5g/kg seed) that is 44.05 per cent as compared to control (86.31 per cent). Highest germination per cent was observed in *T*6 (30g/kg seed) and *T*4 (20g/kg seed) that is 93.33 per cent followed by *T*3 (15g/kg seed) (90.00 per cent) and *T*2 (10g/kg seed), *T*5 (25g/kg seed) (86.67 per cent) which were at par with each other. Treatment *T*6 (30g/kg seed) found best in increasing vigour index (4386.51) followed by *T*5 (25 g/kg seed) (4229.50) and *T*4 (20g/kg seed) (3994.52) as compared to other treatment and control (2258.56).

Combined application of seed treatment + soil application with *Trichoderma* spp. (*T32*) exhibited least mortality (18.14 per cent) followed by seed

priming + soil treatment (22.22 per cent) as compared to only seed treatment (32.22 per cent), soil treatment (33.33 per cent) and seed priming treatment (36.11 per cent) The seed and soil treatment (*T*4) was found best in increasing vigour index (4722.50) as well as germination 96.66 per centage as compared to the other treatment and untreated control.

Combined application of seed priming + soil application with fluorescent *Pseudomonas* formulation (*Pf*-26),exhibited least mortality (10.00 per cent) followed by only seed priming (13.70 per cent), seed + soil treatment (14.44 per cent) as compared to only soil treatment (19.07 per cent) and seed treatment (25.93 per cent). All the treatments were found to enhance germination per cent, but highest was observed in seed priming + soil treatment (100 per cent) followed by seed priming (96.67 per cent), seed + soil treatment (93.33 per cent), seed treatment (90per cent) and soil treatment (90 per cent). The above treatments were found to be significantly superior over control (76.67 per cent). Seed priming with with fluorescent *Pseudomonas* was found best in increasing vigour index (4930.17) followed by seed priming + soil treatment that is (4540.00).

Combined application of seed priming + soil application with *Bacillus* spp. (*B-21*) exhibited significant least mortality (21.48 per cent) followed by seed+ soil treatment (25.19 per cent), seed priming (26.85 per cent) as compared to soil treatment (33.33 per cent) and seed treatment (36.11 per cent). The seed priming and soil treatment was found best in increasing vigour index (4237.18) as well as germination (93.33 per cent) as compared to the other treatment and untreated control.

In vitro evaluation was done to study the efficacy of fungicides at different concentrations viz., 20, 50, 100, 200 and 500 ppm against *Sclerotium rolfsii* on potato dextrose agar (PDA) medium using poisoned food technique. Systemic fungicides like Hexaconazole 5% EC, Propiconazole 25% EC and combi products Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% showed complete inhibition of the pathogen at all the concentrations. Whereas, the non-systemic fungicide Mancozeb 75%WP, Thiram 75% WS and Propineb 70%

WP were found inhibitive only at higher concentrations (100 ppm) against *S. rolfsii*.

In vivo pot experiment was conducted to find out an effective fungicidal seed treatment against collar rot disease of lentil. Seed treatment with systemic fungicides, Hexaconazole 5% EC, Propiconazole 25% EC and Azoxystrobin 35% EC showed zero mortality that is 100 per cent decrease in disease incidence over control. Whereas, maximum mortality was observed in Carbendazim 50% WP (14.97 per cent). Highest germination per centage was observed in seed treatment with Propiconazole 25% EC and Azoxystrobin 35% EC (100 per cent) followed by Benomyl (96.66 per cent). Seed treatment with Hexaconazole 5% EC was found best in increasing vigour index (3330) followed by Benomyl (3286.78) and Propiconazole 25% EC (3220).

Seed treatment with non-systemic fungicide Propineb 70%WP resulted least mortality (10.37 per cent) followed Mancozeb 75%WP (16.66 per cent) and Thiram 75%WS (20 per cent). Whereas maximum mortality was observed in seed treatment with Copper oxychloride (73.21 per cent). Highest germination per cent was observed in seed treatment with Propineb 70%WP and Mancozeb 75%WP (96.66 per cent). The seed treatment with Propineb 70%WP was found best in increasing vigour index (3712.1) followed by Mancozeb 75%WP (3586.53).

Seed treatment with combo fungicide, Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% exhibited zero mortality (100 p cvsd er cent) decrease over control. Whereas, the seed treatment with Metalaxyl 8% +Mancozeb 64% (29.81 per cent) and Carbendazim 12% + Mancozeb 63% WP (38.42 per cent) showed 63.71 and 53.21 per cent decrease, respectively over control.

Highest germination per centage was observed in seed treatment with Tubaconzole 50%+Trifloxystrobin 25% WG, Captan 70%+ Hexaconazole 5% WP and Carboxin 37.5% + Thiram 37.5% (100 per cent) followed by Propiconazole 13% + Difenoconazol (96.66 per cent). Seed treatment with Carboxin 37.5% + Thiram 37.5% and Tubaconzole 50%+Trifloxystrobin 25% WG were found best in increasing vigour index that is 3710 and 3700 respectively

Compatibility of biocontrol agents *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas*(P26), and *Bacillus* spp., (*B-21*) with seven fungicides(Hexaconazole 5% EC, Propiconazole 25% EC, Mancozeb 75% WP, Thiram 75% WS, Propineb 70% WP, Metalaxyl 8% +Mancozeb 64% and Carboxin 37.5% + Thiram 37.5%). Showed that the biocontrol agents were compatible with Propineb 70% WP *in vitro* condition. Therefore, Propineb 70% WP fungicide can be used for combined application with biocontrol agents for integrated management of collar rot of lentil caused by *S. rolfsii.*

The experiment was carried out to assess the efficacy of an integrated management strategy for lentil collar rot by the combined use of biocontrol agents (*Trichoderma* spp., fluorescent *Pseudomonas* and *Bacillus* spp .) and fungicide (Propineb 70%WP) under glass house condition.

Seed treatment with *Pf-26* + *B-21* + Propineb 70%WP exhibited least mortality (13.70 per cent) followed by treatment *T32* + *Pf-26* + *B-21* + Propineb 70%WP (14.07 per cent), *T32* + *B-21* +Propineb 70%WP (14.53 per cent), *T32* + *P-26* + Propineb 70%WP (15.83 per cent), *Pf-* 26 + Propineb 70%WP (17.77 per cent), *T32* + Propineb 70%WP (19.90per cent) and Propineb 70%WP (20.74 per cent) which were statistically at par with each other. These seed treatment showed significantly better results, over other treatments. Maximum mortality was observed in seed treatment with *B-21* (mortality 39.28 per cent).

Highest germination per cent was observed in seed treatment *Pf-* 17 + *B-21* +Propineb, *T32* + *Pf-26* + *B-21* and Propineb 70%WP (96 per cent) followed by *Pf-26*, *T32* + *Pf-26*, *T32* + *B-21*, *P-26* + Propineb 70% WP and *T32* + *Pf-26* + *B-21* + Propineb 70% WP (93.33 per cent)

Thus, the result showed that, the combination of seed dressing formulations of *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas Pf-26* and *Bacillus* spp. (*B-21*) and Propineb 70%WP provide maximum protection to emerging seedlings. The seed treated with *P-26* + *B-21* + Propineb 70%WP resulted the highest germination, effectively help to increase plant growth and minimum per cent disease incidence of collar rot of lentil in pot experiment.

Most of the strain of Trichoderma along with combination of fungicide showed positive response for plant growth promoting activity. Seeds treated with

Trichoderma isolates, showed significant increase in plant hight (53.62cm in T5 to 58.28cm in T7; 53.57cm in control treatment), No. of tiller (2.43 in T3 to 4.68 in T6; 2.25 in control treatment), Number of leaflets (66.62 cm in T7 to 84.40 cm in T4; 65.76 in control treatment), Number of flawer (49.37 in T5 to 53.46 in T7; 40.63 in control treatment), Number of pods (38.83 in T3 to 44.44 in T7; 34.95 in control treatment), Length of leaflets (4.55 cm in T6 to 5.90 cm in T4; 4.52cm in control treatment), Shoot Length (42.35 cm in T2 to 46.82 cm in T4; 41.99 cm in control treatment), Root lengh (16.33 cm in T2 to 20.97 cm in T7; 15.80 cm in control treatment), No. of root nodule3.12 in T3 to 3.75 in T7; 2.53 in control treatment), plant emergence (342.33 in T3 to 471.67 in T7; 307 in control treatment), Disease incidence (28.91 in T3 to 12.59 in T7; 307 in control treatment), Yield / hac. (1696 in T5 to 1762.27 in T7; 1631.87 in control treatment), Among these, seven treatment *viz.*, T1, T2, T3, T4, T5, T6, and T7 showed maximum increase in shoot length ranging from 45.43 cm (T7) to 46.82 cm (T4), root length ranging from 20.03 cm (T4) to 20.97 cm (T7) and yield 1728.33 (T4) to 1762.27 (T7)

During first *rabi* season 2018- 2019, total 139 lentil entries were screened against collar rot pathogen under field conditions. All the entries tested under Lentil LS AVT-1(7), Lentil LS AVT-2(6), Lentil EE IVT(21), Lentil EE IVT(10), Lentil MMLT(13), Lentil Germplasm(52), Lentil Released Variety(30) were susceptible to highly susceptible to collar rot. None entry was resistant or moderately resistant to collar rot.

While during second *rabi* season 2019-20, total 132 lentil entries were screened against collar rot pathogen under field conditions. All the entries tested under lentil AVT 2 Large seed CV(5), Lentil IVT Rice Fallow NEPZ(13), Lentil IVT Large Seed(22), ,lentil germplasm(34) , lentil germplasm(52)

132 lentil germplasm accessions were screened against collar rot pathogen under natural conditions. Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as resistant. These resistant sources can further be exploited in breeding program for the development of disease resistant commercial cultivars.

CONCLUSION

In the light of finding of present investigation, following conclusions were drawn:

During survey made at forteen locations in seven lentil growing districts of Chhattisgarh state, the occurrence and severity of lentil collar rot was very high during crop season. The highly prone area of collar rot was Mungeli district followed by, Bemetara and Raipur district.

Isolates of *S. rolfsii* exhibited a high degree of variability in cultural, morphological characteristics and aggressiveness.

SR 4 (Matia) isolate of *S. rolfsii* was found to be most virulent *in vivo* (sick soil method).

Soil factors (soil type, soil texture and soil pH) strongly influenced the collar rot disease development in lentil. Soil pH 6, Kanhar and clay soil exhibited highest mortality in lentil.

Trichoderma spp. (*T32*), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*B-21*) were found effective biocontrol agents against *S. rolfsii* as they showed strong antagonistic activity in dual culture techniques and *in vivo* pot condition.

Among the botanicals evaluated, *Agave* recorded maximum mycelial inhibition of 100 per cent at all the concentrations tested, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively with a mean of 65.25%. Neem leaves recorded 32.6, 41.1 and 41.3 per cent inhibition at 5, 10 and 15 per cent concentrations with a mean of 38.3%; NSKE 13.2, 17.4 and 22.4 per cent.

Seed treatment with biocontrol agent, *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*B-21*) were found to control collar rot of lentil efficiently under increased dose than the recommended dose.

Seed treatment with biocontrol agent *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*B-21*) alone was effective in controlling collar rot of lentil as compared to control but combined seed treatment and soil application significantly increased the plant vigour index and reduced the incidence of collar rot under greenhouse conditions.

Propineb 70% WP was highly effective fungicide against *S. rolfsii* under *in vitro* dual culture and *in vivo* pot experiment and also showed compatibility with biocontrol agents (*Trichoderma* spp. (*T32*), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*B-21*)) Therefore, Propineb 70% WP fungicide can be used for combined application with biocontrol agents for integrated management of collar rot of lentil caused by *S. rolfsii.*

The combination of seed dressing formulation *Trichoderma* spp. (*T32*), fluorescent *Pseudomonas* (*Pf*-26) and *Bacillus* spp. (*B-21*) and Propineb 70%WP provided maximum protection to emerging seeding. The seed treatment with *Pf*- 26 + *B-21* + Propineb 70%WP resulted the highest germination, increased plant growth and minimum per cent disease incidence of collar rot of lentil in pot experiment.

Among all the treatment of *Trichoderma* along with combination of fungicide the treatment of combination of *Trichoderma harzianum*+ Propineb and *Trichoderma* mutant formulation significantly reduced lentil plant mortality (and was superior to other *Trichoderma* based formulations. Combination of *Trichoderma harzianum*+ Propineb and *Trichoderma* mutant was evaluated and compared with a few other *Trichoderma* strains, fungicides, and combination treatment, at field condition over 2 years. Combination of *Trichoderma harzianum*+ Propineb and *Trichoderma* mutant treatment of seeds significantly improved seedling emergence, reduced disease incidence, and improved yield over both years.

Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as resistant.

SUGGESTIONS FOR FUTURE RESEARCH WORK

- Collar rot has become a serious problem in lentil growing areas of Chhattisgarh. Therefore, regular survey works are needed to be conducted to assess the severity at regular intervals. This helps in devising management practices.
- More advanced techniques such as SSR and RFLP can be applied for better understanding of genetic diversity and pathogenic variability among the isolates of *S. rolfsii*.
- The genotypes, resistant at seedling and vegetative stage, should be utilized in breeding programmes to build disease resistant pyramids due to complex nature of *S. rolfsii*.
- More intensive studies need to be conducted at the molecular level in order to understand the nature of mechanism by which the resistant host defends against infection.
- More attention needs to be focused on more microbes for identifying their resistance imparting and growth promoting abilities.
- Integrated management strategy for collar of lentil that is combined the use of microbial antagonist and fungicide should be taken under field condition.
- Condition influencing the performance and adoptability of the *Trichoderma* and *Pseudomonas* isolates to be worked out.
- *Trichoderma* genomes are a source of candidate genes for developing transgenic plants that are tolerant to biotic and abiotic stresses. Development of engineered plants will be benefited by *Trichoderma* genome mining.
- There is a need to construct future research programmes that aim to promote fluorescent *Pseudomonad* as a potential bio-pesticide for augmentative biological control of many diseases of agriculture and horticultural importance.

➢ The success of these PGPR agents will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms.

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