STUDIES ON *Alternaria solani* CAUSING EARLY BLIGHT DISEASE IN TOMATO (*Lycopersicon esculentum* Mill.)

M. Sc. (Ag.) Thesis

by

Pankaj Kumar

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, RAIPUR
FACULTY AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA,
RAIPUR (Chhattisgarh)

2017

STUDIES ON *Alternaria solani* CAUSING EARLY BLIGHT DISEASE IN TOMATO (*Lycopersicon esculentum* Mill.)

Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Pankaj Kumar

IN PARTIAL FULFILLMENT OF THEREQUIREMENTS FOR THE DEGREE OF

Master of Science

in

Agriculture

(Plant Pathology)

V.V. ID No. 20151622549 ID No. 120115135

JULY, 2017

CERTIFICATE – I

This is to certify that the thesis entitled **"Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill.)"** submitted in partial fulfillment of the requirement for the degree of **Master of Science in Agriculture (Plant Pathology)** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Pankaj Kumar** 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 has been published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

21/07/2017

Date: 21/07/2017 **Chairman**

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman : (Dr. Shyam Singh)

Member : (Dr. G.K. Awadhiya)

Member : (Dr. Hemant Kumar Panigrahi)

Member : (Dr. (Smt.) G. Chandrakar)

Member : (Dr. R.K. Dantre)

CERTIFICATE-II

This is to certify that the thesis entitled **"Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill.)"** submitted by **Pankaj Kumar** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Plant Pathology** has been approved by the external examiner and Student's Advisory Committee after oral examination.

Signature External Examiner

Date: **(Name .....)**

Major Advisor _____

Head of the Department _____

Faculty Dean _____

Approved/Not approved

Director of Instructions _____

ACKNOWLEDGEMENT

Research needs the close co-operation of the friends and colleagues and the guidance of experts in the field to achieve something worthwhile with light patience, vigour and dedication of the person.

"A journey is easier when you travel together; interdependence is certainly more valuable than independence". I bow to Almighty "GOD" who keeps ever burning before my vagrant steps the kindly light of hopes and always showered blessing on me without whose endless benevolence and blessing this tedious task could not have been accomplished.

With a sense of high resolve and reverence, I would like to give my sincerest thanks to my Chairman of my advisory committee ***Dr. Shyam Singh****, Assistant Professor, Department of Plant Pathology, S.K. College of Agriculture and Research Station, Kawardha (Indira Gandhi Agriculture University, Raipur, C.G.), for his precious instruction, extra efforts, broad and profound knowledge, unique supervision, his patient instruction, sparing his valuable time has given me a great inspiration and help at every step during my thesis and pleasure to conduct this thesis under his supervision. With a great reverence, I express my sincere thanks to respected members of my advisory committee Dr. G.K. Awadhiya, Professor, Department of Plant Pathology, Dr. Hemant Kumar Panigrahi Assistant Professor, Department of Fruit Science, Dr. (Smt.) G. Chandrakar, Professor, Department of Mathematics, Statistics & Computer Science, Dr. R. K. Dantre, Professor, Department of plant pathology, College of Agriculture, Raipur, for their critical suggestions, keen co-operation and kind help rendered as and when needed.*

I wish to record my sincere thanks to Dr. S.K. Patil, Hon'ble Vice Chancellor, Dr. S.S. Shaw, Director of Instructions and Dr. J.S. Urkurkar, Director Research Services, Sri. S.K.Verma Registrar, Dr. M. P. Thakur, Director Extension Services, IGKV, Raipur, Dr. O.P. Kashyap Dean, College of Agriculture Raipur, Dr. R.K.. Dwivedi Dean, College of Agriculture Kawardha, IGKV, Raipur, for providing necessary facilities, technical and administrative supports for conductance of this research work.

I pay my sincere thanks to Dr. A. S. Kotasthane, Professor and Head, Department of Plant Pathology, College of Agriculture, Raipur (C.G.).for his excellence guidance during course of investigation and providing necessary facilities, for their kind help and valuable suggestions during the course of investigation.

I am highly obligated to all teaching staff members of Department of Plant Pathology, namely Dr. V.S. Trimurty (Retd. Professor), Dr. N. Khare, Dr.C. S. Shukla, Dr. K.P. Verma, Dr. C.P. Khare, Dr. N. Lakpale Dr. P. K. Tiwari and Shri

H. K. Singh Department of Plant Pathology, College of Agriculture Raipur (CG), IGKV for their co-operation, valuable suggestions and encouragement during the course of study.

I would like to express my sincere gratitude to Dr. M. Pandey (Librarian, Nehru Library, Raipur) and Shri Anjeet Jangre, Department of Agronomy, S.K. College of Agriculture and Research Station, Kawardha for giving me their kind helps during my present study.

I would also like to thank Mr. Siyaram sahu, Mrs. pushpa sahu, Ukiya dadi, Ghanshyam and other technical and non-technical staff members of Department of Plant pathology, IGKV, Raipur and Mr. Tulsi Bagh for the necessary facilities they provided to me during my thesis work.

Words can hardly express the heartfelt gratitude to my beloved father Shri Rameshwar Prasad Dilliwar, Maa Smt. Mongra Devi Dilliwar and Mr. SomeshwarDeshmukh, Mathura Deshmukh whose selfless love, filial affection, obstinate sacrifices and blessing made my path easier. My most cordial thanks goes to my elder brother Mr. Sunil and bhabhi Smt. Sarika, sister Lata, Manju and jiju Hemant, Kulesh and my all family members whose obstinate sacrifice, filial affection and blessing made my path easier.

I render my heartiest appreciation and gratitude to my lovely wife Smt. Vidya Dilliwar and daughter Ojasvi (Pihu) who took trouble adjusted with me and encourage me during the course of study.

I will be failing in my duties if I don't convey my sincere thanks to Ajani , Meghchand, Sandeep, Shekhar and my batchmates Pankaj , Abhisek, Parikshit, Praveen, Nirmal, Dheeraj, Nohar, Veer, Parshuram, Avinash, Devika, Leena, Lekha, Santoshi, Yuvraj and Kamlesh.

I wish to extend my heartiest thanks to Smt. MaltiShyam Singh, Yajvendra, Jeetendra, Rupesh, Pramod, Nandkumar, Deepak, Prafull, Awdhesh, Basant, Adit nandan, Kirti, Sachin, Shashikant, Suryanarayan, Bodish, Deepak ukey Ankit and Meenu for their filial affection, constant encouragement, and sincere prayers, so as of enable me to complete this thesis. I would also like to convey cordial thanks to all those who helped me directly or indirectly to fulfill my dreams.

At last but not the least I would thank the almighty God for all his blessings and showerings he offered me.

(Pankaj Kumar)

Department of Plant Pathology

College of Agriculture, IGKV, Raipur

(Chhattisgarh)

LIST OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

CD	Critical difference
cm	Centimeter
DAS	Days after Sowing
EC	Emursifiable concentreation
et al.	*et alii*
Fig	Figure
g	Gram
hrs	Hours
i.e.	That is
kg	Kilogram
kg/ha	Kilogram per hectare
lit	Liter
m	Meter
mw	Metrological week
m2	Meter square
ml	Milliliter
No	Number
NS	Non-significant
NSKE	Neem seed kernel extract
PDC	Percent disease control
PDI	Percent disease index
ppm	Parts per million
SEm	Standard error of mean
SN	Serial Number
SMW	Standard metrological week
µm	Micrometer
WP	Wettable Powder
%	Per cent
/	Per
@	At the rate of
HAI	Hours after incubation

THESIS ABSTRACT

a) Title of the Thesis	:	"Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill)
b) Full Name of the Student	:	Pankaj Kumar
c) Major Subject	:	Plant Pathology
d) Name and Address of the Major Advisor	:	Dr. Shyam Singh, (Asst.Professor) Department of Plant Pathology, S.K. College of Agriculture and Research Station,Kawardha Dist.- Kabirdham Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)
e) Degree to be Awarded	:	M. Sc. (Ag.) Plant Pathology

Signature of Major Advisor

Signature of the Student

Date: 21/07/2017

Signature of Head of the Department

ABSTRACT

The present investigation on "Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill) was carried out in the Department of Plant Pathology, S.K.College of Agriculture and research station (IGKV, Raipur), Kawardha, during 2016-17.

Among the several disease of tomato, early blight of tomato is one of the most destructive diseases, which cause heavy losses in fruit yield on tomato. The symptoms of early blight of tomato were first observed as small brown water

symptoms of early blight of tomato were first observed as small brown water soaked lesion on the older leaf. Symptoms also reported on stem and petioles as brown to dark brown elongated target board type spots on leaf, stem and fruit. Survey carried out during *Rabi* 2016-17 revealed that severity of early blight of tomato was more in Mungeli district followed by Rajnandgaon and Kabirdham district. In case of village the highest PDI was noticed in Kalegondi village of Rajnandgaon district, while the lowest PDI was recorded in Arasnara village of Durg district. Maximum AUDPC was recorded in Rajnandgaon district followed by Mungeli. However, least AUDPC was found in Durg district. *Alternaria solani* was identified as the pathogen of early blight disease on the basis of morphological characters and pathogenicity test. The conidia were observed solitary straight and slightly flexuous oblong or muriform or ellipsoidal tapering to beak, pale or olivaceous brown colour, length 28-75µm and 15-20µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. Among the isolates of *A.solani*, different culture media were exhibited variation in respect of colony colour, substrate colour, growth pattern, margin colour, topography, thickness of mycelium and sporulation. *Alternaria solani* produce grayish, dark brown, grayish black to olive green colony colour in different isolate on different medium. Growth pattern were also varied among the isolates from circular smooth to irregular rough. Margin colour of colony was varied from whitish to brown among the isolates. Isolates produce Aerial, submerge to merge topography. Margin colour of colony was varied from whitish to brown among the isolates of *A. solani.* Water agar media produce maximum sporulation in all seven isolates. Among the fourteen solid culture media, potato dextrose agar medium produce maximum mycelia growth followed by oat mea agar media, while water agar medium was produce minimum mycelium growth. However, in liquid media oatmeal broth gave higher biomass followed by potato dextrose agar media. Minimum biomass was obtained in mineral broth. The fungus preferred neutral pH level (6.0-7.0) for better growth and sporulation. The pathogen showed maximum growth and sporulation at 25-30°C temperature.

Among the fungicides, most effective fungicide was found Hexaconazole 5%EC followed by Thiafluzamide 24% SC under *in vitro* condition. Among the plant extacts, *Allium sativum* gave maximum inhibition of mycelial growth followed by *Crotalaria juncea* and *Azadirachta indica*, while *Euphorbia hirta* was showed least mycelium inhibition. Out of the different bio-agents tested, *P. fluorescens* was found most effective followed by *Trichoderma harzianum* under *in vitro* condition. Under field conditions, integrated effect of fungicides, *P. fluorescens* and NSKE was studied to determine effectiveness of integrated management strategy. All the treated plots showed significantly less percent disease index than untreated plot.T5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) was found the most effective followed by T6=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS). While, treatment T_7=Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas florescence* @ 1x 10^9 was found less effective over the control. The number of fruits per plant was found maximum in the plot which sprayed with T_5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS). The field evaluation of integrated management indicate that the Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) found most effective for the management of early blight disease, produce maximum fruit yield and net return.

शोध सारांश

शोध का नाम	:	टमाटर (*लाइकोपेर्सिकॉन एस्कुलेन्टम* मिल.) में *अल्टरनेरिया सोलानी* द्वारा उत्पन्न अगेती अंगमारी बीमारी पर अध्ययन
विद्यार्थी का पुरा नाम	:	पंकज कुमार
मुख्य विषय	:	पादप रोग विज्ञान
मुख्य सलाहकार का नाम व पता	:	डॉ. श्याम सिंह (सहायक अध्यापक) पादप रोग विज्ञान विभाग, संत कबीर कृषि महाविद्यालय एवं अनुसंधान केन्द्र, कवर्धा जिला कबीरधाम (छ. ग.)
सम्मानित किये जाने वाली उपाधि	:	एम. एस. सी. (कृषि), पादप रोग विज्ञान

मुख्य सलाहकार हस्ताक्षर

दिनांक : 21/07/2017

विद्यार्थी के हस्ताक्षर

विभागाध्यक्ष के हस्ताक्षर

शोध सारांश

वर्तमान शोध कार्य जिसका शीर्षक टमाटर (*लाइकोपर्सिकान एस्कुलेन्टम* मिल.) में अल्टरनेरिया सोलानी द्वारा उत्पन्न अगेती अंगमारी बीमारी पर अध्ययन है, जो कि संत कबीर कृषि महाविद्यालय एवं अनुसंधान केन्द्र कवर्धा, जिला कबीरधाम, इंदिरा गांधी कृषि विश्वविद्यालय, रायपुर (छ.ग.) के पादप रोग विज्ञान के प्रयोग शाला एवं प्रक्षेत्र में किया गया था।

टमाटर की फसल में फफुंद के द्वारा कई बीमारियां उत्पन होती है पर फसल के शुरूआती समय में अगेती अंगमारी का प्रकोप बहुत होता है जिसके कारण टमाटर की उपज में भारी नुकसान होता है। शुरूआती समय में यह बिमारी पहले पुराने पत्तों पर छोटे भूरे रंग के घांवों (धब्बे) के रूप में उत्पन्न हुआ बाद में उपरी पत्ती, तना एवं फल पर गहरे भूरे रंग के लम्बी लक्ष्यबोर्ड प्रकार के लक्षण प्रसारित हुऐ। रबी वर्ष 2016–17 में टमाटर फसल उगाये जाने वाले पांच जिलों में अक्टुबर 2016 से फरवरी 2017 तक सर्वे

किया गया जिसमें सभी बीस टमाटर प्रक्षेत्रों का रोग की तीव्रता एवं प्रतिशत रोग सुचकांक दर्ज किया गया। मुंगेली जिले में रोग की औषत प्रतिशत रोग सुचकांक सबसे अधिक मात्रा में थी उसके बाद राजनांदगांव और कबीरधाम जिले में पाया गया। अल्टरनेरिया सोलानी को उसके रूपात्मक पात्रों और रोगजन्यता परिक्षण के आधार पर अगेती अंगमारी के रोग जनन के रूप में पहचाना गया। इसके बिजाणु एकांत सीधे और थोड़ा लहरदार, म्युरीफार्म, पीला या जैतुन के रंग जैसा भूरा 8–10 अनुक्रमिक और 0–4 अनुधैय प्रभेद के साथ 28-75 µm लंबा और 15-20 µm मोटा संरचना प्रदर्शित किया। सात विभिन्न स्थानों से प्राप्त वियोजनों द्वारा विभिन्न 14 माध्यमों पर उसके उपनिवेश रंग, वृद्धि का तरीका, किनारे का रंग, स्थलाकृति, कवक जाल की मोटाई, बीजाणु की संख्या एवं सबस्ट्रेट रंग के संबध में विभिन्नताएं प्रदर्शित की गई। अल्टरनेरिया सोलानी विभिन्न माध्यमों पर जैतुन हरी रंग, गहरे भूरे रंग, भूरे से काले रंग, प्रदर्शित करते है एवं किनारे रंग सफेद से लेकर भूरे रंग का पाया गया, चौदह माध्यमों मे आलू डेक्सट्रोज अगर माध्यम में सबसे अधिक कवक जाल का विकास देखा गया । जबकि तरल माध्यम में ओटमील माध्यम से प्राप्त सुखा कवक जाल (बायोमास) का वजन सबसे ज्यादा पाया गया। अल्टरनेरिया सोलानी के विकास और बीजाणु उत्पन्न होने के लिए तटस्थ पी. एच. स्तर (6.0-7.0) पाया गया एवं 25-30^{0}c तापमान पर अधिकतम कवक जाल वृद्धि देखा गया। रोगकारक के रोग थाम के लिए प्रयोगशाला में किये गये परीक्षण में प्रयोग किये गये फफुंदनाशकों में हेक्साकोनाजोल 5% EC द्वारा अधिकतम (97%) कवक जाल निषेध पाया गया। विभिन्न 6 प्लांट एक्सेट्रेक्ट में से 5% पर एलियम सटाईवम (40%) एंव क्रोटोलारिया जंसिया (38%) में सबसे ज्यादा कवक जाल का निषेध पाया गया। विभिन्न जैव कारकों का अल्टरनेरिया सोलानी के विरूदध परीक्षण किया गया जिसमें स्युडोमोनास फ्लूरेसेन्स सबसे ज्यादा प्रभावी पाया गया इसके बाद ट्राइकोडर्मा हार्जिएनम प्रभावी पाया गया। प्रक्षेत्र परिस्थितियों में एकीकृत प्रबंधन रणनीति की प्रभावशषीलता को निर्धारित करने के लिए फफुंदनाशक, स्युडोमोनास फ्लूरेसेन्स एवं NSKE का एकीकृत प्रभाव का अध्ययन किया गया। सबसे प्रभावी संयोजन $\mathbf{T_5}$ = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)पाया गया, इसके बाद $\mathbf{T_6}$=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) जबकि $\mathbf{T_7}$=Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 नियत्रण पर सबसे कम प्रभावी पाया गया। प्रति पौधे फल की संख्या $\mathbf{T_5}$ = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)में अधिकतम मिला था। एकीकृत प्रंबधन के सम मुल्यांकन से स्पष्ट है कि अगेती अंगमारी बीमारी के लिए $\mathbf{T_5}$ = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) प्रभावी प्रबंधन एवं अधिकतम फल उत्पादन व शुद्ध मुनाफा प्राप्ति के लिए सबसे प्रभावी पाया गया।

* * * * * *

CHAPTER–I
INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most remunerative and widely grown vegetables in the world. It is a small annual or short lived perennial herb belonging to the family Solanaceae, probably native of 'Peru-Equador'. It is a regular kitchen component of Indian diet which is used as raw fruit and also as cooked processed products like soup, ketchup, sauce, pickle, pastes and powder. The pulp and juice of tomato is very digestible, promoter of gastric secretion and blood purifier, additionally it nutrients and metabolites (Folate, potassium, and vitamins A and C) that are important for human health. Tomato seed contains 2.4 percent oil which has great medicinal value. Tomato fruits content about 95 percent water and 5 percent other component mainly carbohydrates and fibers. Details of nutrients contents in fresh tomato fruit are given in table 1.1.

Table 1.1: Nutrient value of tomato in fresh fruit ($100gm^{-1}$).

Constituents	Value	%	Constituents	Value	%
Energy	18 Kcal	1.0%	Vitamin K	7.9 µg	6.5%
Carbohydrates	3.90 g	3.0%	Folates	15.0 µg	4.0%
Protein	0.90 g	1.6%	Thiamine	0.037 mg	3.0%
Total Fat	0.20 g	0.7%	Calcium	10.0 mg	1.0%
Cholesterol	0.00 mg	0.0%	Iron	0.30 mg	4.0%
Dietary Fibre	1.20 g	3.0%	Magnesium	11 mg	3.0%
Vitamin A	833 IU	28.0%	Manganese	0.15 mg	6.5%
Vitamin C	13 mg	21.5%	Phosphorus	24 mg	3.0%
Vitamin E	0.54 mg	4.0%	Zinc	0.17 mg	1.5%

(**Source:** USDA National Nutrient database)

Tomato cultivation has become more popular since mid nineteenth century because of its varied climatic adaptability and high nutritive value. Tomato is being extensively grown as an annual plant all over the world. Tomato has ranks second next to potato in world acreage but it has rank first among processing crops. It is cultivated in an area of 4.73 million hectares all over the world with production of 163.96 million tones and an average yield of 34.66 tones ha^{-1}. In India, it is grown in a wide range of climate across states of Andhra Pradesh, Odisha, Karnataka, Maharashtra, West Bengal, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh and Chhattisgarh, accounting total production of 18732 thousand tones from an area of 774 thousand hectares with an average productivity of 24.20 tones ha^{-1} during 2015-16 (Anonymous, 2016). In Chhattisgarh, it is cultivated throughout the year during rainy, winter and summer seasons. It is occupies an area of 55.081 thousand hectares with production of 938.567 thousand tones and productivity of 17.04 tones ha^{-1} during 2015-16 (Anonymous, 2016).

Tomato is highly sensitive to abiotic stresses especially extreme temperature, salinity, drought, excessive moisture and environmental pollution and biotic stresses. Tomato plants are suffered with large number of biotic stresses including insect pests and diseases from the time of emergence to harvest. Tomatos suffer with various diseases cited by fungi, bacteria, viruses, nematodes etc. in several countries (Mark *et al*., 2006). More than 200 diseases have been reported to infect tomato in the world (Atherton and Rudich, 1986). Large number of fungal diseases such as early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), Powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Collar rot (*Sclerotium rolfsii*), and Damping off (*Pythium sp.)* are causes severe losses in tomato. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones *et al.*, 1991).

Alternaria solani has ability to survive for a long time on the diseased plant deberies in soil in the absence of main host (Moore and Thomas, 1942 and Basu, 1971). Rotem (1998) reported that *Alternaria solani* may survive for more than ten years in the soil on plant debris and seeds at optimum temperatures. *Alternaria solani* survived on also other solanaceous cultivated crops such as potato, pepper, egg plant and weed host.

Alternaria solani infect all the aboveground parts of the plant at all stages of growth and development (Peralta *et al*., 2005; Verma and Verma, 2010). The plants are more susceptible to infection by the pathogen during fruiting period (Cerkauskas, 2005; Momel and Pemezny, 2006). Appearance of rotting on either green or ripe fruit was observed when most of the tomato fruits gate infection with *A. solani* (*Morris et al.,* 2000; Blancard *et al.,* 2012). The maximum incidence of fruit rot disease has been observed in ill drained and low laying fields. *A. solani* produced a wide range of symptoms on leaves, stems, petiole, twig and fruits under favorable conditions which are leading to defoliation, drying off of twigs and premature fruit drop (Mathur and Shekhawat,1986). Initial symptoms of the early blight disease appear as brown to dark leathery necrotic spots on the lower leaflets (Locke, 1949). Spots are oval or angular in shape ranging from 0.3 to 0.4cm diameter with usually narrow chlorotic zone around the spot (Walker, 1952). Symptoms progress from lower to upper leaves. As the spots mature, concentric rings raised and depressed brown tissue are evident. Heavily infected plants often become defoliated.

The pathogen *Alternaria solani* belongs to phylum: Ascomycota, class: Deuteromycetes, order: Moniliales, family: Dematiaceae (Jones and Grout, 1986). According to morphological characters and phylogenetic analysis, *Alternaria solani* bear large, long- beaked and non catenated spores (Simmons, 2000). The mycelium consisted of septa, branched, light brown hyphae which turned darker with age. The conidiophores are short, 50-90 µm long and dark in colour. Conidia are 120-296 ×12-20 µm in size, beaked, muriform, dark colour and born singly. Conidia contained 5-10 transverse septa and 1-5 longitudinal septa (Singh, 1987*). A. solani* have ability to sporulate asexually and produce conidia, however sexual stage of the fungus still unknown.

A. solani is a soil inhabiting fungus and it can also come from other host through air and splashing rain. The germinating spores of *A. solani* penetrate susceptible tissue directly or through wounds and soon produce new conidia that are further spread by wind, splashing rain, *etc.* (Agrios, 2005). The disease can occur over a wide range of climatic conditions, but it is most prominent in areas where received heavy dew deposition, heavy rainfall precipitation and high relative humidity. In severe rainfall, high humidity and fairly high temperatures 24-29^{0}C are more favorable for disease development (Peralta *et al*. 2005).

The early blight was the most catastrophic diseases incurring loss under field and post harvest stages causing 50 to 86 percent reduction in fruit yield (Mathur and Shekhawat, 1986). Every one percent increase in disease intensity can reduce yield by 1.36 per cent, and complete crop failure can occur when the disease is most severe. Yield losses up to 79 per cent have been reported in the field by Sherf and MacNab (1986). Saha and Das (2012) reported losses in yield 0.75 to 0.77 tons ha^{-1} with 1per cent increase in disease severity. Infection accompanied by the production of toxins by *A. solani,* including some non-host specific toxins called alternaric acid, zinniol, altersolanol and macrosporin. The toxins act on the host protoplast to disturb physiological processes that sustain plant health (Agrios, 2005).

Once early blight is established in the crop, it is very difficult to be controlled (Smith and Kotcon, 2002). Fungicide treatments are generally the most effective control measures, but are not economically feasible in all areas of the world and may not be effective under weather conditions favorable for disease epidemics (Herriot *et al*., 1986). Beside this *Alternaria solani* has low sensitive with fungicides because of its production of dark brown to black pigment called melanin which enhanced survival and competitive abilities of the pathogen under certain environmental conditions (Bell and Wheeler, 1986). However, in the recent years, huge use of fungicides in agriculture has been the subject of growing concern for both environmentalist and public health authorities. Now days various botanical and bio-control agents available which can reduce populations of foliar pathogens but there effect are very slow. Plant extracts have shown the antimicrobial activity against fungal pathogens under *in vitro* and *in vivo* conditions (Kagale *et al.,* 2004). Bio-control agents are used for the control of soil

borne, foliar and post harvest diseases in various crops in the field, in commercial green house and storage depots (Abeysinghe, 2009; Jegathambigai *et al*., 2010). Root colonizing bacteria, especially *Pseudomonas spp*., can efficiently control diseases caused by soil borne pathogens (Maurehofer *et al*., 1994). Any one of the above control measure is alone unable to suppress disease in sustainable crop production.

Besides, these botanicals and bio-agents are environmentally safe and these are major components in integrated disease management programs (Bowers and Locke, 2004). An integrated control will have to be considered with bio-control agents, botanicals as well as chemicals for the management of disease. Thereby, novel approach requires low amount of chemicals to reduce pollution hazards as well as the cost of management. So, the possibilities of controlling plant disease by the integration of several methods have been the subject of extensive research. An integrated control will have to be considered with bio-agents, botanicals as well as chemicals for the management of airborne pathogens. Integrated disease management is a good strategy for the control of the early blight disease in tomato.

In recent year, the disease has assumed serious problematic disease in Chhattisgarh. There is not much information available on status of intensity, variability and management of *Alternaria solani* in this region. It is very essential to determine the efficacy of different doses of fungicides, botanicals and bio-control agents and their integrations against early blight of tomato. Therefore, keeping in view of above facts present experiments were conduct on "Studies on *Alternaria solani* causing early blight disease in Tomato (*Lycopersicon esculentum* Mill.)" The objectives of the present investigations are taken up as follows:

1. Survey on severity of early blight of tomato in different districts of Chhattisgarh.
2. Cultural and pathogenic variability of *Alternaria solani* isolates.
3. *In vitro* evaluation of fungicides, bio-control agents and plant extracts against *Alternaria solani.*

4. Development of integrated management strategies for the control of early blight in tomato.

CHAPTER – II

REVIEW OF LITERATURE

Early blight disease on tomato caused by *Alternaria solani* is an important and widely distributed disease throughout the world, wherever tomato is grown. The studies with respect to status of early blight in Chhattisgarh on tomato and its management are taken into consideration. Reviewing of literatures on present general picture of the disease information is presented here:

2.1. Historical Background

The genus *Alternaria* was first recognized by Nees in 1817 and Berkeley (1836) identified the causal fungus on plants belonging to family Brassicaceae as *Macrosporium brassicae* Berk. which was later renamed as *Alternaria brassicae* (Berk.) Sacc.

The early blight disease was first described by Ellis and Martin (1882) from U.S.A. on potato and the causal organism was identified as *Macrosporium solani*. Later Jones and Grout (1897) transferred the fungus to the genus *Alternaria* on the basis of formation of spores in catenulate (in chains) in culture. The name early blight was ascribed to the disease by Jones (1893) because of the sever attacks on early maturing cultivars than medium or late maturing cultivars.

Sherf and MacNab (1986) reported that first symptoms of early blight are small, dark, necrotic lesions that usually appear on the older leaves and spread upward of the plants. As lesions enlarge, they commonly have concentric rings with a target-like appearance, and they are often surrounded by a yellowing zone. In severe epidemics, *A. solani* can cause premature defoliation, which weakens the plants and exposes the fruit to injury from sunscald. Large, dark, and sunken lesions may appear on the stems of seedlings at the ground line, causing partial girdling known as collar rot.

Butler (1903) reported *Alternaria solani* for first time in India on *Solanum tuberosum* from Farukhabad district of Uttar Pradesh.

Madden *et al*. (1978) reported that the early blight caused by *A. solani* is principal foliar disease of tomato in North Eastern USA. The disease characterized by dark lesions with concentric rings that appear first on the lower leaves.

Jones and Grout (1986) reported that the pathogen *Alternaria solani* belongs to phylum Ascomycota, class Deuteromycetes, order Moniliales, family Dematiaceae, genus *Alternaria* and species *solani.*

Maiero *et al*. (1990) observed that the *A. solani* causes two distinct phases of disease on tomato plants *i.e.* early blight and collar rot.

2.2. Survey of early blight

Kamble *et al.* (2009) found major disease of tomato was early blight incited by *A. solani* under agro climatic conditions of Konkan region of Maharashtra. Early blight disease intensity was ranged between 20.78 to 42.30 percent in Raigad district and 35.12 to 55.75 percent in Thane district.

Chaurasia *et al*. (2013) conduct regular survey in local Tomato growing field of Tikamgarh (M.P.) and observed that most of the tomato fruits have been suffered by fruit rot disease caused by *Alternaria solani.* The green fruits was found very less susceptible which showed some resistant against *Alternaria solani.* While, semi ripe" fruits was found most susceptive for the pathogen and maximum rotting was recorded in semi ripe fruits. The ripe fruits were found moderately susceptible.

Munde *et al*. (2013) conducted roving survey on early blight of tomato disease during *Rabi* season, 2008 – 2009 at Thane, Raigad, Ratnagiri and Sindhudurg districts of Maharashtra and percent disease incidence was ranged from 26.33 to 50.77 percent. The maximum percent disease incidence (50.77%) was observed in Wada Tahsil of Thane district and minimum percent disease incidence (26.33%) in Goregaon Tahsil of Raigad districts.

Sahu *et al*. (2013) conducted survey in *Rabi* 2012-13, to know the seasonal occurrence of tomato diseases and current status of early blight diseases in Abhanpur, Aarang and Dharsiwa block of Raipur district of Chhattisgarh. In entire

growing season disease severity was more in Abhanpur block (51.31%) followed by Aarang block (49.35%) and least in Dharsiwa block (44.24%).

Ahmad *et al.* (2014) survey was conducted in Peshawar division to determine the incidence of early blight on tomato caused by *Alternari solani*. High disease incidence (100%) was recorded in Pathwarbala, Sufaid Sang, Kanderysadin and Shahibala and lowest in Regiaftezai and Malakandhir (50%).

Rani *et al*. (2015) conducted survey during 2011 and 2012 to diagnose the symptoms of early blight disease of tomato (*Lycopersicum esculentum* L.) in all vegetable growing areas of Jammu Division of Jammu and Kashmir. The disease intensity and incidence were varied from 21.66 to 34.13% and 10.48 to 18.56%, respectively.

Pachori and Sharma (2016) conducted field survey during *Kharif* season 2014-15 at Gwalior, Bhind and Morena Districts of Madhya Pradesh to determine status of early blight on tomato. The percent disease incidence was ranged from 27.50% to 63.36%. The highest percent disease was observed in research farm (63.36%) of Gwalior District and minimum percent disease incidence was observed in village Daboha (27.5%) of Bhind District.

3.3 Symptomatology

Walker (1952) reported that the spots were oval or angular in shape ranging from 0.3 to 0.4 cm diameter in size with usually narrow chlorotic zone around the spot. Symptoms were progress from lower leaves to upper leaves. Leaf spots begin as small brown areas on lower leaves. As the spots mature, concentric rings of raised and depressed brown tissue were evident. Heavily infected plants often become defoliated.

Ramakrishnan *et al*. (1971) observed cankerous spots on tomato stems of seedling causing by *A. solani*. They were especially injurious when they occurred at the juncture of the stem and side branches. Tomato fruit, both green and ripe, may also become infected with the fungus. Infection generally begins at the calyx end. Brown leathery areas were formed at infection sites. They contain the same concentric rings found in leaf spots.

Datar and Mayee (1981) observed that *Alternaria solani* was attacked on green and ripe fruits at the stem end growth cause cracks and other wounds.

Sherf and MacNab (1986) observed the first symptoms of early blight as small, dark, necrotic lesions that usually appear on the older leaves which subsequently spread upward as the plants become older. In severe epidemics *A. solani* can cause premature defoliation, which weakens the plants and exposes the fruit to injury from sunscald.

Peralta *et al.* (2005) reported that early blight is the major disease symptom caused by the fungus *Alternaria solani* (Ellis & Martin) Sorauer. This disease can lead to complete defoliation in severe case in regions where received heavy rainfall, high humidity and fairly high temperatures (24°–29°C).

Chaerani *et al.* (2006) noticed symptoms on tomato due to early blight as collar rot on stem of seedlings, lesions on stem of adult plant and rotting on fruits.

Foolad *et al*. (2008) described early blight symptoms on potato and tomato foliage as small, dark, circular lesions becoming distinctly zonate as they develop. Stem lesions was occur on diseased tomato plants as roughly circular, sunken, dark and zonate.

Blancard *et al.* (2012) observed the appearance of rot spots on green and ripe fruit of tomato.

2.4 Pathogen

Neergaard (1945) reported that *Alternaria solani* as large spores producing group of fungus and characterized by separate conidia borne singly on simple conidiophores within the genus *Alternaria.*

Joly (1959) studied on morphological variations of *Alternaria* species and later during 1964 divided in three sections and proposed a simple key for identification and determination of the most common species. Further, Ellis and Gibson (1975) noticed the conidia of *Alternaria solani* are muriform and beaked.

Ellis (1971) studied the morphological characters of *Alternaria solani* and reported that the conidia are dark muriform, pale golden or olivaceous brown,

smooth and usually 150– 300 µm in length and 15–19 µm thick in the broadest part, with 9–11 transverse septa and 1–4 longitudinal or oblique septa; sometimes branched 2.5–5 µm thick tapering gradually.

Bose and Som (1986) observed the septate, branched, light brown hyphae which turned darker with age. The conidiophores were short measuring 50 to 90 µm long and dark colour. Conidia were 120-296 x 12-20 µm in size, beaked, muriform, dark colour and borne singly. However in culture they formed short chains.

Singh (1987) reported that the conidia contained 5-10 transverse septa and 1-5 longitudinal septa. The mycelium was septate, branched, light brown hyphae which turned darker with age.

2.5 Losses due to Early blight disease

Yield losses up to 79% due to early blight were reported from Canada, India, USA, and Nigeria (Datar and Mayee 1981; Sherf and MacNab 1986; Gwary and Nahunnaro 1998). Collar rot caused by *A. solani* can cause seedling losses in the field from 20 to 40% (Sherf and MacNab 1986).

Meitei *et al.* (2012) reported the loss in yield due to the early blight disease was 2.15% in highly resistant genotype and 42.75% in highly susceptible genotype.

Saha and Das (2012) conducted experiment to assess the crop loss in relation to disease severity due to early blight in the year 2007-08 and 2008-09 in west Bengal and revealed that loss in yield was 00.76 tone ha^{-1} for every 1% increase in disease severity.

2.6 Isolation, inoculation and pathogenicity of *Alternaria solani*

Barksdale (1968) and Dhiman *et al*. (1980) used spore suspension containing $2x10^4$ spore ml^{-1} for proving pathogenicity of early blight of tomato caused by *A. solani*. Further, they atomized the culture suspension on three leaf stage seedlings at the rate of 30 ml per seedling for successful inoculation.

Coffey *et al*. (1975) showed that the early blight severity was gradually increased on young tomato plants with increased as conidial concentration from 5 x 10^3 to 8 x 10^4 conidia ml^{-1}. A positive relationship between inoculum concentration and symptom development has also been demonstrated for other *Alternaria* species by Vloutoglou (1994).

Vloutoglou and Kalogerakis (2000) studied the effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria solani*) and on shedding of leaves in tomato plants. They reported the main effect of early blight was premature defoliation which was linearly related to the percentage of leaf area showing symptoms. They also observed that the 4-6 hrs of wetness is sufficient to initiate the disease on plants and as wetness duration increased up to 24 h, there was an increase in the percentage leaf area showing symptoms and in the percentage of defoliation.

Arunakumara (2006) conducted pathogenicity test by inoculating of spore suspension and homogenized mycelium bits (2 x 10^4 spore ml^{-1}) of *A. solani* on foliage of 30 days old tomato seedlings.

Tippeswamy *et al*. (2010) confirmed the pathogenicity of early blight of tomato by spraying 1x10^4 conidial suspension of *A. solani* on 30 days old seedlings, before flowering (60 days) and after flowering (90 days).

2.7 Cultural and Morphological Variability

Perez and Martinez (1995) studied on variability in four isolates of *Alternaria solani* with respect to morphological characters like colony growth, colony diameter, mycelium colour, colony texture, pigmentation and conidia size on culture medium and concluded that *Alternaria solani* exhibited variability among the isolates.

Arunakumara (2006) postulated that the *Alternaria solani* produce uniformed, beaked, single separate conidia on simple conidiophores. Like other members of the genus *Alternaria*, *A. solani* has transverse and longitudinal septate conidia, multinucleate cells and dark-coloured (melanized) cells. Morphological

and pathological variations among *A. solani* isolates had been enormously studied by many researchers (Khan, 2002, Pérez, 2003 and Varma *et al*., 2007).

Hubballi *et al*. (2010) studied on cultural variability of *A. alternata* isolates on different culture media and reveal that the colony and substrate colour, margin, topography, zonation, pigmentation, colony diameter (mm) and sporulation varied among the isolates.

Alhussaen (2012) studied morphology and physiology characteristics of *Alternaria solani* for identification and variability. The mycelia width range between 0.8-1.5 µm and conidia were 35-75 µm in length and 10-20 µm in width and 2-7 transverse septa and 1-4 longitudinal septa. This study pointed that there was a variation in the population of *Alternaria solani* isolated from Jorden Vally.

Nikam *et al.* (2015) studied on pathogenic variability in tomato cultivar Pusa Ruby among the eight isolates of *A. solani*. The test isolates could grow better on the basic culture medium potato dextrose agar; however, highest mycelial growth was recorded on the isolate AsLt (88.50 mm), followed by AsBd (82.36 mm) and AsHl (78.40 mm), with excellent sporulation. All of the eight test isolates exhibited a wide range of variability in respect of their mycelial and conidial dimensions and septation.

Pachori *et al.* (2016) determined variability among collected fifteen isolates of *Alternaria solani* isolated from different locations of Bhind, Morena and Gwalior districts of Madhya Pradesh. Variability was observed in conidial morphology, cultural variability (colour of mycelium, growth of mycelium) on PDA media.

2.8 Pathogenic Variability

Castro *et al*. (2000) conducted an experiment on study of variability of *A. solani* under green house conditions based on the inoculation of 7 isolates on 14 tomato genotypes and reveal that all the isolates showed different degree of virulence on 14 tomato genotypes, demonstrating the existence of high level of variability in the fungus.

Singh *et al.* (2014) reported that out of 10 isolates, only three major groups were recorded on the basis of SAS analysis likewise Group-A were highly virulent (MF-4 and PN-4), Group-C indicate virulent (BG, AF-2, EC-1 and RF-1) and Group-E were less virulent (BHU-1, IIVR, SF-1 and BX-2) with all the five varieties.

Stammler *et al.* (2014) various experiments were performed to analyze the virulence of *Alternaria solani* and *Alternaria alternata* on tomatoes and potatoes. *A. solani* isolates were showed highly virulent while *A. alternata* isolates showed low or no symptoms after inoculation.

Rahmatzai *et al.* (2016) reveal that the isolate AS1 of *A. solani* was mostly virulent with maximum level of disease incidence (53.5%) and disease severity (32%), whereas, the AS2 isolate noted the least early blight incidence of 27% and disease severity of 18%. AS1 isolate produced properly clear early blight symptoms on leaves, stem and even fruits and was found to be virulent causing sever disease in tomato plants.

3.9 Effect of culture media on Growth and cultural characters of *Alternaria solani* isolates

Several literature reports showed PDA is a good medium for the growth and sporulation of *A. solani* (Bonde 1929; Neergaard 1945 and Rotem 1966). Barksdale (1968) reported that potato dextrose agar and lima bean agar media were the best media for growth and sporulation of *A. solani.*

Kulkarni (1998) reported that *Alternaria solani* attained maximum growth after ninth and seventh days of incubation in Richard's and potato dextrose agar medium.

Arunakumara (2006) studied growth characters of *Alternaria solani* isolated from tomato on nine solid media *viz.* potato dextrose agar, corn meal agar, Richards's agar, malt extract agar, bean meal agar, oat meal agar, glucose peptone agar, Czapek's agar and host leaf extract agar and maximum fungus growth was obtained on PDA followed by corn meal agar after 9 days of inoculation.

Chohan *et al.* (2015) postulated that the maximum growth of *Alternaria solani* was obtained at 25°C on PDA medium at 6.5 pH level under continuous light condition. On PDA medium the pigmentation varied from creamy yellow, brown black to olivacious brown, while on HLEA medium it was light brown.

Yadav *et al*. (2015) studied on five grain based media (wheat, sorghum, barley, maize and pearlmillet) to assess the effect of moisture, light quality, darkness quality and UV light on conidial production of *A. solani.* Excellent colonization with sporulation (4.50×10^3) was found with sorghum grains at 25±2°C after 30 DAI (days after incubation). The varying moisture content of substrates gave diversified colonization and sporulation of test pathogen on sorghum grain-based media.

Koley and Mahapatra (2015) tested the growth of the fungi under culture in twelve different liquid and solid media and compared with each other. Potato dextrose agar and oat meal agar media solid media and Richard's broth and Sabouraud's broth liquid media appeared be better than other media for growth of tomato early blight causing fungi.

Kumar *et al.* (2015) conducted an experiment on effect of culture media on various cultural characters of *Alternaria solani*. *Alternaria solani* grow well on Czapek dox agar medium and Jenson medium but maximum average radial growth of 54.7 mm was recorded on PDA at 25 ^{0}C temperature with pH 7.5.

Rahmatzai *et al*. (2016) reveal that maximum mycelial growth of AS1 was noted with Sabouraud's Agar medium (9 cm) followed by host agar medium (8.7cm) and PDA (7.9cm). While, the maximum linear growth of AS2 was recorded with Richard's Agar (9cm) followed by Czapeck's Agar (8.6cm) and Sabouraud's Agar (8.5cm). Isolates of *A. solani* showed highly variation in pigmentation, sporulation and feature of mycelial growth such as colony surface, growth margin and zonation.

3.10 Effect of pH on growth of *Alternaria solani*

Samuel and Govindaswamy (1972) demonstrated that good mycelial growth and sporulation of *A. solani* was between pH 4.0 to 8.0 and pH 5.0 was the best for mycelia growth and pH 7.0 for sporulation.

Gemawat and Ghosh (1980) observed that the *A. solani* was capable to grow on wide range of pH (4.0 to 9.5) and maximum growth and sporulation were observed at 6.3 pH.

Alhussaen (2012) observed that the optimum pH level for the growth of *Alternaria solani* grow *in vitro* was 6 to 7. Maximum growth of *Alternaria solani* was recorded at 6.5 pH level on PDA medium under continuous light condition by Chohan *et al.* 2015).

3.11 Effect of temperature on growth of *Alternaria solani*

Kaul and Saxena (1988) reported that the maximum growth of five isolates of *A. solani* at 25°C followed by 20, 15, 10 and 5°C with least growth at 35°C. *A. solani* germinated most rapidly in darkness when ambient temperature was near 25°C (Stevenson and Packer, 1988). The temperature requirement for *A. solani* was reported from 5-35°C by several workers (Bonde, 1929; Verma, 1970 and Gemawat and Ghosh, 1979).

Kemmitt (2002) reported that warm, humid (24-29°C) environmental conditions are conducive to infection in tomato in the presence of free moisture at optimum temperature range of 28-30°C, conidia germinate in approximately 40 min.

Arunakumara (2006) postulated that the *A. solani* produced maximum growth at 25 to 30°C temperature followed by 25°C, 20°C, 35°C, 15°C, 40°C, 10°C.

Rodrigues *et al.* (2010) studied conidial production and reveal that the fungal colonies were maximum grown in V8 medium at 25^{o}C in the dark with agitation for seven days at 25 ± 2^{o}C under near ultraviolet light and 12 h-photoperiod.

Hubballi *et al* (2010) reported that the growth of *A. alternata* was higher at temperature range of 25 - 30°C. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of *A. alternata* compared to continuous light and dark.

Arunakumara *et al.* (2015) an experiment was conducted to study the impact of abiotic and nutritional factors on the growth of *Alternaria solani* causing early blight of potato at College of Horticulture, Bidar during 2013-14. Optimum pH for the growth of A. solani was in the range of 6.5 to 7.0. The fungus thrived well at a temperature of 25°C followed by 30°C.

2.12 *In vitro* efficacy of plant extracts, bio-agents and fungicides against *Alternaria solani*

2.12.1 *In vitro* efficacy of plant extracts against *Alternaria solani*

Nashwa *et al.* (2012) studied on antimicrobial activity of six plant extracts from *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander), and *Allium sativum* (Garlic) for controlling *Alternaria solani*. Leaf extracts of *D. stramonium, A. indica,* and *A. sativum* @ 5% inhibited highest mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively), while *O. basilicum* @ 1% and 5% and *N. oleander* @ 5% inhibited lowest mycelial growth of the pathogen.

Ganie *et al.* (2013) evaluated five plant extracts *viz.*, *Artimesia absinthium* L., *Datura stramonium* L., *Urtica dioica* L., *Juglans regia* L. and *Mentha arvensis* L. against *Alternaria solani* causing early blight of potato under *in vitro* condition. Among plant extracts, *D. stramonium* proved superior to all other botanicals which exhibiting 61.12% mycelial growth inhibition of *A. solani*.

Sahu *et al*. (2014) evaluated antifungal activities of 9 plant extracts against *Alternaria solani* causing early blight of tomato. All tested plant extracts produced some antifungal activities, whereas *Azadirachta indica* (neem), *Datura stramonium* (datura) and *Withania somnifera* (ashwagandha) showed significant antifungal activities. The leaf extract of *W. somnifera* was most effective in inhibiting the mycelial growth of *A. solani* (62.56%) followed by *D. stramonium* (34.65%) and *A. indica* (25.27%).

Roopa *et al*. (2014) tested bio-efficacy of ten botanicals against *Alternaria solani* casing early blight of tomato. Among the ten plant botanicals evaluated,

Jatropa leaf extract @ 10 percent was found most effective in inhibiting the mycelial growth of *A. solani* (62.78%).

Rahman *et al.* (2015) evaluated some botanical against *A. porri*. *Adhatoda vasica* extract @ 5% showed the maximum (91.11%) inhibition of mycelial growth of *A. porri* followed by *Azadirachta indica* (60 %) and *Ocimum sanctum* (55.33%).

Maya and Thippanna (2015) tested bio-efficacy of aqueous extracts of ten locally available botanicals *viz*. *Accacia catechu, Cassia fistula, Cassia tora, Eupatorium odoratum, Melia azardichita, Pongamia pinnata, Psidium guajava, Tamarindus indica, Vitex nigundo and Zinger officinalea* against *A. solani* using poison food technique. Maximum mycelial inhibition of 80.70% was recorded in Rhizome extracts of *Zinger officinalea* followed by *M. azardichita* (73.64%), *P. guajava* (71.75%), *P. pinnata* (68.92%) and *E. odoratum* (63.71%) and least mycelial inhibition (13.74%) was recorded in *Cassia tora*.

Sadana, and Didwania (2015) studied on bio-efficacy of fifteen plant extracts (*Polyalthia longifolia, Azadirachta indica, Datura stramonium, Ocimum sanctum, Calotropis procera, Crotalaria juncea, Eucalyptus obliqua, Cassia fistula, Agele marmelos, Croton bonplonadium, Pergularia daemia, Cleome viscose, Phyllanthus amarus, Bauhinia purpurea, Euphorbia hirta)* against *A. solani* under *in vitro* conditions. Fresh aqueous extract of *Eucalyptus obliqua* @ 15% was most effective which exhibited 88 percent inhibition of mycelial growth of *A. solani* strain A1 followed by *Datura stamonium, Azadirachta indica, Calotropis procera* and *Polyalthia longifolia*.

Koley *et al. (*2015) reported growth inhibitory activity of botanicals against *Alternaria solani* causing early leaf blight of tomato. Aqueous leaf extract of *Datura stramonium* was the best followed by *Azadirachta indica* oil and *Lantana camara* leaf extract showing fungus growth inhibition of 57.03%, 51.35% and 48.02%, respectively. The efficiency of the botanicals was significantly highest at 15% concentration for all the botanicals as compare to lower concentration 5% and 10%.

Rani *et al*. (2017) evaluated fungicides and plant extracts against *Alternaria solani.* Among plant extracts, maximum mycelial growth inhibition was exhibited

by *Datura stramonium* (20%) followed by *Lantana camara* (20%) and *Azadirachta indica* (20%).

2.12.2 *In vitro* efficacy of bio-control agents against *Alternaria solani*

Babu *et al.* (2000a) evaluated the efficacy of six *Trichoderma* species on early blight of tomato. Among the six species of *Trichoderma, T. harzianum* exerted the highest inhibition of the mycelial growth (50.22%) of the pathogen over control fallowed by *T. viride.*

Babu *et al*. (2000b) reported that all the six *P. fluorescens* used were significantly inhibited the growth of *A. solani* compared to control.

Kota (2003) postulated that the *Trichoderma harzianum* and *T. virens* were highly inhibited the growth of *A. alternata* under *in vitro* condition.

Kumar *et al.* (2005) observed that in dual culture, all the three antagonist *viz. Trichoderma virens, T. harzianum and T. viride* over grow the colony of *Alternaria alternata* but *T. viride* parasitized the test fungus earliest.

Dalpati *et al.* (2010) evaluated four different bio-agents *viz. Trichoderma harzianum, T. viride, Pseudomonas fluorescens* and *Bacillus subtilis* against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*. Among the four bio-agents, *T. harzianum* was found superior as compared to others by inhibition the growth of 76.66% followed by *Bacillus subtilis* (73.66%).

Zafar *et al.* (2013) tested five *Trichoderma* species (*viz. T. viride, T. virens, T. harzianum, T. koningii* and *T. pseudokoningii*) against *Alternaria solani.* Culture filtrate of all the species of *Trichoderma* retarded the growth of *A. solani* but *T. viride and T. harzianum* most strongly suppressed the growth of *Alternaria solani*.

Koley *et al.*,(2015) determined the efficacy of six bio-control agents against fungus *Alternaria solani* causing early leaf blight of tomato. *Bacillus subtilis* showed the highest growth inhibition (52.77%) of *A. solani* over the control followed by isolate 2 and isolate 1 of *Pseudomonas fluorescens* with 47.22% and 45.55% of growth inhibition, respectively.

Rani *et al*. (2017) evaluated fungicides and plant extracts against *Alternaria solani*. Amongst the bio agents tested using dual culture technique, *Trichoderma harzianum* showed maximum growth inhibition of the pathogen and appeared to be the most effective.

2.12.3 *In vitro* efficacy of fungicides against *Alternaria solani*

Choulwar and Datar (1994) studied the tolerance of *A. solani* to fungicides like Mancozeb, Captofol, Thiophenate methyl and Carbendazim. These were tested at 1000, 1500, 2000, 2500 ppm *in vitro*. The results indicated that *A. solani* could tolerate 2500 ppm of all the fungicides tested.

Mallikarjun (1996) evaluated eight fungicides against *A. alternata* causing leaf blight of turmeric *in vitro* condition. Propiconazole (tilt) was found superior in inhibiting the growth of the fungus.

Dubey *et al*. (2000) conducted a study to investigate the effect of combination of different fungicides to inhibit the growth of *Alternaria alternata* which is responsible for *Alternaria* blight of broad bean and concluded that Contaf (Hexaconazole 5 EC) inhibited cent percent growth of *Alternaria alternata* under *in vitro* conditions.

Arunakumara (2006) tested *in vitro* efficacy of mancozeb and chlorothalonil (0.1, 0.2 and 0.3% conc.) and Azoxystrobin and Pyraclostrobin (0.05, 0.1 and 0.15% conc.) against *Alternaria solani* causing early blight of tomato and reported that the maximum inhibition of fungal growth in Azoxystrobin (78.60%) followed by Pyraclostrobin (78.02%), Mancozeb (63.20%%) and Chlorothalonil (51.54%).

Singh and Singh (2006) tested efficacy of seven fungicides viz., chlorothalonil, copper oxychloride, azoxystrobin, propineb, copper hydroxide, mancozeb at 2500, 2000, 1000, 500 and 250 ppm and hexaconazole at 1000, 500, 200, 100 and 50 ppm against *A. alternata* causing blight of tomato. All the fungicides significantly reduced the radial growth of the fungus. However, hexaconazole was very effective as it caused 100% growth inhibition.

Sharma and Gaur (2009) evaluated nine fungicides against *A. alternata* under *in vitro* condition. Among the tested fungicides Prochloraz found most effective in inhibiting mycelial growth (95.3%) followed by Propineb (65.8%), SAAF (60.5%) and Mancozeb (57.8%).

Mesta *et al.* (2009) evaluated mancozeb, captan, and chlorothalonil (0.1, 0.2 and 0.3% conc.); hexaconazole and difenoconazole (0.05, 0.1 and 0.15% conc.) under *in vitro* conditions against *Alternaria helianthi* and maximum inhibition of fungal growth was recorded in hexaconazole (72.87%) followed by difenoconazole (72.61%), mancozeb (58.29%), chlorothalonil (51.54%) and captan (50.43%).

Patel and Choudhary (2010) tested the efficacy of different systemic and contact fungicides against early blight of tomato. Among systemic fungicides, Difenconazole inhibited maximum growth of *A. solani*, whereas in contact fungicides, Mancozeb gave highest percent inhibition.

Hassan *et al.* (2014) determined the efficacy of commonly available fungicides at six different concentrations against *Alternaria solani*. Results showed that Chlorothalonil has better effectiveness as compared to others followed by Clipper and Antracol.

Sadana and Didwania (2015) tested seven fungicides (Mancozeb, Captan, Thiram, Coppersulphate, Carbendazim, Zineb and Copperoxychloride) against *Alternaria solani* and highest reduction in the disease was achieved by applying Mancozeb (1500ppm) that caused 86.4 percent inhibition of mycelial growth of *Alternaria solani*.

2.13 *In vivo* efficacy of fungicides, plant extracts and bio-agents against *Alternaria solani*

2.13.1 *In vivo* efficacy of plant extracts against *Alternaria solani*

Babu *et al*. (2000a) reported the effect of plant extracts, oils and Neem products (Neem leaf, neem seed kernel and neem cake) on tomato early blight in the field. *Acacia concinna* pod extract was found most effective with lowest percent disease index (23.1%) followed by neem oil (30.9%).

Hassanein *et al*. (2010) evaluated the effect of neem (*Azadiracta indica*) leaf extract against *Alternaria solani*, the causal agents of early blight of tomato and revealed that the concentration of 20% aqueous neem leaf extract sprayed on tomato plants showed lower incidence of early blight from 53.2 to 42.5% after two weeks and from 100 to 79.2% after 4 weeks.

Nashwa *et al.* (2012) studied on antimicrobial activity of six plant extracts from *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander), and *Allium sativum* (Garlic) for the control of *Alternaria solani* and highest reduction of disease severity was achieved by the extracts of *A. sativum* at 5% concentration and *D. stramonium* at 1% and 5% concentration.

2.13.2 *In vivo* efficacy of bio-control agents against *Alternaria solani*

Ahmed (2011) studied two strains of growth promoting rhizobacteria (PGPR), *Pseudomonas putida* MG4, and *Pseudomonas fluorescens* MG18 selected as inducers of systemic resistance for biological control of leaf spot caused by *Alternaria solani* and bacterial speck caused by *Pseudomonas syringae* in tomato. The two bacterial isolates afforded reduced disease intensity and elicited systemic protection against the two studied pathogens. The two PGPR stimulated a systemic response in tomato by inducing high rates of enzyme activity of phenylalanine ammonialyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and chitinase as well as the accumulation of high level of phenolics. The combined effect of these factors induced drastic decrease in the degree of infection of the two pathogens.

Sabriye Yazici *et al*. (2011) tested twenty three bacterial isolates to investigate their ability to protect the tomato plant against early blight disease. *Paenibacillus macerans-GC* subgroup A, *Serratia plymuthica*, *Bacillus coagulans*, *Serratia marcescens-GC* subgroup A, *Bacillus pumilis –GC* subgroup B and *Pantoea agglomerans* bacterial isolates reduced the disease severity of early blight significant when compared with control.

Moges *et al.* (2012) evaluated the antagonistic effect of some rhizospheric bacteria (bio-control agent) against *A. solani*. Five local antagonistic bacteria such as *Pseudomonas fluorescens* TK-1, *P. fluorescens* TK-3, *Bacillus subtilis TK-4*, *P.*

fluorescens TK-8, and *P. fluorescens* TK-10 were selected. Experiment revealed that the *P. fluorescens* TK-3 was found best bio-control agent with increase plant height by 35.20% and biomass by 52.28%. The efficacy test results clearly indicated that the indigenous strain, *P. fluorescens* TK-3 followed by *Bacillus subtilis TK-4* was an efficient bio-control agent against *A. solani* with antagonistic activity.

Sundaramoorthy and Balabaskar (2012) developed an effective eco-friendly strategy to manage early blight disease of tomato caused by *Alternaria solani* using endophytic and plant growth promoting rhizobacteria. Strains of *Bacillus subtilis* (EPCO16 and EPC5) and *Pseudomonas fluorescens* (Pf, Py15 and Fp7) were tested alone and in combination for their effectiveness against early blight. Significant reduction was found in early blight incidence of tomato due to the combined application of EPCO16+Pf1.

Bellishree *et al.* (2015) tested efficacy of four bio-agents, *Trichoderma harzianum, Chaetomium* sp., *Bacillus subtilis* and *Pseudomonas fluorescence* to mitigate *Alternaria solani.* Subsequently *B. subtilis* and *T. harzianum* reduced the disease severity of early blight significantly when compared with control.

2.13.3 *In vivo* efficacy of fungicides against *Alternaria solani*

Vloutoglou *et al.* (2001) tested the efficacy of Mancozeb (0.14% a.i.), Iprodione (0.075% a.i.), Prochloraz (0.025% a.i.), Chlorothalonil (0.15% a.i.) and Azoxystrobin (0.025% a.i.) on spray inoculated tomato plants with spore suspension of *Alternaria solani* as a protective and curative application. They were found effective when the fungicides were applied one day prior to inoculation with reduction in disease severity by 91-100% and defoliation by 100% as compared to untreated control. Prochloraz, Azoxystrobin and Iprodione showed the greatest curative activity, especially when they were applied to one day prior to inoculation (reduction in disease severity by 71-98%, in defoliation by 75-100%).

Bernat (2004) reported the efficacy of a newer fungicide Pytonconsento 450SC in controlling early blight (*Alternaria solani* and *A. alternata*). The fungicide effectively reduced the development of early blight (40-61%) compared with the untreated control.

Patel and Choudhary (2010) reported the efficacy of foliar spray of contact fungicide Mancozeb 75WP (0.2%) against *A. solani* which was gave maximum fruit yield (245.30qha^{-1}). Among systemic fungicides Difenconazole (0.1%) was effective in controlling the disease.

Horsfield *et al.* (2010) conducted series of experiments to evaluate fungicide use strategies for the control of early blight (*Alternaria solani*). Significant reduction in foliar disease of potatoes in Australia was reported with the application of Azoxystrobin and Difenoconazole.

Sahu *et al.* (2013) conducted an experiment to evaluate the efficacy of some newer molecules like Pyraclostrobin, Boscalid, and their combination Maccani, Pristine and commonly used chemicals *viz*., Mancozeb, Copper Oxychloride and Chlorothalonil against early blight of tomato. All fungicide treatments reduce the disease severity as compared to untreated check. Pristine 38%WG @ 64+126g a.i./ha was significantly reduced the disease (31.88%) followed by maccani 16%WG @ 60+180g a.i./ha (33.31%).

2.14 Integrated management of early blight caused by *Alternaria solani*

Afroz *et al.* (2008) an experiment conducted to determine the effective of eco-friendly management practices against major fungal diseases of tomato. There were 10 treatments *viz.* T1 = BAU - Biofungicide + Sanitation + Neem (3), T2= MOC (Mustard oil cake) + Neem (2) + Karmacha, T3 = BAU-Biofungicide + Neem + Karmacha (2), T4 = BAU-Biofungicide + Karmacha (2) + Mahogony, T5 = BAU-Biofungicide + MOC + Neem + Karmacha + Mahogony, T6 = MOC + Karmacha + Mahogony (2), T7 = BAU - Biofungicide + MOC + Neem + Mahogony (2), T8 = MOC + Sanitation + Neem (3), T9 = BAU - Biofungicide + MOC + Karmacha + Mahogony + Sanitation and T10 = control. Among the above treatments, treatment T6, T7, T8 and T9 exhibited more or less equally effective against the early blight disease.

Sallam (2011) studied the effect of six plant extracts and some fungicides against *Alternaria solani in vivo*. The greatest reduction of disease severity was achieved by Redomil Plus 74.2% followed by *A. sativum* @ 5% and the smallest reduction was obtained when tomato plant was treated with *O. basilicum* @1 and

5% (46.1 and 45.2%, respectively). Fungicide, *D. stramonium* and *A. sativum* at 5% increased in fruit yield 85.7, 76.2 and 66.7% compared to infected control.

Ganie *et al.* (2013) developed an effective management strategy for early blight of tomato. Seed treatment with Mancozeb 75WP (0.3 %) + foliar spray with Hexaconazole 5 EC (0.1%) + foliar spray with dhatura (5.0%) + foliar spray with *Trichoderma harzianum* (1×10^7 spore ml^{-1}) were highly effective in reducing the disease severity.

Tewari and Vishunavat (2012) evaluated fungicides along and with cultural practices to develop an effective management strategy for early blight of tomato. Thiram was found to be the best for seed treatment as it enhanced the germination and reduced seedling infection. Cultural practices (inter cropping with marigold, mulching and stacking) when integrated with fungicides reduced the percent disease index and increased the yield.

Soni *et al.* (2015) evaluated bionano formulation (Cu-chitosan) in integration with fungicide and botanicals to develop effective management strategies against early blight of tomato caused by *Alternaria solani.* Under pot study the integration of three component; Cu- chitosan 0.1% as seed treatment with spray of Mancozeb @ 0.25% and neem oil @ 2% was found best that gave maximum efficacy of disease control (43.01 and 50.81%) with minimum PDI mean (27.50 and 30.38%), respectively, at first and second spray of the treatment as compare to inoculated control.

Rani *et al*. (2017) developed integrated disease management module for early blight of tomato. Fungicides, plant extracts and bio agents were integrated in different treatments and applied in field with varying spray schedules consecutively for two seasons. It was observed that treatment comprising of Mancozeb (0.25%), Datura (50%) and *T. harzianum* S.T ($1x10^7$ spores ml^{-1}) reduced disease intensity up to 84.00% followed by treatment comprising of Mancozeb (0.25%) and *T. harzianum* S.T ($1x10^7$ spores ml^{-1}) which reduced disease intensity to 82.33%.

* * * * * * *

CHAPTER–III
MATERIALS AND METHODS

The experiments pertaining to the "Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill.)" were carried out during *Rabi* 2016-17 at S.K. College of Agriculture and Research Station (Indira Gandhi Agriculture University, Raipur), Kawardha (District – Kabirdham) – 491995 (C.G.). The details of materials used and the methodology adopted in the present investigation are briefly described below:

3.1 Experimental site

All the experiments *in vitro* and *in vivo* were conducted in the laboratory of the Department of Plant Pathology, S.K. College of Agriculture and Research Station, Kabirdham (C.G.) during *Rabi* 2016-17. It is situated at 22.02 degree north latitude and 81.25 degree east longitude.

3.2 Climate

Kabirdham district of Chhattisgarh has sub humid agro climatic condition. The average rainfall of this region is 800-850 mm, out of which about 88 percent is received during rainy season (June-September) and 12 percent during winter season (October-February). The rainfall pattern has a great variation during rainy season from year to year. Month May is the hottest and December is the coldest month of the year. During summer season highest day temperature is range between 29^0C to 45^0C.

3.3 Materials and instruments used in the investigation

The glassware's, plasticware's, blotting paper and chemicals of standard trade marks were used during the course of the investigations. All the glassware's, plasticware's, polythene bags, ethyl alcohol, formalin, chemicals, different fungicides and other materials were obtained from Department of Plant Pathology, S.K. College of Agriculture and Research Station, Kawardha (Distt.-Kabirdham),

Chhattisgarh. During the course of investigation the following tools/instruments were used for various purposes:

1. Autoclave for sterilization.
2. BOD incubator for incubation of pathogen.
3. Compound microscope for identification of pathogen.
4. Hot air oven for glassware's sterilization.
5. Laminar Air Flow for isolation and purification of pathogen.
6. Electronic Digital Balance for weighting of chemicals and other materials.
7. Forceps, needles, blades, cork borer & inoculation needle.
8. Spirit lamp for sterilization.
9. Microwave oven for preparation and melting of media.
10. pH meter for measurement of pH of culture media.
11. Micro pipette etc.

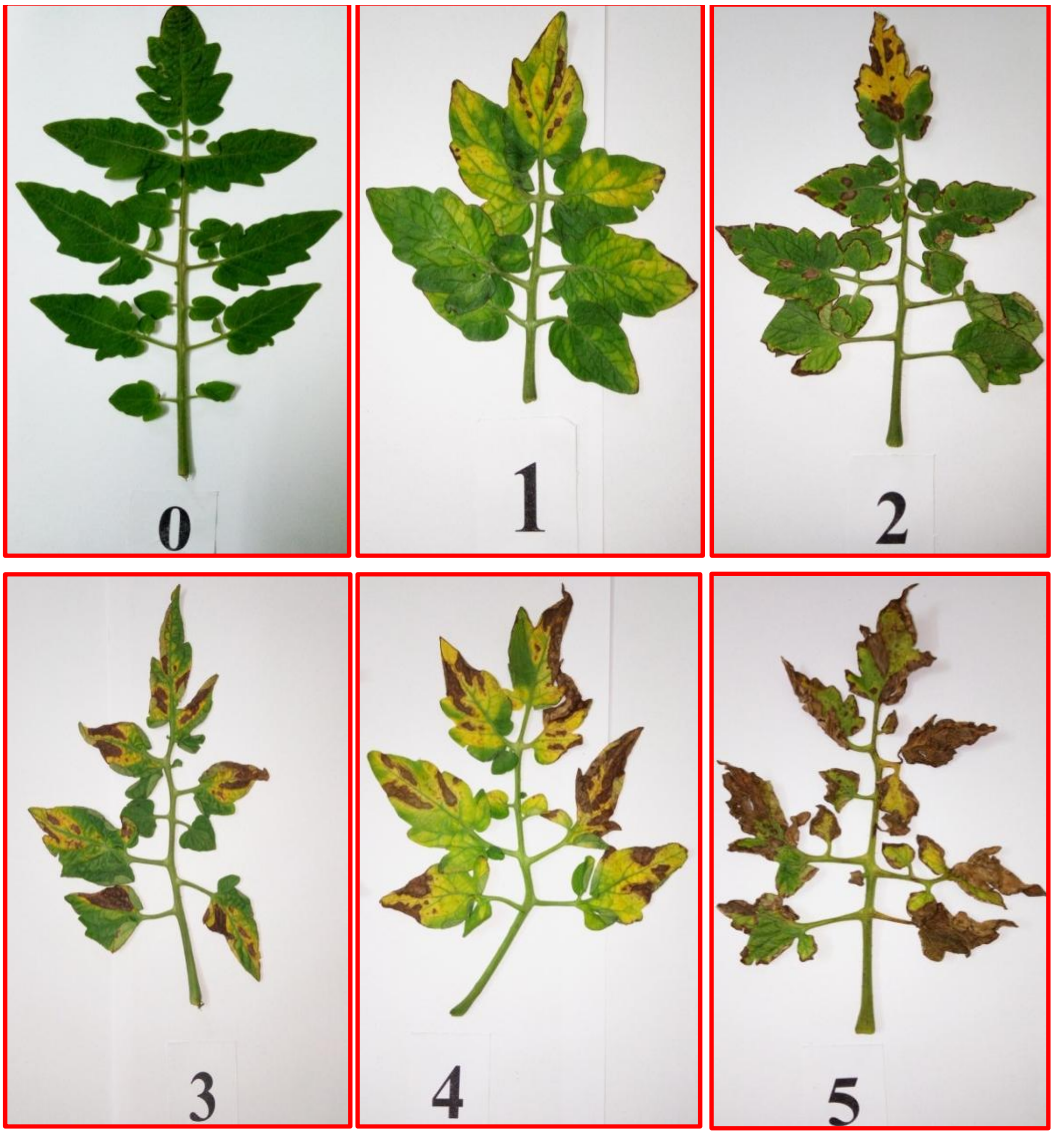
3.4 Survey on severity of early blight of tomato in different districts of Chhattisgarh

An intensive roving survey was conducted during *Rabi* 2016-17 in five districts of Chhattisgarh *viz.* Kabirdham, Durg, Bemetara, Rajnandgaon and Mungeli to know the intensity of early blight disease on tomato. Four villages were randomly selected from each district where the tomatoes were grown. Details are given in table 3.1.

Table 3.1: Details of districts and village selected for survey

S. No.	District	Village
1.	Mungeli	Sangwakapa
2.		Ramgarha
3.		Saleghori
4.		Dendori
1.	Kabirdham	Khadhauda
2.		Samnapur
3.		Mohganw
4.		Mainpuri
1.	Bemetara	Lalpur
2.		Patharikala
3.		Pipariya
4.		Bhalesar
1.	Rajnandgaon	Kalegondi
2.		Mushra
3.		Dhara
4.		Bundela
1.	Durg	Khapari
2.		Kodiya
3.		Arasnara
4.		Kutha

Disease intensity of early blight was recorded in same tomato fields in the months of October, November, December, January and February 2016-17. Five places were marked in the field (four from each corner and one in the center). Severity of early blight was assessed from 20 plants excluding those plants in the border lines as per procedure described by Enikuomehin and Peter (2002). Disease severity was measured by using 0-5 scale with modification described by Pandey *et al.* (2003) (Plate 3.1).



Rating	Reaction Description
0	Free from infection
1	One or two necrotic spots on a few lower leaves of plants, covering nearly 1-10% of the surface area of the plant
2	A few isolated spots on leaves, covering nearly 11-25% of the surface area of the plant
3	Many spots coalesced on the leaves, covering 26-50% of the surface area of the plant
4	Irregular, blighted leaves and sunken lesion with prominent concentric rings on the stem petiole, fruit, covering 51-75% leaf area of the plant.
5	Whole plants blighted, leaf and fruits starting to fall, covering more than 75% leaf area of plant.

Plate 3.1: Description of disease rating scale for early blight

Table 3.2: Description of disease rating scale for early blight (Pandey *et al.*, 2003)

Rating	Reaction Description
0	Free from infection
1	One or two necrotic spots on a few lower leaves of plants, covering nearly 1-10% of the surface area of the plant
2	A few isolated spots on leaves, covering nearly 11-25% of the surface area of the plant
3	Many spots coalesced on the leaves, covering 26-50% of the surface area of the plant
4	Irregular, blighted leaves and sunken lesion with prominent concentric rings on the stem petiole, fruit, covering 51-75% leaf area of the plant.
5	Whole plants blighted, leaf and fruits starting to fall, covering more than 75% leaf area of plant.

Percent Disease Index (PDI) was worked out by using formula given by Wheeler (1969).

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of individual disease ratings}}{\text{Total No.of plant examined X Maximum No.of disease rating}} \text{X } 100$$

Area under the disease progress curve (AUDPC) was calculated by using following formula:

$$\text{AUDPC} = \sum_{i-1}^{n} \left[0.5(x_{i+1} + x_i)[t_{i+1} + t_i]\right]$$

Where,

$x_i =$ Cumulative disease severity expressed as a proportion at the i^{th} observation

$t_i =$ Time (days after planting) at the i^{th} observation

$n =$ Total number of observations

3.5 Studies on Cultural and pathogenic variability of *Alternaria solani* isolates

3.5.1 Collection of diseased sample

Tomato leaves showing typical early blight symptoms were collected from growing tomato plants from different tomato growing district *viz.* Kabirdham, Durg, Bemetara, Rajnandgaon, Mungeli, Bilaspur and Raipur district of Chhattisgarh during crop season.

3.5.2 Isolation and identification of pathogen

Standard tissue isolation technique was followed to obtain *A. solani* culture described by Naik *et al.* (2010). The leaves were microscopically examined to confirm the presence of the fungus. After confirming for the presence of fungal spores, isolation was done by following standard tissue isolation method as described below.

1. The infected leaves were brought to laboratory and cut into small bits measuring about 2mm.
2. Surface of spaceman was sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 1 minute and wash thrice with sterile distilled water.
3. Then one piece of specimen was transferred on Potato Dextrose Agar (PDA) medium in the center of Petri dish.
4. These petri dishes ware incubated under B.O.D. incubator at 25±2°C.
5. Pure culture of the *A. solani* was obtained by hyphal tip culture and single spore culture technique.
6. The isolates collected from various places were designated as follows:

Table 3.3: Description of *A. solani* isolates isolated from different place

Sl. No	Location	Designation
1.	Village - Arasnara, District - Durg	**AS_1**
2.	Village - Pipariya, District - Bemetara	**AS_2**
3.	Village - Saleghori, District - Mungeli	**AS_3**
4.	Village – Sakari, District - Bilaspur	**AS_4**
5.	Village - Kalegondi, District - Rajnandgaon	**AS_5**
6.	Village - Khadhauda, District - Kabirdham	**AS_6**
7.	Village – Horticulture Farm, IGKV, Raipur	**AS_7**

3.5.3 Purification of *A. solani* isolates

The culture was purified by both hyphal tip method (Pathak, 1972) and single spore technique described by Johnston and Booth (1983). As soon as the mycelial growth was observed in Petri plates, advancing hyphal tips growing out of tissue segments were cut off with sterilized inoculation needle and transferred to potato dextrose agar slants for further growth. In case of single spore technique 2 to 3 drops from spore suspension prepared from 10 days old culture with sterilized distilled water. Later 1 ml of suspension was spread on the surface of plain agar medium in Petri plates and incubated at 25±2°C for 24 h. The plates were observed for germinating spores under stereoscopic microscopic and finally germinating spores were lifted by inoculation needle and transferred aseptically to potato dextrose agar slants for further growth.

3.5.4 Maintenance of cultures of *A. solani* isolates

The pure cultures obtained were maintained by repeated sub-culturing at an interval of 30 days for further studies. The stock cultures of all isolates grown on PDA slants were stored at 5°C in refrigerator.

3.5.5 Identification of *Alternaria solani* causing early blight disease in tomato

Spores of *Alternaria solani* were taken from the pure culture and mounted on the clear glass slide. Spores were mixed with thoroughly with lactophenol in order to obtain a uniform spread over slide and covered with cover slip. The spores and

hyphae of the fungus were observed under compound microscope. *Alternaria solani* was identified following the cultural and morphobiometric characteristics criteria as per described by Ellis (1971).

3.5.6 Pathogenicity test of early blight pathogen

In order to confirm the identification of early blight disease and its causal agent, the pathogenicity test was carried out under open condition in pot. Four 30 days old seedlings of Pusa Ruby tomato variety were transplanted into the pots. Three replications were maintained for each isolate and three seedlings of tomato were growing in each replication. For each isolate of the *A. solani*, 10-days old culture grown on PDA was added to 100 ml sterile distilled water and shake properly to release conidia. The suspension was filtered using cheese cloth to remove PDA. The conidial suspension at $3x10^6$ spore's ml^{-1} of the isolates was prepared. The spore suspension was sprayed on tomato plants 20 days after transplantation of seedlings and the inoculated tomato plants were sprayed with sterilized water for 2 days to retain optimum humidity. The symptoms were observed on *A. solani* isolates inoculated plants and compared with the original symptoms of early blight. The pathogen was re-isolated from artificially inoculated tomato leaves and the morphological and cultural characteristics were compared with original pathogen.

3.5.7 Studies on cultural variability among the *A. solani* isolate causing early blight disease in tomato

The variability of representative isolates of *A. solani* was studied on various parameters of cultural characters such as colony colour, substrate colour, topography, thickness of mycelium mat, colony diameter, dry weight of mycelium, type of margin, and sporulation on potato dextrose agar (PDA). All culture parameters of each isolate on PDA medium were recorded 10 days after incubation by visual observation except colony diameter and sporulation. Colony diameter was recorded in each isolate at 48, 96, 144, 192 and 240 hours after inoculation. Linear growth of the colony was measured with the help of fine transparent plastic scale in millimeter. In case of sporulation, sporulation was observed from 10 days old culture of each isolate by making the spore suspension. A single block of 5 mm diameter was cut out from the fungal colony near the margin by sterilized cork

borer and was transferred to 5 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on a slide and average spore count of three microscopic fields under low power (10X) objective of the microscope. Sporulation was categorized as below:

No. of spore per microscopic field	Designation
0	- (nil)
1-10	+ (poor)
11-20	++ (moderate)
21-30	+++ (good)
31 and above	++++ (excellent)

3.5.8 Studies on morphological variability among *A. solani* isolate causing early blight disease in tomato

Potato dextrose agar (PDA) medium were prepared as suggested by Ainsworth (1961) and used for the cultural studies of *A. solani* isolates. Twenty millimeter of medium was poured into 90mm diameter petri plates. After solidification 5mm discs from 7 days old cultures of each isolate of *A. solani* were cut by using a cork borer and were placed at the centre of the plate. Each set of the experiment was replicated thrice and the plates were incubated at 25±2^{o}C for 10 days. The size of conidia, beak length of conidia, width of conidia, number of horizontal and vertical septa were recorded from each isolates at 10 days after inoculation. The photographs of the conidia were taken and measurement of conidia was done with the help of ocular and stage micrometer.

3.5.9 Studies on pathogenic variability among *A. solani* isolate causing early blight disease in tomato

Pathogenic variability of seven isolates of *A. solani* was studied by applying detached leaf technique. The spore suspension (3 x 10^{6} spore ml^{-1}) of each isolate was separately prepared from 10 days old pure culture of the representative isolates. Healthy growing nine tomato plants were inoculated at room temperature by spraying of spore suspension of each isolate. Observations on first appearance

of symptoms, disease severity and typical symptoms were recorded. Based on these characteristics, the test isolates were categorized as highly virulent, moderately virulent and a mildly virulent. To study the virulence potential variability of each isolate, the disease severity was continuously measured at 7 days intervals after inoculation. Disease severity was recorded using 0-5 disease rating scale described by Pandey *et al.* (2003) with little modifications. Percent disease index (PDI) for each isolate were calculated as per formula described earlier.

3.6 Effect of different culture media on *A. solani* isolates

3.6.1 Effect of solid culture media on *A. solani* isolates

The experiment was conducted to find out the effect of different culture media on cultural characteristic of *A. solani* isolates. Twenty ml of each medium was poured in each 90mm petri plates. Five mm disc of fungus culture was cut with the help of sterilized cork borer from the margin of 7 days old pure culture of *Alternaria solani* grown on PDA. One disc of the culture was placed in inverted position in the centre of each petri plates. The petri plates were incubated at 25 ± 2^0C. Three replications were maintained for each medium and each isolate. The following solid media were used during the investigation. Details of composition and preparation procedure of culture media are given below:

1. Potato Dextrose Agar Medium (PDA)
2. Tomato Leaf Extract Agar Medium (TLEA)
3. Corn meal Agar Medium (CMA)
4. Malt extract Agar Medium (MEA)
5. Glucose Peptone Yeast Agar Medium(GPYA)
6. Asthana and Hawker's Medium (AH)
7. Yeast Powder- Soluble Starch Agar Medium (YPSS)
8. Oat meal Agar Medium (OMA)
9. Waksman's Medium (WM)
10. Potato + $CaCo_3$ Agar Medium (PDA+ $CaCo_3$)
11. Martin's Medium (MM)
12. Czapek's Dox Agar Medium (CDA) Mineral Agar Medium (MA)

Water Agar Medium (WA)

1. Potato Dextrose Agar Medium:

Composition:

Potato	:	200g
Dextrose	:	20g
Agar	:	20g
Distilled water	:	1000 ml

Procedure: Potato slices were boiled in 500 ml distilled water for 30 minutes and filtered through muslin cloth. Agar (20 g) was melted by adding 500ml distilled water. Both were mixed thoroughly and added 20 g of dextrose. Finally volume was made 1000ml using distilled water and autoclaved at 15 lb psi for 20 minutes.

2. Tomato Leaf Extract Agar Medium:

Composition:

Healthy tomato leaves (Green)	:	200 g
Agar	:	20 g
Distilled water	:	1000 ml

Procedure: Green tomato leaves (200 g) were boiled in 1000 ml of water for 30 minutes. Extracts was collected by filtering through muslin cloth. The agar was added in the filtrate. Finally volume was made to 1000 ml and sterilized in autoclave at 15 lb psi for 20 minutes.

3. Corn meal Agar Medium:

Composition:

Corn meal	:	20 g
Agar	:	15 g
Distilled water	:	1000 ml

Procedure: Added the 20 g corn meal to the 1000 ml water and simmer for 30 min. Filtered through double layers of cheesecloth. Added the 15 g agar powder to the filtrate and boiled up to milting of agar. Then maintain the 1000 ml volume with distilled water, then autoclave for 20 min at 15 lb psi on slow exhaust.

4. Malt extract Agar (Blakeslee's formula):

Composition:

Malt extract	:	20g
Glucose	:	5g
Peptone	:	1g
Agar	:	20g
Distilled Water	:	1000 ml

Procedure: Suspended 20 g malt extract, 5g glucose, 1 g peptone and 20 g agar-agar in one liter of distilled water. Mixed well and heated with frequent agitation up to boiling point for milting of agar. Sterilized in an autoclave at 121^{o}C (15 lb psi) for 20 minutes.

5. Glucose Peptone Yeast Agar:

Composition:

Glucose	:	40g
Peptone	:	5g
Yeast extract	:	5g
Agar	:	15g
Distilled Water	:	1000 ml

Procedure: Suspended 40 g glucose, 5g peptone, 5g yeast extract and 15g agar-agar in 1000 ml distilled water. Milt the agar by heating the suspension. Then media was sterilized at 121°C in autoclave for 20 minutes.

6. Asthana and Hawker's Medium:

Composition:

Potassium Nitrate (KNO_3)	:	3.50g
Potassium Monobasic Phosphate (KH_2PO_4)	:	1.75g
Magnesium Sulphate ($MgSO_4.7H_2O$)	:	0.75g
Glucose	:	5g
Agar	:	20g
Distilled Water	:	1000ml

Procedure: Suspended all the components except agar in 500 ml distilled water. Added the agar in 500 ml and heated for milting of agar. Then both the mixed together and make the volume 1000 ml by adding distilled water. Later media was sterilized at 121°C in autoclave for 20 minutes.

7. Yeast Powder- Soluble Starch Agar:

Composition:

Potassium Monobasic Phosphate (KH_2PO_4)	:	1gm
Magnesium Sulphate ($MgSO_4.7H_2O$)	:	0.5g
Starch (Soluble)	:	15g
Yeast extract	:	4g
Agar	:	15g
Distilled Water	:	1000ml.

Procedure: All the above ingredients except agar were dissolved in 500 ml water and agar was milted in 500 ml distilled water. Both solutions were mixed thoroughly and the volume was made to 1000 ml by adding distilled water and then sterilized.

8. Oat meal Agar Medium:

Composition:

Rolled oats	:	40 g
Agar	:	20g
Distilled water	:	1000 ml

Procedure: Rolled oat was boiled in 500 ml distilled water for 30 minutes, filtered through muslin cloth and agar was melted in 500 ml distilled water. Two solutions were mixed and the volume was made to 1000 ml by adding distilled water and then sterilized.

9. Waksman Medium:

Composition:

Potassium Monobasic Phosphate (KH_2PO_4)	:	1g
Magnesium Sulphate ($MgSO_4.7H_2O$)	:	0.5g

Glucose	:	10g
Peptone	:	5g
Agar	:	20g
Distilled Water	:	1000ml

Procedure: All the above ingredients except agar were dissolved in 500 ml water and agar was milted in 500 ml distilled water. Both solutions were mixed thoroughly and the volume was made to 1000 ml by adding distilled water and then sterilized in autoclave at 15 lb psi for 20 minutes.

10. Potato+$CaCo_3$ Agar Medium:

Composition:

Potatoes	:	20g
$Caco_3$	:	3g
Agar	:	20g
Distilled Water	:	1000 ml.

Procedure: As described in PDA.

11. Martin's Medium:

Composition:

Glucose	:	10g
Peptone	:	5g
Potassium Monobasic Phosphate (KH_2PO_4)	:	1g
Magnesium Sulphate ($MgSO_4.7H_2O$)	:	0.5g
Yeast extract	:	0.5g
Streptomycin sulfate	:	30mg
Agar	:	15g
Distilled Water	:	1000 ml.

Procedure: All the above ingredients except agar were dissolved in 500 ml water and agar was milted in 500 ml distilled water. Both solutions were mixed thoroughly and the volume was made to 1000 ml by adding distilled water and then sterilized in autoclave at 15 lb psi for 20 minutes.

12. Czapek's Dox Agar:

Composition:

Sodium nitrate (NaNo3)	:	2g
Potassium dihydrogen phosphate (K2HPO4):		1g
Magnesium sulphate (MgSO4. $7H_2O$)	:	0.5g
Ferrous sulphate (FeSO4. 7 H2O)	:	0.01g
Sucrose (C12 H22O11)	:	20g
Agar	:	20g
Distilled water	:	1000 ml

Procedure: Agar was melted in 500 ml distilled water and rest of the ingredients was thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml and then autoclaved.

13. Mineral Salt Agar Medium:

Composition:

Sodium Nitrate ($NaNO_3$)	:	2g
Magnesium Sulfate ($MgSO_4$)	:	0.5g
Potassium Chloride (KCl)	:	0.5g
Ferrous sulphate (FeSO4. 7 H2O)	:	0.01g
Potassium Monobasic Phosphate (KH_2PO_4)	:	0.14g
Potassium dihydrogen phosphate (K2HPO4):		1.2 g
Yeast extract	:	0.02g
Agar	:	15g
Distilled Water	:	1000 ml.

Procedure: Agar was melted in 500 ml distilled water and rest of the ingredients was thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml. Adjusted the pH to 7.2. and then autoclaved.

14. Water Agar:

Composition:

Agar	:	20g

Distilled Water : 1000ml.

Observation recorded: Following observations were recorded in each isolates of *A. solani* on different culture media:

1. Colony diameter at 48 hrs intervals
2. Colony colour
3. Substrate colour
4. Growth pattern
5. Margin colour
6. Topography
7. Thickness of mycelium mat
8. Sporulation

3.6.2 Effect of different liquid culture medium on *A. solani* isolates

The experiment was conduct to find out the best broth for biomass production of *Alternaria solani*. Hundred mililiter of different liquid medium (Broth) were taken in sterilised flask. Then these flasks were sterilized at 15 lb psi (121°C) for 20 minutes in autoclave and allowed to cool. One disc of 5 mm of seven days old *A. solani* culture was inoculated in each flask. Three replication were maintained for each treatment and kept for incobation at 25±2°C. Flasks were harvested after 10^{th} day of inoculation. The culture was filtered through Whatman No.1 filter paper. Before filtering, the filter papers were dried to a constant weight by drying in hot air oven at 50°C. The mycelial mat on the filter paper was thoroughly washed with distilled water and dried in hot air oven at 50°C. The filter paper with mycelium mat was weighed in a digital electronic balance. The thirteen liquid media (Broth) were used during the investigation for determine the biomass production of *A. solani* isolates. Composition and preparation procedure of broth are similar to solid media except in addition of agar in solid media. The names of liquid media used in the experiment are given below:

1. Potato Dextrose Broth
2. Oat Meal Broth
3. Tomato Leaf Broth
4. PDA + $CaCO_3$ Broth

5. Corn Meal Broth
6. Glucose Peptone Yea extracts Broth
7. Malt Extracts Broth
8. YPSS Broth
9. Czapek Dox Broth
10. Martin's Broth
11. Mineral Broth
12. Waksman's Broth
13. Asthana & Hawkar's Broth

Observation recorded: The observation of dry biomass different isolates was recorded on different liquid culture media. The weight of dry mycelium mat was recorded and the data were statistically analyzed.

3.7 Studies on effect of physiological factor on *Alternaria solani*

3.7.1 Effect of pH on *Alternaria solani*

The effect of pH levels such as 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were tested to determine influence of pH levels on growth characteristics of *Alternaria solani* isolate AS–6 on potato dextrose agar medium. The pH of the medium was adjusted before sterilization with the help of digital pH meter by addition of 0.1N HCL and 0.1N NaOH. Twenty ml of medium was poured in each Petri plates. Culture discs of 5mm were cut with the help of sterilized cork borer from the margin of 7 days old pure culture of *Alternaria solani* isolate grown on PDA. One disc of the culture was placed in inverted position in the centre of each petri plates. These petri plates were incubated in the BOD incubator at 25 ± 2^{0}C. The three replications were maintained for each pH level.

Observation recorded: Observation on mycelium diameter was measured with the help of scale at the time of full growth of fungus was observed in any one of the petri plates inoculated. Dry weight of mycelium was also recorded as per standard procedure. Numbers of spore were also counted per microscopic field with the help of compound microscope as per procedure described earlier.

3.7.2 Effect of temperature on *Alternaria solani*

The experiment was conducted to find out the most suitable temperature for growth of *Alternaria solani* isolate AS–6. Twenty ml of medium was poured in each petri plates. Five mm discs were cut with the help of sterilized cork borer from the margin of a 7 days old pure culture of *Alternaria solani* isolate AS-6 grown on PDA. One disc of the culture was placed in inverted position in the centre of each petri plates. The petri plates were incubated at 15^0C, 20^0C, 25^0C, 30^0C, 35^0C and 40^0C. Three replications were maintained for each treatment.

Observation recorded: Observation on radial growth of mycelium was measured by the help of scale at the time of full growth observed in any one of the petri plates inoculated. Numbers of spore were also counted per microscopic field by the help of compound microscope.

3.8 *In vitro* evaluation of fungicides, bio-control agents and plant extracts against *Alternaria solani*

3.8.1 *In vitro* evaluation of fungicides against *Alternaria solani* causing early blight disease in tomato

The poisoned food technique (Falck, 1907) was followed to evaluate the efficacy of six systemic fungicides against *A. solani* at five concentrations. Fungicides were added to the potato dextrose agar medium before sterilization as per treatment detail. Five mm disc of *A. solani* isolate AS-6 was taken from seven days old culture and placed at center of petri dish. The activity of fungicides were recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control. Treatment details are given below:

Treatments details

Fungicides:

F_1 = Trifloxystrobin 25% w/w + Tebuconazole 50%WG

F_2 = Difenconazole 25% EC

F_3 = Hexaconazole 5 % SC

F_4 = Propineb 70 WP

F_5 = Azoxystrobin 23%SC

F_6 = Thiafluzamide 24%SC

F_7 = Control

Concentrations : 50, 100, 250, 500 and 1000 ppm.

Replications : Three

Design : CRD

3.8.2 *In vitro* evaluation of bio-control agents against *Alternaria solani* causing early blight disease in tomato

Bio-control agents were evaluated for their efficacy against *A. solani* using dual culture technique. Twenty ml of PDA was poured into 90 mm diameter petri dishes and allowed to solidify. Five mm disc of *A. solani* was taken from seven days old culture and placed at one end of petri dish. Respective antagonistic organism (5mm disc) was also inoculated at the opposite side of same petri plate. In case of bacterial antagonist *A. solani* was placed at center of the petri plates and bacterial culture was streaked triangular at the centre of Petri plates. Each treatment was replicated five times and incubated for at 25 ± 2^0C. The activity of antagonistic organisms were recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control. Details of bio-control agents are as follows:

Details of bio-control agents:

B_1 =*Trichoderma harzianum*

B_2 =*Tricoderma viride*

B_3 =*Pseudomonas fluorescens*

B_4 = Control

3.8.3 *In vitro* evaluation of plant extracts against *Alternaria solani*

Tested efficacy of plant extracts against *Alternaria solani* using poisoned food technique under *in vitro* conditions (Nene and Thapliyal, 1993). Fresh healthy plant parts of 100 g (leaves/fruit/bulb) were collected from field, then they were washed with tap water, air dried and crushed in 100 ml of sterile water. Potato dextrose agar medium was used as nutrient medium and required quantity of each plant extract was added separately to get a required concentration of the plant extract. The plant extract were thoroughly mixed with PDA medium and sterilized

at 121^0C for 20 minutes. Twenty milliliter of poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification. Simultaneously without plant extract PDA was poured in petri dishes as control. Actively growing periphery of the 7 day old culture of *A. solani* was carefully cut using a cork borer and transferred aseptically to the centre of each petri dish containing the poisoned/non-poison solid medium. The plates were incubated at 25±2°C. Each treatment was replicated three times. Details of treatments are given below:

Treatment details:

P_1 = *Datura stramonium* (Dhatura) green fruit extract

P_2 = *Azadirachta indica* (Neem) seed kernel extract

P_3 = *Allium sativum* (Garlic) bulb extract

P_4 = *Eucalyptus spp.* (Eucalyptus) dry leaf extract

P_5 = *Crotalaria juncea* (Sunhemp) seed extract

P_6 = *Euphorbia hirta* (Bara Dudhi) whole plant extract

P_7 = Control

Concentrations : 1, 2, 3, 4 and 5%

Replications : Three

Design : CRD

Observation recorded: The radial growth of the fungus on the poisoned medium was recorded at time of mycelium growth reached 90 mm in control. Per cent inhibition of mycelium growth of the fungus was calculated by using the formula described by Vincent (1927).

$$I = \frac{(C - T)}{C} X\ 100$$

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treated (fungicide/ botanicals/ bioagents).

3.9 Development of integrated management strategies for the control of early blight disease in tomato

3.9.1 Soil of the experimental field

Soil of the experimental field was clay in nature (Vertisols) locally known as Kanhar. The soil was slightly acidic with a pH of 6.5. It had low nitrogen and medium phosphorus and potassium contents.

3.9.2 Field preparation and application manure and fertilizers

Field preparation was done with the help of cultivator. Prior to ploughing well decomposed FYM @ 10t ha^{-1} was incorporated uniformly in the soil. Recommended dose of fertilizers *viz.* 150:60:60 NPK were given through urea, single super phosphate and murate of potash, respectively.

3.9.3 Nursery raising

Nursery beds of 10 x 1 m^2 were prepared in well ploughed and leveled field per treatment. A well rotten FYM @ 5kg per nursery bed was added to soil and mixed properly. Seed of variety were treated with Propineb @ 3g Kg^{-1} was sown in lines. For control it was sown without treatment. The beds were covered with paddy straw (mulch). The beds were irrigated by hand sprinkler in morning and in the evening.

3.9.4 Experiment layout

The experiment was laid out in Randomized Block Design with eight treatments and three replications (Fig 3.1).

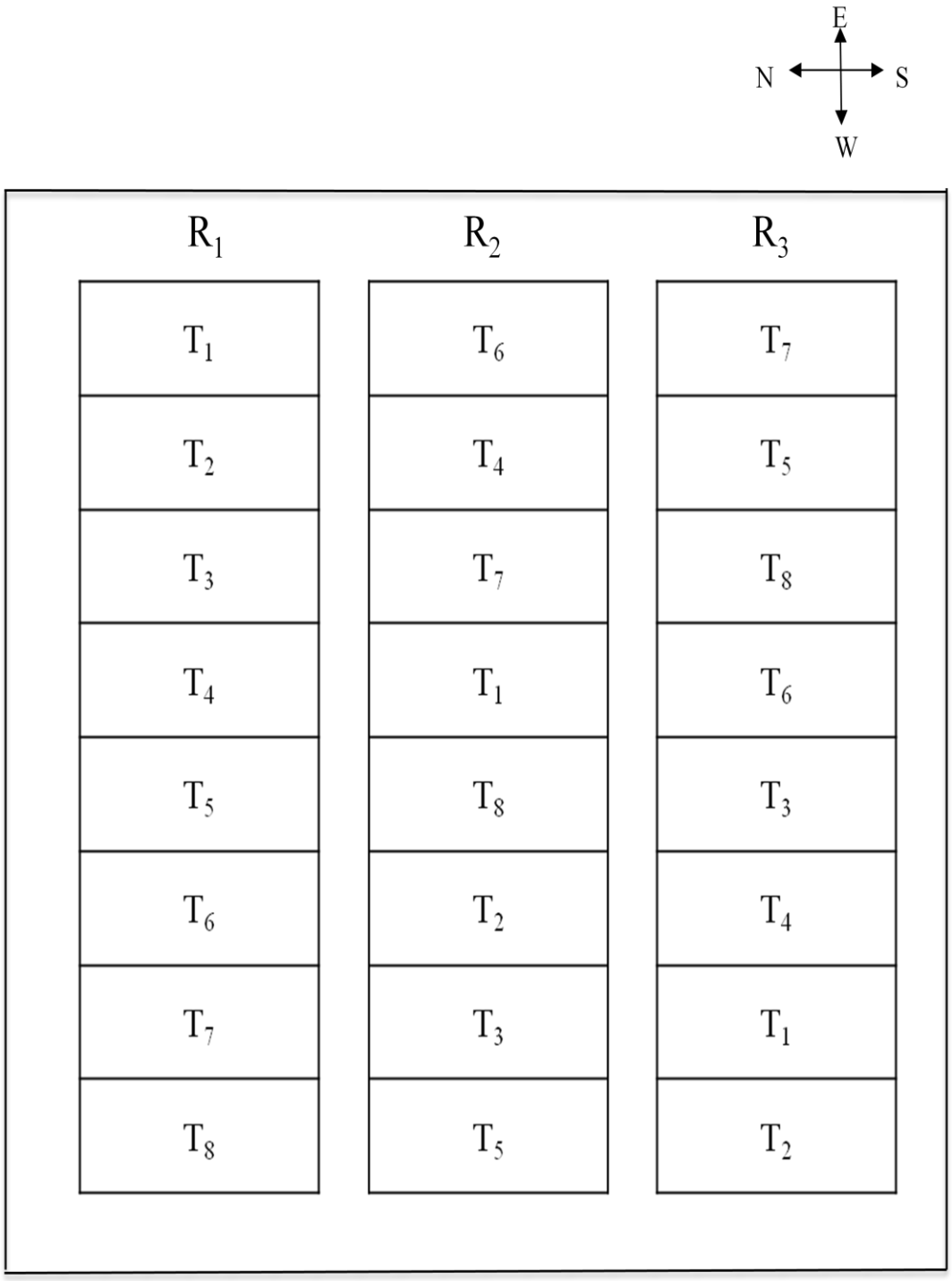


Figure 3.1: Layout of field experiment on management of early blight in tomato

3.9.5 Experimental details

Number of treatments : Eight

Treatment Details:

T_1 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS)

T_2 = Propineb @ 3g /kg (ST) + (Trifloxystrobin 25% + tebuconazole 50% WG@ 0.05%) (FS)

T_3 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas florescence* @ 1x 10^9 (FS)

T_4 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + *Pseudomonas florescence* @ 1x 10^9 (FS)

T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS)

T_6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)

T_7 = Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas florescence* @ 1x 10^9

T_8 = Control (water only)

Variety	:	Pusa Ruby
Replications	:	Three
Design	:	RBD
Spacing	:	60 x 45 cm
Plot size	:	4.8 M x 3.60 M

Dates of sowing and treatment applications

1.	Date of sowing	:	11.09.16
2.	Date of transplanting	:	15.10.16
3.	Date of first foliar spraying	:	15.11.16
4.	Date of second foliar spraying	:	22.11.16

3.9.6 Time and method of application

Seed of variety Pusa Ruby ware treated with Propineb @ 3g Kg^{-1} seed before sowing. After the transplanting foliar spray (FS) of fungicide/*P. fluorescens*/plant extracts were applied as per treatment details. First spray of

respective fungicides was given after 30 days of transplanting in all the treatments except in treatment T_7 where sprayed NSKE @ 5% instead of fungicide. In case of treatment T_3, T_4 and T_7, *Pseudomonas fluorescens* was sprayed as a second spray after 7 days of first spray, whereas in treatments T5 and T6 NSKE @ 5% was sprayed as a second spray. In control plot plants were sprayed with water as first and second spray.

Observation recorded:

1. **Disease intensity**

   Ten plants were selected randomly in each treatment and observation on severity of the disease on the foliage was recorded at 15, 30, 45, 60, 75 and 90 days after first spray using 0-5 disease rating scale (Pandey *et al.*, 2003). Percent Disease Index (PDI) and area under disease progress curve (AUDPC) was calculated using formulas described earlier.

2. **Fruit yield**

   Picking of fruits was done at the time of ripening. Total ten picking in management trial were done. Total weight of tomato fruit harvested per plant, per plot from all the pickings was calculated. Finally the yield tons per hectare was work out. The per cent avoidable number of fruits, fruit yield losses were calculated in all the treatments as follows:

$$\text{Avoidable loss } (\%) = \frac{T-C}{T} X\ 100$$

   Where,

   T = No. of fruits/fruit yields in treatment.

   C = No. of fruits/fruit yields in control.

3. **Economics and statistical analysis**

Cost benefit ratio of different treatments were worked out as per the rates of input applied for the disease management and wages prevailing during the course of the study.

3.10 Statistical analysis

Present experimental data was analyzed statistically by techniques of analysis of variance applicable RBD. The significance of treatments was tested by

F-test value. Critical Value at 5% level of significance was worked out for comparison and statistical interpretation of significant treatment means. The standard error of difference was given in each case for significant treatment effect, critical difference of different treatment combinations per interaction at 5% level of probability was calculated, wherever F-test was significant.

* * * * * * *

CHAPTER–IV
RESULTS AND DISCUSSION

Among the foliar diseases of tomato, early blight caused by *Alternaria solani* is one of the major diseases. Hence, investigation on **"Studies on *Alternaria solani* causing early blight disease in Tomato (*Lycopersicon esculentum* Mill.)"** was carried out during *Rabi* 2016-17 at the S.K. College of Agriculture and Research Station (IGKV, Raipur), Kawardha, C.G. During present investigation, field observations were recorded to collect information on the occurrence of disease on tomato in five district of Chhattisgarh around Kabirdham district. Laboratory studies on cultural and pathogenic variability among the *Alternaria solani* isolates and efficacy of fungicides, bio-control agents and plant extracts against *Alternaria solani* were undertaken. Further, in field, development of integrated management strategies for the control of early blight in tomato was also studied. The results obtained are presented in different sections under various sub-heads.

4.1 Survey on severity of early blight of tomato in different districts of Chhattisgarh

An intensive roving survey was conducted during *Rabi* 2016-17 in Kabirdham, Durg, Bemetara, Rajnandgaon and Mungeli district of Chhattisgarh during month of October, November, December 2016, January and February 2017 to know the status of early blight disease on tomato. The data pertaining to percent disease index (PDI) have been presented in Table 4.1 and revealed that the PDI was lowest (3.33%) in village Khapri and Arasnara of district Durg during month of October which was gradually increased with increasing of crop age throughout the cropping period. It was peak (92.33%) in village Kalegondi of district Rajnandgaon during month of February (last stage of crops).

Survey data have been presented in table 4.1 indicated that the PDI was ranged from 3.33 to 8.67%, 8.67 to 22.67%, 17.67 to 38.00, 30.67 to 71.00 and

40.67 to 92.33 in the month of October, November, December, January and February, respectively in various village of different district. In district Mungeli, maximum PDI of (87.33%) was observed in Dendori village followed by Sangwakapa (83.33%) and minimum PDI was observed in Saleghori village (75.00%) during month of February. In Kabirdham district, maximum PDI (85.33%) was recorded in Mainpuri village followed by Khadhauda (80.33%) and minimum PDI was observed in village Mohgaon (65.67%) during month of February. However, in Bemetara district, maximum (PDI was ranged from 3.67 to 6.67, 10.67 to 19.33, 20.67 to 33.56, 39.67 to 66.00 and 61.33 to 89.33% in the month of October, November, December, January and February), respectively in different villages. In case of Rajnadgaon district, maximum severity of early blight of 8.67, 22.67, 36.67, 71.00 and 92.33% during month of October, November, December, January and February, respectively in village Kalegondi and minimum PDI of 4.33, 12.00, 20.33, 40.00 and 58.67% during month of October, November, December, January and February, respectively in Bundela village. While, in Durg district, PDI was ranged from 3.33 to 7.67, 8.67 to 18.67, 17.67 to 38.00, 30.67 to 68.33 and 40.67 to 88.33 in the month of October, November, December, January and February, respectively.

The data indicated that the disease appeared in severe form in Mungeli district followed by Rajnadgaon during *Rabi*, 2016-17. Among the surveyed district, least average PDI of 4.75, 12.34, 24.75, 44.92 and 58.00 percent were recorded in Durg district during month of October, November, December, January and February, respectively. However, highest PDI of 7.42, 30.58, and 80.58 percent was recorded in Mungeli district during month of October, December and February, respectively. It was highest in the month of November (17.58%) and January (55.92%) in Rajnandgaon (Fig. 4.1). In case of AUDPC, least AUDPC (4845) was found in Durg district followed by Bemetara (5646), Kabirdham (5776) and Mungeli (6046) while, highest AUDPC was found in Rajnandgaon district (6063) (Fig. 4.2).

Table 4.1: Severity of early blight disease on tomato in different districts of Chhattisgarh during *Rabi* 2016-17

S. No.	Village	Variety	Area (ha.)	Early blight Intensity (%)				
				Oct.	Nov.	Dec.	Jan.	Feb.
A. District –Mungeli								
1.	Sangwakapa	Karishma	0.45	8.67	18.33	33.67	54.67	83.33
2.	Ramgarha	Laxmi 5005	0.63	7.33	21.00	29.33	46.33	76.67
3.	Saleghori	US- 440	0.79	6.00	15.33	31.00	43.67	75.00
4.	Dendori	Abhishekh	1.82	7.67	14.67	28.33	52.33	87.33
B. District –Kabirdham								
1.	Khadhauda	Arihant 7007	0.40	5.67	15.33	27.33	53.67	80.33
2.	Samnapur	Laxmi 5005	0.48	8.33	18.67	23.67	50.33	73.67
3.	Mohgaon	US- 440	0.76	4.67	13.67	20.33	46.67	65.67
4.	Mainpuri	Laxmi 5005	0.38	3.67	15.00	30.67	53.67	85.33
C. District –Bemetara								
1.	Lalpur	Laxmi 5005	0.52	5.33	16.33	27.33	50.33	82.67
2.	Patharikala	US- 440	0.82	3.67	10.67	25.67	41.56	63.67
3.	Pipariya	US- 440	0.73	6.67	19.33	33.56	66.00	89.33
4.	Bhalesar	Karishma	1.61	4.00	12.33	20.67	39.67	61.33
D. District – Rajnandgaon								
1.	Kalegondi	Laxmi 5005	0.36	8.67	22.67	36.67	71.00	92.33
2.	Mushra	962	0.83	5.33	15.33	23.00	49.33	65.33
3.	Dhara	Kunal	1.26	7.67	20.33	28.67	63.33	83.67
4.	Bundela	Kunal	1.27	4.33	12.00	20.33	40.00	58.67
E. District – Durg								
1.	Khapari	US- 440	1.28	3.33	12.67	24.00	45.00	57.67
2.	Kodiya	Kunal	1.69	4.67	9.33	19.33	35.67	45.33
3.	Arasnara	Karishma	7.12	3.33	8.67	17.67	30.67	40.67
4.	Kutha	US- 440	0.76	7.67	18.67	38.00	68.33	88.33

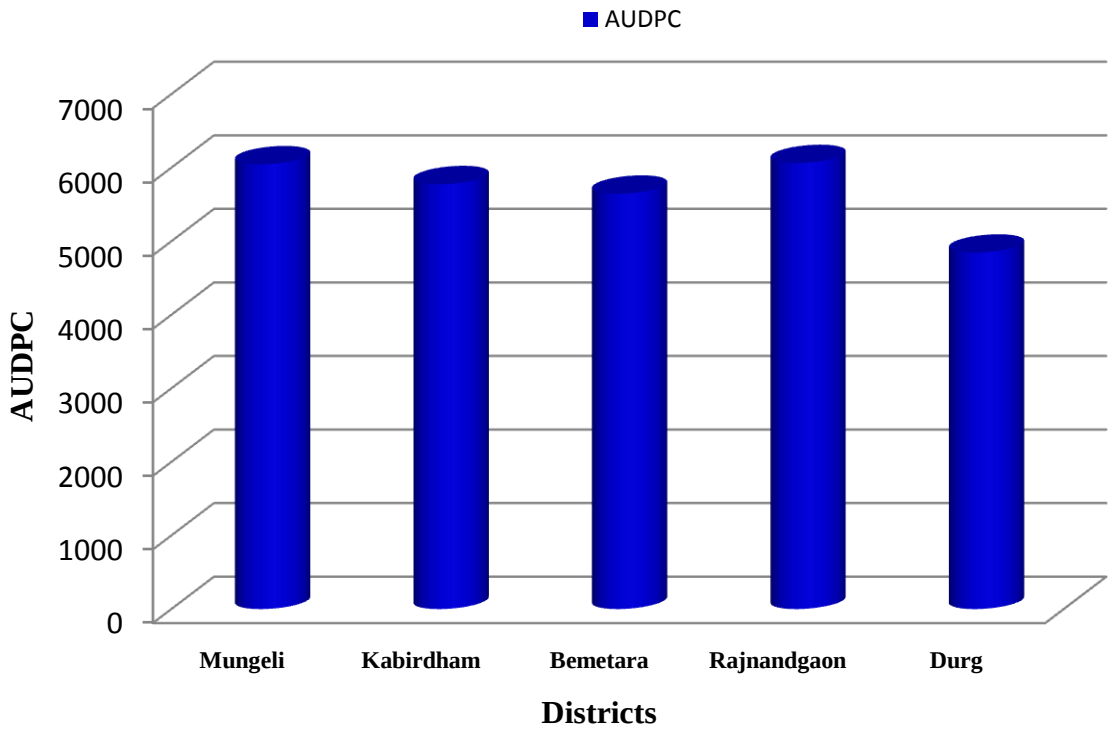


Fig. 4.2: AUDPC on tomato in different districts of Chhattisgarh during *Rabi* 2016-17

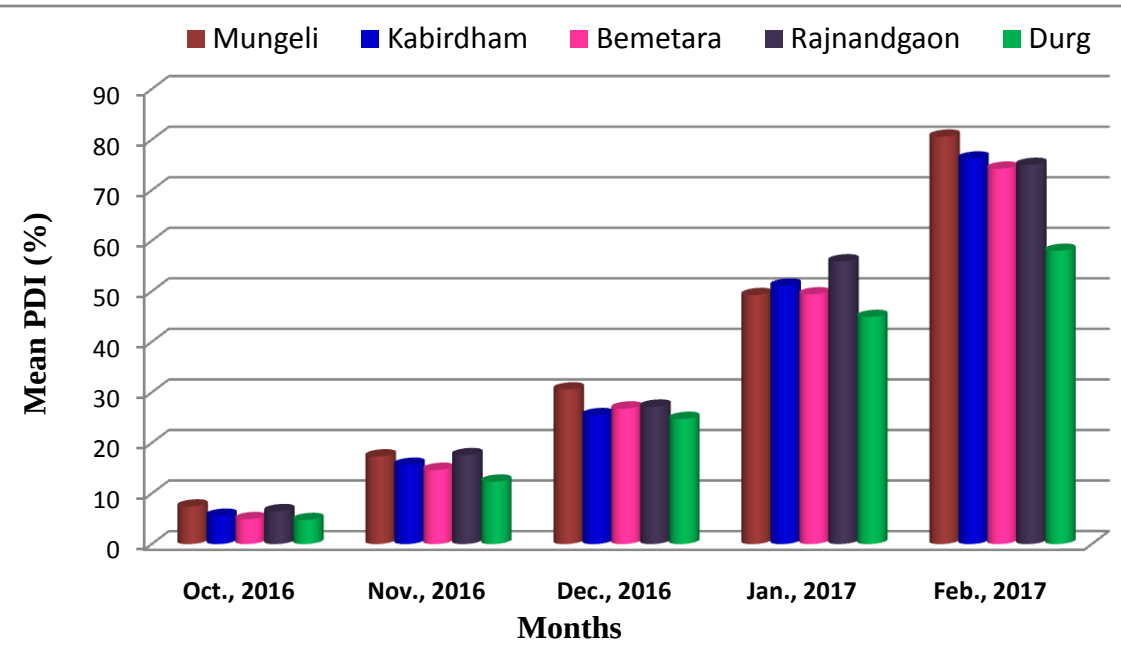


Fig. 4.1: Average early blight intensity on tomato in different districts of Chhattisgarh during *Rabi* 2016-17

Present finding was supported with the finding of Sahu *et al*. (2013). They conducted survey in *Rabi* 2012-13 to know the status of early blight diseases in Abhanpur, Aarang and Dharsiwa block of Raipur district of Chhattisgarh. In entire growing season disease severity was more in Abhanpur block (51.31%) followed by Aarang block (49.35%) and least in Dharsiwa block (44.24%). Survey was also conducted by Kamble *et al.* (2009) in Konkan region of Maharashtra. They found major disease was early blight incited by *A. solani* in tomato and early blight disease intensity was ranged between 20.78 to 42.30 percent in Raigad district and 35.12 to 55.75 percent in Thane district. Rani *et al*. (2015) conducted survey during 2011 and 2012 to diagnose the symptoms of early blight disease of tomato (*Lycopersicum esculentum* L.) in all vegetable growing areas of Jammu Division of Jammu and Kashmir. The disease intensity and incidence were varied from 21.66 to 34.13% and 10.48 to 18.56%, respectively. Pachori and Sharma (2016) conducted field survey during *Kharif* season 2014-15 at Gwalior, Bhind and Morena Districts of Madhya Pradesh to determine status of early blight on tomato. The percent disease incidence was ranged from 27.50% to 63.36%. The highest percent disease was observed in research farm (63.36%) of Gwalior District and minimum percent disease incidence was observed in village Daboha (27.5%) of Bhind District.

4.2 Symptomatology

The first symptoms of the early blight disease caused by *Alternaria solani* on tomato was appeared as small brown water soaked lesions on the older leaf (Plate 4.1a). Older leaves got infection first and later it was progressed upward (Plate 4.1b). The spots were oval or angular in shape ranged from 1 to 5 mm diameter. Narrow chlorotic zone was also noticed around the spot (Plate 4.1c). Later stage spots were enlarged and concentric rings were formed in the center (Plate 4.1d). In advance stage colour of the spots were changed from brown to dark brown and finally adjacent spots coalesced to form large irregular spots. Severe attacked of the disease caused drying and defoliation of foliage. When plants were old, symptoms appeared on stem and petioles as brown to dark brown elongated target board type spots (Plate 4.1e). These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepals and fruits resulting in pre-mature dropping of

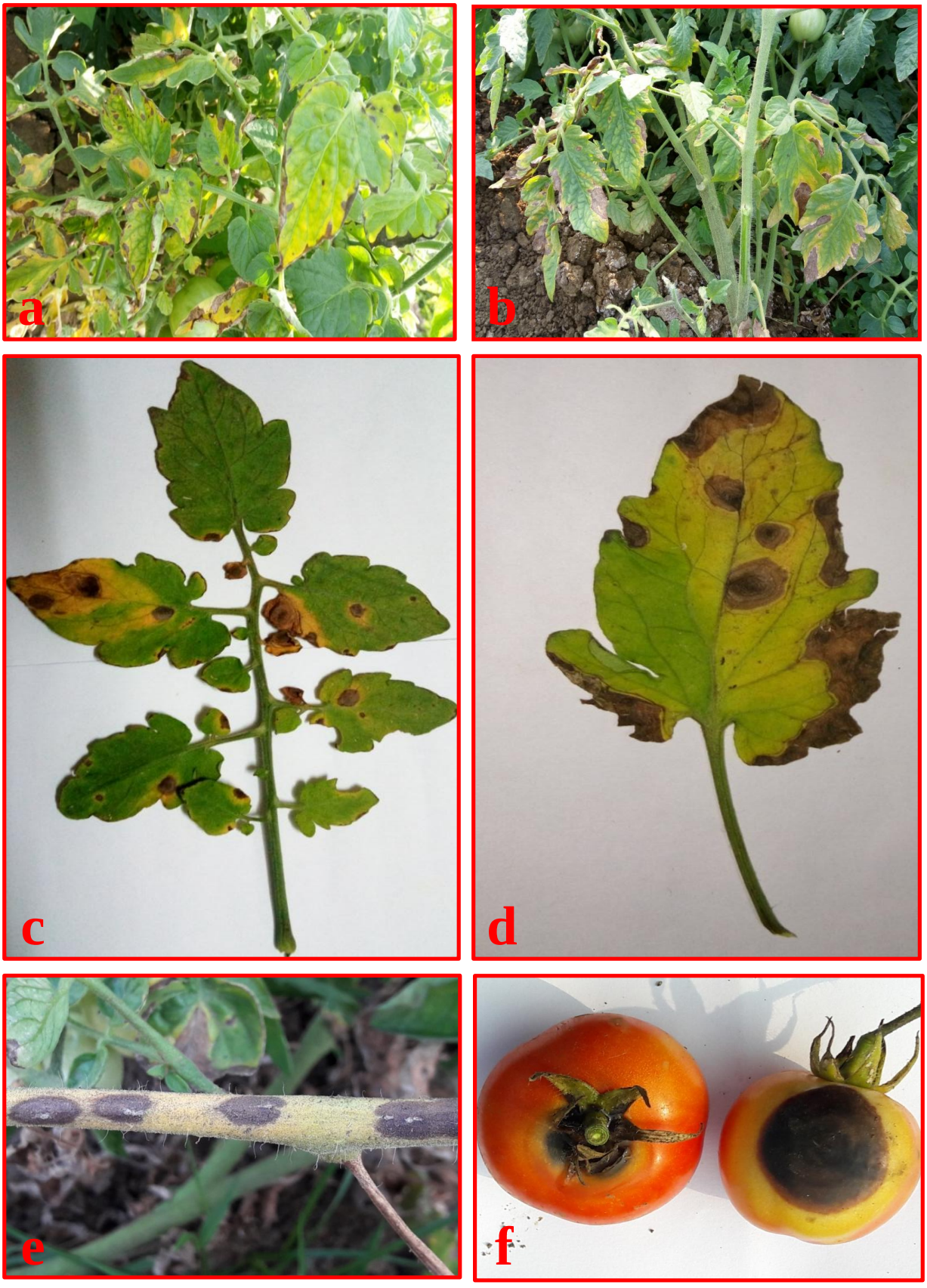


Plate 4.1: Symptoms of early blight of tomato on leaves, stem and fruits

fruits. The symptoms on fruits appeared first at stem end as black or brown sunken spots both on green and ripe fruits which enlarged within eight days involving most of the fruits, finally the fruits were rotted (Plate 4.1f).

Similar kinds of symptoms were also reported by Ramakrishnan *et al*. (1971). They observed cankerous spots on tomato stem of seedling causing by *A. solani*. Tomato fruit, both green and ripe, may also become infected with the fungus. Infection generally begins at the calyx end. Brown leathery areas were formed at infection sites. They contain the same concentric rings found in leaf spots. Walker (1952) reported that the spots were oval or angular in shape 0.3 or 0.4 cm diameter in size with usually narrow chlorotic zone around the spot. Symptoms progress from lower to upper leaves. Leaf spots begin as small brown areas on lower leaves. These areas enlarge surrounded by a border of yellow host tissue. As the spots mature, concentric rings of raised and depressed brown tissue were evident. Heavily infected plants often become defoliated. Peralta *et al.* (2005) reported that early blight disease can lead to complete defoliation in severe case in regions. Chaerani *et al.* (2006) noticed symptoms on tomato due to early blight as collar rot on stem of seedlings, lesions on stem of adult plant and rotting on fruits. Foolad *et al*. (2008) also described early blight symptoms on potato and tomato foliage as small, dark, circular lesions becoming distinctly zonate as they develop. Stem lesions was occur on diseased tomato plants as roughly circular, sunken, dark and zonate. Appearance of rot spots on green and ripe fruit of tomato were also reopted by Blancard *et al*. (2012).

4.3 Isolation and identification of the *Alternaria solani* isolates

Tomato leaves showing typical early blight symptoms of dark brown spots with concentric rings surrounded by discoloured tissue were collected from infected field. The standard tissue isolation technique was followed to obtain *Alternaria solani* culture. Identification of the isolated fungus was carried out based on the morphological characters. The conidiophores were formed singly and in groups, they were straight or flexuous and brown to olivaceous brown in colour. The conidia were observed solitary straight and slightly flexuous oblong or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 150-300

µm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks of conidia were flexuous, pale and sometimes branched.

The description of this fungus agreed with the description given for *A. solani* by Common Wealth Mycological institute, Kew, Surrey, England (Ellis, 1971). Thus, the pathogen causing early blight of tomato has been identified as *A. solani* Ellis and Martin (Jones and Grout, 1986).

4.4 Pathogenicity test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and such culture was used for pathogenicity test following by Koch's Postulate. The pathogenicity test was carried out as described in material and methods by inoculation with spore suspension with homogenized mycelial bits of *A. solani* on foliage of 30 days old Pusa Ruby variety of tomato. Fifteen days after inoculation, symptoms were appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. Hence, the causal agents of the disease were confirmed as *Alternaria solani.*

4.5 Cultural and pathogenic variability of *Alternaria solani* isolates

4.5.1 Studies on cultural variability among the *Alternaria solani* isolates

4.5.1.1 Variability in colony diameter among the isolates of *A. solani* on PDA medium

The data on variability in colony diameter have been presented in Table 4.2 indicated that the significant differences in colony diameter was recorded among the isolates of *Alternaria solani*. Maximum radial growth of the fungus (22.67mm) was recorded in isolate AS-6 which was at par with isolate AS-1 (22.50 mm), AS-2 (22.21 mm), AS-4 (21.17 mm) and AS-5 (20.83 mm) and significantly higher over isolate AS-3 (20.40 mm) and AS-7 (18.17 mm). Same trend was found after 96 hours of incubation. However, 144 hours after incubation, maximum colony diameter was observed in isolate AS-6 (68.78mm). It was at par with AS-1

(67.40mm), AS-2 (66.40mm), and AS-4 (65.40mm) and significantly higher over rest of the isolates. Same trend was found after 192 and 240 hours after incubation (Plate 4.2).

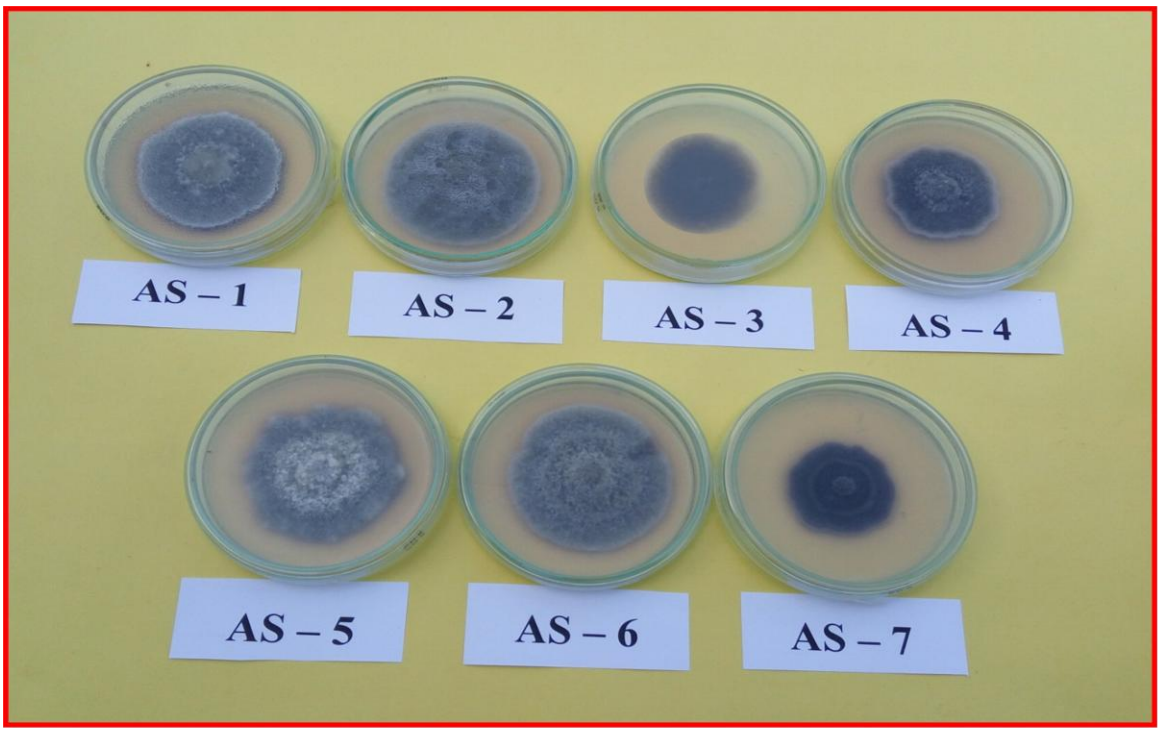
Table 4.2: Variability in colony diameter among the isolates of *A. solani* on PDA medium:

Isolates	Colony diameter (mm)				
	48 HAI	**96 HAI**	**144 HAI**	**192 HAI**	**240 HAI**
AS-1	22.50	49.25	67.40	79.57	89.35
AS-2	22.21	47.53	66.40	78.57	88.50
AS-3	20.40	42.56	63.00	74.40	83.43
AS-4	21.17	47.17	65.40	78.57	86.17
AS-5	20.83	46.84	63.40	76.57	83.83
AS-6	22.67	50.26	68.78	82.17	90.00
AS-7	18.17	32.60	46.00	55.33	65.50
CV (%)	**5.26**	**6.31**	**5.67**	**7.15**	**6.78**
SEm±	**0.78**	**1.13**	**1.29**	**1.55**	**1.60**
CD at 5%	**2.17**	**3.16**	**3.59**	**4.34**	**4.48**

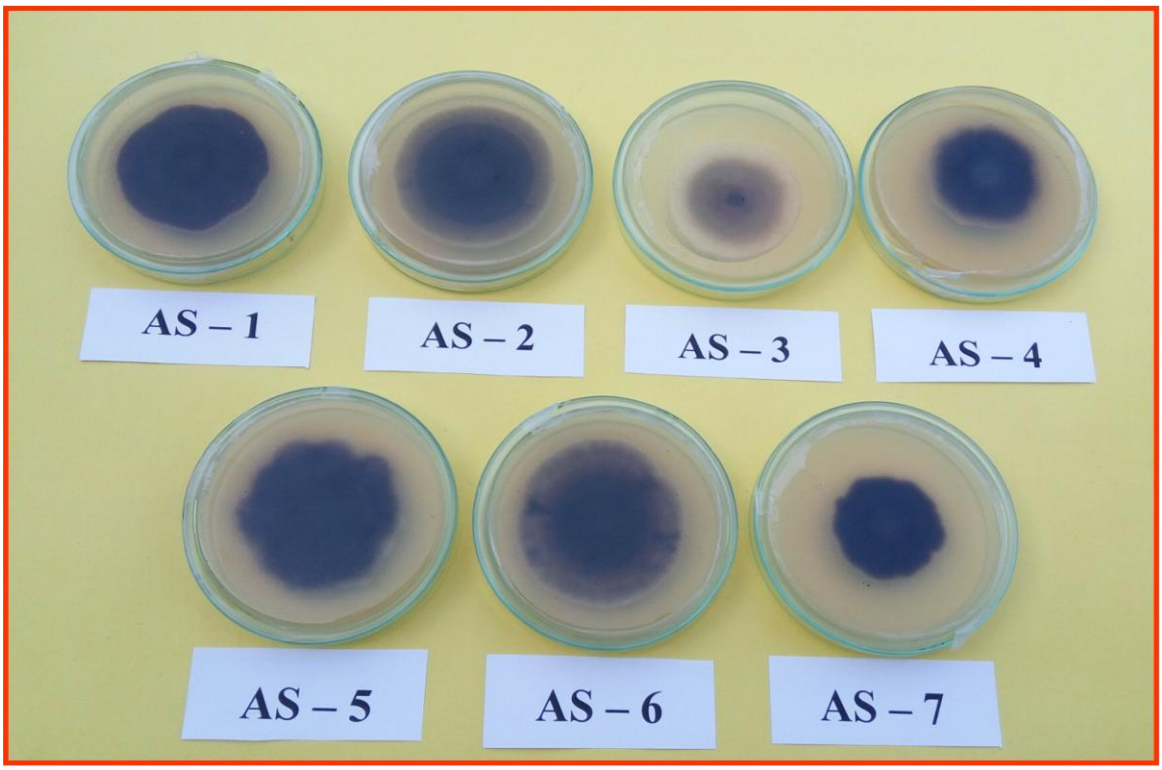
HAI= hours after incubation

4.5.1.2 Variability in cultural characters among the isolates of *Alternaria solani* on PDA medium

Cultural variability data have been presented in Table 4.3 indicated that the variation in colony colour, substrate colour, growth pattern, margin colour, topography, thickness of mycelium mat and sporulation were observed among the isolates of *Alternaria solani*. In case of colony colour, greenish brown colour colony was observed in isolates AS-1, grayish brown in isolate AS-2, AS-4 and AS-5, gray colour in AS-6, grayish black in AS-3, while dark brown colony colour was observed in isolate AS-7. In case of substrate colour, black colour substrate was observed in isolate AS-1 and AS-7, brown to black in isolate AS-2 and AS-3, grayish black in isolate AS-4, dark brown to black in AS-5 and dark brown in AS-6.Growth pattern were also varied from circular smooth to irregular rough. Isolate AS-3 was gave circular smooth colony, whereas circular rough colony was observed in isolate AS-2 and AS-6. Irregular smooth colony growth was noticed in isolate AS-7 and irregular rough in isolate AS-1, AS-4 and AS-5. In case of margin colour of colony, whitish margin was appeared in isolate AS-1, brown in AS-7, whitish gray in AS-2, AS-4 and AS-6, whitish brown in AS-3 and gray in AS-5. Arial topography was observed in isolate AS-1, AS-2, AS-3, AS-4, AS-5 and AS-6. While, merged topography was observed in isolate AS-7. In case of thickness of mycelium mat on PDA medium, very thick mycelium mat was observed in all the isolates except isolate AS-7 which showed thick mycelium mat. In case of sporulation, excellent sporulation was noticed in isolate AS-3, AS-5, AS-6 and AS-7, however poor sporulation was observed in isolate AS-1, AS-2 and AS-4 (Plate 4.2).



Colony characters of different isolate of *Alternaria solani* on PDA



Substrate colour of different isolate of *Alternaria solani* on PDA

Plate 4.2: Variability in cultural characters among the isolates of *Alternaria solani* on PDA medium

Table 4.3: Cultural variability among the isolates of *Alternaria solani* on PDA medium

Isolates	Cultural characters of *Alternaria solani* isolates						
	Colony colour	**Substrate colour**	**Growth Pattern**	**Margin Colour**	**Topography**	**Thickness of mycelium mat**	**Sporulation**
AS-1	Greenish brown	Black	Irregular rough	Whitish	Aerial	Very thick	++
AS-2	Grayish brown	Brown to black	Circular rough	Whitish gray	Aerial	Very thick	++
AS-3	Grayish black	Brown to black	Circular smooth	Whitish brown	Aerial	Thick	++++
AS-4	Grayish brown	Grayish black	Irregular rough	Whitish gray	Aerial	Very thick	++
AS-5	Grayish brown	Dark brown to black	Irregular rough	Gray	Aerial	Very thick	++++
AS-6	Gray	Dark brown	Circular rough	Whitish gray	Aerial	Very thick	++++
AS-7	Dark brown	Black	Irregular smooth	Brown	Merged	Thick	++++

- = (0 spore) No sporulation, + = (1-10 spore per microscopic field) Poor sporulation, ++ = (11-20 spore per microscopic field) moderate sporulation, +++ = (21-30 spore per microscopic field) good sporulation, ++++ = (more than 30 spore per microscopic field) excellent sporulation

4.5.2 Studies on morphological variability among the isolates of *Alternaria solani* on PDA medium

The microscopic examination on septation in conidia have been presented in table 4.4 revealed that the highly septation were found in conidia of all the isolates on PDA medium. In case of horizontal septation, highest horizontal septa (2-11) was recorded in isolate AS-7, followed by AS-2 (3-10), AS-6 (3-9) and AS-4 (3-7), while least horizontal septa (3-6) was observed in isolate AS-1 and AS-5. However, maximum vertical septa (1-3) were observed in isolate AS-3, AS-4 and AS-6 and minimum vertical septa (0-2) was found in isolate AS-1, AS-2, AS-5 and AS-7. Conidia length with beak was varies from 39.17 - 74.35 µm in isolate AS-6, 29.86 -73.23 µm in AS-7, 34.34 -73.14 µm in AS-2, 33.61 -71.43 µm in AS-4, 32.12 - 68.63 µm in AS-1, 30.53 -63.14 µm in AS-5 and 28.12 -58.35 µm in AS-3. In case of beak length, maximum length (7.64 -14.78µm) was recorded in isolate AS-4, while minimum length of beak (4.18 -7.62µm) was recorded in isolate AS-3. Variation in width of conidia was also observed among the isolates of *A. solani*. Width of conidia was maximum (8.82 – 24.23 µm) in isolate AS-3. While, minimum width of conidia (8.13 – 19.48 µm) was found in isolate AS-7 (Plate 4.3).

The result obtained in the present study agreed with the findings of Alhussaen (2012) who reported that the mycelia width between 0.8-1.5 µm and conidia are 35-75 µm in length and 10-20 µmin width and 2-7 transverse septa and 1-4 longitudinal septa. Similar result was also obtained by Naik *et al*. (2010) describe *Alternaria solani* isolate from tomato plant and those results were agreed with the result presented in this study. Similar type result is also obtained by Ellis (1971), Kumar *et al.* (2012), Kaul and Saxena (1988) and Marak *et al*. (2014).

Table 4.4: Morphological variability among the isolates of *Alternaria solani* on PDA medium

Sl. No.	Isolates	No. of horizontal septation	No. of vertical septation	Length of conidia with beak (µm)	Beak length (µm)	Width of conidia (µm)
1	AS-1	3 – 6	0 – 2	32.12 - 68.63	6.68 -13.23	8.12 - 21.36
2	AS-2	3 – 10	0 – 2	34.34 -73.14	5.76 -12.37	7.91 -19.56
3	AS-3	2 – 7	1 – 3	28.12 -58.35	4.18 -7.62	8.82 -24.23
4	AS-4	3 – 7	1 – 3	33.61 -71.43	7.64 -14.78	9.32 -22.86
5	AS-5	3 – 6	0 – 2	30.53 -63.14	4.25 -8.87	7.05 -20.56
6	AS-6	3 – 9	1 – 3	39.17 -74.35	7.23 -14.16	8.63 -21.63
7	AS-7	2 – 11	0 – 2	29.86 -73.23	3.63 -13.65	8.13 -19.48

4.5.3 Studies on pathogenic variability among the isolates of *Alternaria solani*

Experimental data regarding first appearance of symptoms, PDI and AUDPC of different isolates of *Alternaria solani* on variety Pusa Ruby under pot culture have been presented in table 4.5. In case of appearance of symptoms, symptoms was early noticed in isolate AS-6 (3-4 DAI) followed by isolate AS-5 and AS-7 (3-5 DAI), AS-3 and AS-4 (4-5 DAI), AS-2 (6-7 DAI), while delay symptoms were appeared in isolate AS-1(6-8 DAI). Percent disease intensity was recorded after 42 days of inoculation reveal that the highest PDI was observed (88.89 %) in isolate AS-6 followed by AS-5 (84.44%), while least PDI was observed in isolate AS-1 (Plate 4.4a,b). Area Under Disease Progress Curve (AUDPC) was calculated from the disease intensity recorded at 7 days intervals up to 42 days after inoculation are illustrated in Fig. 4.3. Isolate AS-6 showed maximum AUDPC (2302) followed by AS-5(2147), AS-7(1836), AS-3(1672), AS-4(1423), AS-2(1260). While, minimum AUDPC was observed in isolate AS-1(996).

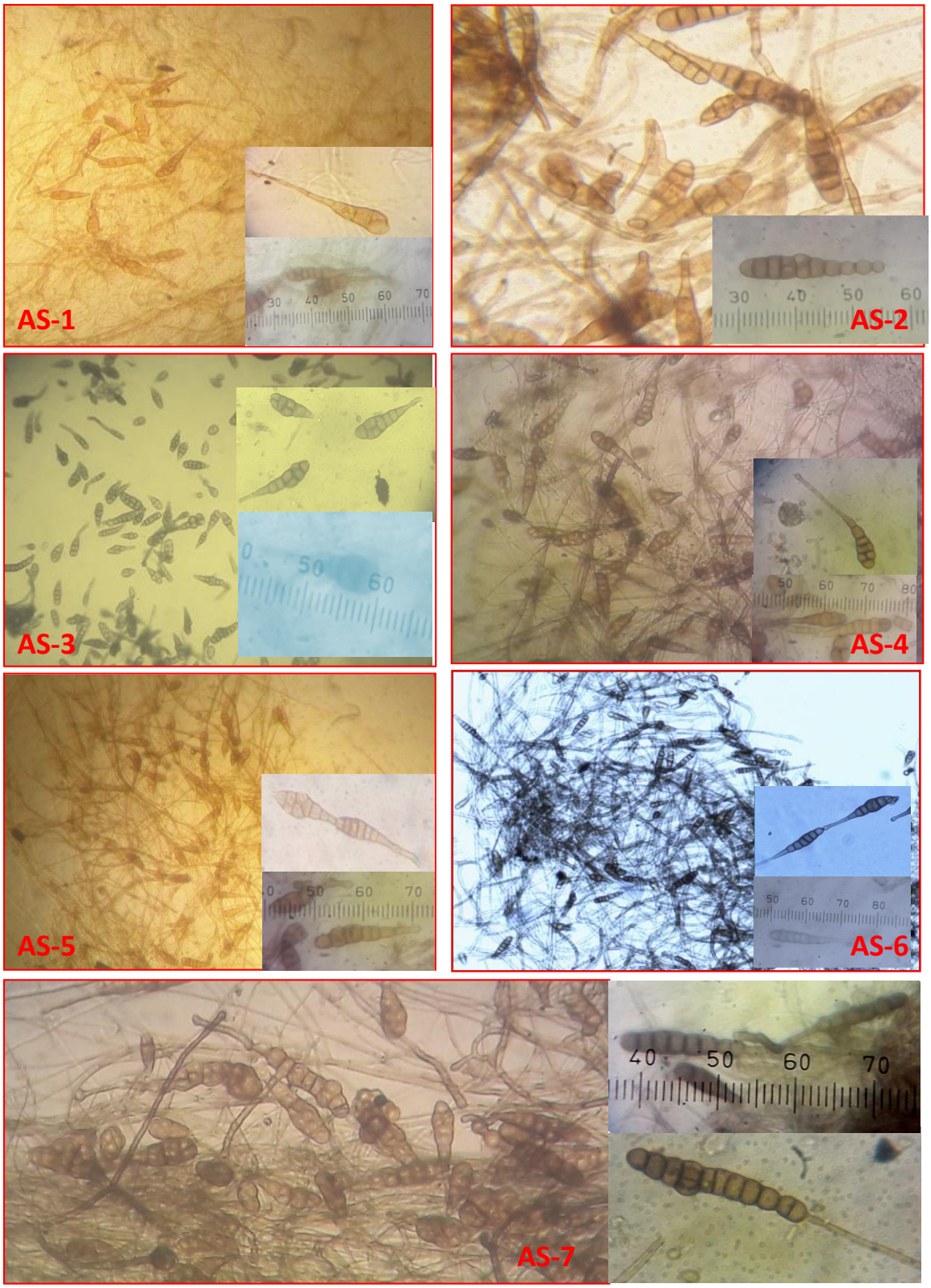


Plate 4.3: Morphological variability among the isolates of *Alternaria solani* on PDA medium

Present investigation indicated that the most virulent was found isolate AS-6 followed by AS-5 and AS-7. However, isolate AS-1, AS-2 and AS-4 was found moderately virulent. Similar findings were also reported by Castro *et al*. (2000) conducted an experiment on study of variability of *A. solani* under green house conditions based on the inoculation of 7 isolates on 14 tomato genotypes and reveal that all the isolates showed different degree of virulence on 14 tomato genotypes, demonstrating the existence of high level of variability in the fungus. Singh *et al.* (2014) reported that out of 10 isolates, only three major groups were recorded on the basis of SAS analysis likewise Group-A were highly virulent (MF-4 and PN-4), Group-C indicate virulent (BG, AF-2, EC-1 and RF-1) and Group-E were less virulent (BHU-1, IIVR, SF-1 and BX-2) with all the five varieties. Similar results were also reported by Rahmatzai *et al.* (2016). The isolate AS1 of *A. solani* was mostly virulent with maximum level of disease incidence (53.5%) and disease severity (32%), whereas, the AS2 isolate noted the least early blight incidence of 27% and disease severity of 18%. AS1 isolate produced properly clear early blight symptoms on leaves, stem and even fruits and was found to be virulent causing sever disease in tomato plants.

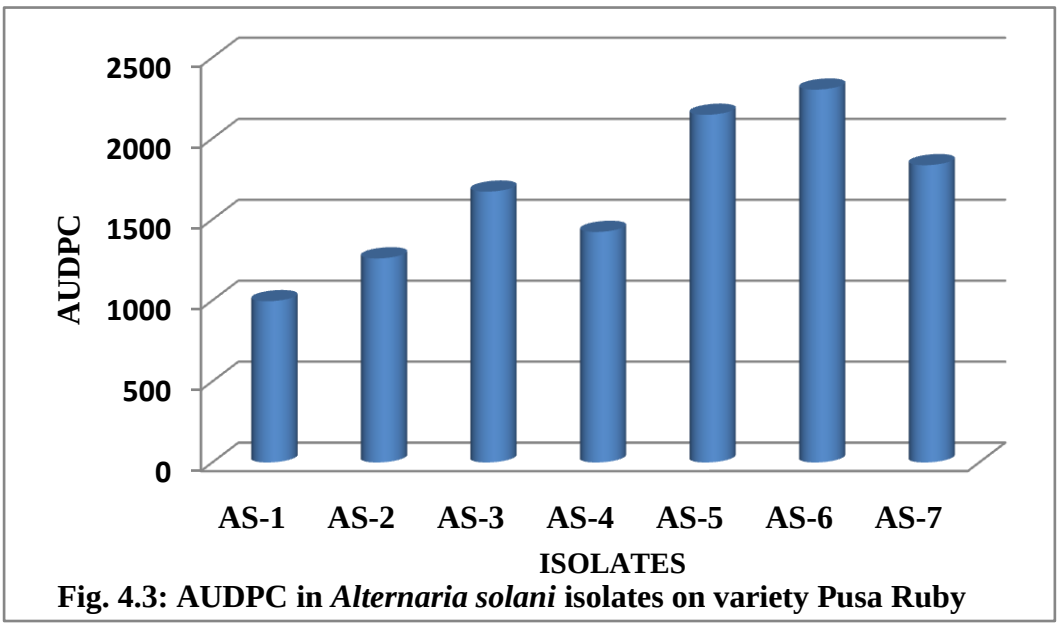


Fig. 4.3: AUDPC in *Alternaria solani* isolates on variety Pusa Ruby

Plate 4.4a: Pathogenic variability among the isolates of *Alternaria solani* on variety Pusa Ruby

Plate 4.4b: Pathogenic variability among the isolates of *Alternaria solani* on variety Pusa Ruby

Table 4.5: Pathogenic variability among the isolates of *Alternaria solani* on variety Pusa Ruby

Isolates	Symptoms appearance (Days after inoculation)	PDI at 42 days after inoculation	AUDPC
AS-1	6-8	40.00	995.56
AS-2	6-7	48.49	1260.00
AS-3	4-5	64.44	1672.22
AS-4	4-5	55.56	1423.33
AS-5	3-5	84.44	2146.67
AS-6	3-4	88.89	2302.22
AS-7	3-5	75.56	1835.56

4.6 Studies on effect of culture media on different isolates of *A. solani*

The cultural characteristics of *A. solani* isolated from tomato was studied on 14 solid media as described in material and methods and the results of the study are presented below that the pathogen showed highly significant difference in its mycelium growth on different media (Plate 4.5 a,b,c,d).

4.6.1. Effect of culture media on colony diameter of different isolates of *A. solani*

4.6.1.1 Effect of culture media on colony diameter of different isolates of *A. solani* after 48 hrs of incubation:

The data have been presented in Table 4.6 indicated that the colony diameter of *Alternaria solani* isolates were significantly influenced by culture media. Among the different media, PDA media was found better which showed maximum mean radial growth of fungus (21.14mm). It was at par with Oat meal agar media (20.40mm) and significantly superior over rest of culture media. Whereas, minimum mean colony diameter was observed in water agar media (11.59mm). In case of isolates, maximum colony diameter (17.57mm) was observed in isolate AS-1 followed by AS-6 (17.33mm), AS-2 (16.93mm), AS-4 (16.16mm), AS-5 (15.66mm), AS-3 (15.55mm) and minimum colony diameter was recorded in isolate AS-7 (12.76mm). Interaction between media and isolates was found significantly. Isolate AS-6 was showed maximum mycelium growth (22.67mm) on PDA followed by AS-1 (22.50mm) on PDA media, while minimum mycelium growth was observed in isolate AS-7 (9.00 mm) in mineral agar.

4.6.1.2 Effect of culture media on colony diameter of different isolates of *A. solani* after 96 hrs of incubation

Experimental data have been presented in Table 4.7 showed that the mycelium growth of *Alternaria solani* varied within media to media and isolate to isolate. In case of media, highest mean colony diameter was observed on PDA media (45.17 mm) after 96 hrs of incubation followed by oatmeal agar media (43.72 mm), while minimum mycelium growth was observed in water agar media (19.43 mm). Among the seven isolates, more colony growth was observed in AS-1 (34.67mm diameter) which was at par with isolate AS-2 (34.27mm diameter) and AS-6 (34.42mm diameter) and significantly higher over rest of the isolates. While, isolate AS-7 was showed minimum colony diameter (20.59mm). Interaction was found significant. Maximum radial growth was observed in isolate AS-6 on PDA media (50.26mm) followed by AS-1on PDA media (49.25 mm), AS-6 (49.17mm) on OMA and minimum mycelium growth was observed in isolate As-7 on water agar media (13.00 mm).

Table 4.6: Effect of culture media on colony diameter of different isolates of *A. solani* after 48 hrs of incubation

Media	Colony diameter after 48 hrs incubation (mm)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Agar	22.50	22.21	20.40	21.17	20.83	22.67	18.17	**21.14**
Oat Meal Agar	21.83	20.83	20.33	19.67	20.17	22.00	18.00	**20.40**
Asthana & Hawker's	19.00	17.67	17.67	18.17	16.83	18.00	16.33	**17.67**
Corn Meal Agar Media	19.50	18.00	18.67	18.50	17.17	20.17	16.83	**18.41**
PDA + $CaCo_3$	18.33	16.50	13.83	17.50	16.67	17.00	10.33	**15.74**
Tomato Leaf Agar	20.63	20.00	19.17	19.50	19.83	21.67	17.83	**19.80**
Czapek Dox Agar	17.17	12.83	11.17	11.67	12.03	14.50	9.50	**12.70**
Waksman's Agar	16.33	17.33	13.33	15.83	15.00	16.00	10.00	**14.83**
Malt Extract Agar	16.33	17.83	17.50	17.33	16.33	16.17	14.83	**16.62**
Mineral Agar	16.00	12.17	12.50	12.67	12.17	13.33	9.00	**12.55**
YPSS	15.67	13.83	11.83	12.00	12.00	14.67	9.17	**12.74**
Martin's Agar	15.17	17.83	16.50	14.50	14.33	16.00	9.67	**14.86**
Glucose Peptone Yeast Agar	13.83	16.17	15.33	16.33	15.33	17.67	9.50	**14.88**
Water Agar	13.67	13.83	9.50	11.33	10.50	12.83	9.50	**11.59**
Mean	**17.57**	**16.93**	**15.55**	**16.16**	**15.66**	**17.33**	**12.76**	

	CV (%)	**SEm±**	**CD at 5%**
Factor(A)		0.294	0.82
Factor(B)	6.41	0.208	0.58
Factor(A X B)		0.777	2.17

Table 4.7: Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 96 hrs of incubation

Media	Colony diameter after 96 hrs incubation (mm)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Agar	49.25	47.53	42.56	47.17	46.84	50.26	32.60	**45.17**
Oat Meal Agar	47.33	46.67	47.17	38.67	45.67	49.17	31.33	**43.72**
Asthana& Hawker's	37.83	39.00	38.67	35.00	32.00	39.17	26.00	**35.38**
Corn Meal Agar Media	43.83	40.33	40.50	35.83	33.50	40.00	28.17	**37.45**
PDA + $CaCo_3$	36.67	32.67	30.00	34.17	30.00	32.67	16.00	**30.31**
Tomato Leaf Agar	47.19	43.33	45.27	38.25	42.37	43.27	29.27	**41.28**
CzapekDox Agar	32.83	46.67	21.56	20.52	21.50	25.83	14.47	**26.20**
Waksman's Agar	30.50	29.83	29.50	28.83	24.33	29.00	15.00	**26.71**
MaltExtract Agar	29.83	32.67	37.67	34.80	30.00	31.50	24.33	**31.54**
Mineral Agar	28.00	29.17	19.17	22.83	21.67	24.83	14.27	**22.85**
YPSS	27.50	22.00	21.33	17.50	21.33	27.73	14.33	**21.67**
Martin's Agar	26.83	21.50	31.83	23.67	24.00	28.67	15.00	**24.50**
Glucose Peptone Yeast Agar	24.67	21.67	34.50	29.33	27.83	37.83	14.50	**27.19**
Water Agar	23.17	26.67	16.17	16.50	18.50	22.00	13.00	**19.43**
Mean	**34.67**	**34.27**	**32.56**	**30.22**	**29.97**	**34.42**	**20.59**	

	CV (%)	**SEm±**	**CD at 5%**
Factor(A)	6.14	0.41	1.16
Factor(B)		0.29	0.82
Factor(A X B)		1.10	3.06

4.6.1.3 Effect of culture media on colony diameter of different isolates of *A. solani* after 144 hrs of incubation

The data pertaining to the effect of different culture media on *A. solani* isolates after 144 hrs of incubation have been presented in Table 4.8 reveal that mycelium growth of *A. solani* were influenced by different culture media in all the isolates. Mean colony diameter observed was maximum (62.91mm) in PDA medium followed by Oatmeal agar medium (60.55mm), while minimum growth was observed in water agar medium (26.92mm). Maximum colony diameter (50.63mm) was observed in isolate AS-6 which was significantly higher over rest of the isolates, whereas minimum colony diameter was observed in AS-7(29.31mm) after 144 days of incubation. Interaction between isolate and culture media was found significant. Isolate AS- 6 showing maximum growth (68.78mm) on PDA which was at par with on oatmeal agar medium (68.17mm), isolate AS-2 on PDA (66.40mm) and OMA (66.50mm) and isolate AS-1, AS-3 on PDA and significantly higher over rest of the combination.

4.6.1.4 Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 192 hrs of incubation

Results indicated that the mycelium growth was influenced by different culture media in all the isolates of *Alternaria solani*. Among the different culture media, maximum mean colony diameter observed was 79.57, 78.57, 74.40, 78.57, 76.57, 82.17 and 55.33mm on PDA media in isolate AS-1, AS-2, AS-3, AS-4, AS-5, AS-6 and AS-7, respectively. PDA medium was showed significantly higher growth of the fungus as compare to other culture media. Among the isolates, isolate AS-6 was showed significantly higher colony diameter (61.79mm) followed by AS-1(58.91mm), AS-2 (57.79mm) and minimum colony diameter was observed in isolate AS-7 (36.76 mm). Interaction between media and isolates was found significant. Isolate AS-6 showed maximum mycelium growth (82.17 mm), while minimum mycelia growth was observed in isolate AS-7 (24.17 mm) in mineral agar media (Table 4.9).

Table 4.8: Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 144 hrs of incubation

Media	Colony diameter after 144 hrs incubation (mm)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Agar	67.40	66.40	63.00	65.40	63.40	68.78	46.00	**62.91**
Oat Meal Agar	64.67	66.50	63.33	53.33	62.17	68.17	45.67	**60.55**
Asthana & Hawker's	60.67	60.53	52.50	48.50	45.33	57.83	34.83	**51.46**
Corn Meal Agar Media	62.33	61.33	55.50	49.00	47.33	58.17	39.50	**53.31**
PDA + $CaCo_3$	58.00	54.33	41.83	48.33	43.83	49.17	26.67	**46.02**
Tomato Leaf Agar	63.17	63.83	58.00	51.67	60.83	66.67	41.83	**58.00**
Czapek Dox Agar	46.50	34.50	33.67	29.17	30.17	36.67	19.83	**32.93**
Waksman's Agar	45.00	49.17	40.83	37.33	34.50	44.33	24.33	**39.36**
Malt Extract Agar	40.67	45.33	51.83	47.83	43.00	46.83	30.00	**43.64**
Mineral Agar	37.17	31.67	29.67	31.00	30.50	33.00	19.17	**30.31**
YPSS	36.67	30.83	34.00	23.50	29.83	40.00	20.23	**30.72**
Martin's Agar	29.83	30.33	31.00	35.17	34.00	44.33	21.17	**32.26**
Glucose Peptone Yeast Agar	28.50	42.83	47.00	38.83	39.33	56.00	20.00	**38.93**
Water Agar	27.17	28.83	25.00	21.00	26.50	38.83	21.13	**26.92**
Mean	**47.70**	**47.60**	**44.80**	**41.43**	**42.19**	**50.63**	**29.31**	

	CV (%)	**SEm±**	**CD at 5%**
Factor(A)		0.50	1.38
Factor(B)	5.22	0.35	0.98
Factor(A X B)		1.31	3.65

4.6.1.5 Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 240 hrs of incubation

Data regarding effect of different culture media on colony diameter of different isolates of *Alternaria solani* after 240 hrs of incubation have been presented in table 4.10 reveal that the most suitable culture media was found PDA which exhibited maximum diameter of 89.35, 88.50, 83.43, 86.17, 83.83, 90.00 and 65.50mm in isolate AS-1, AS-2, AS-3, AS-4, AS-5, AS-6 and AS-7, respectively. In case of medium maximum colony diameter (83.83mm) was observed on PDA which was significantly superior over rest all the media. Among the seven isolates of *A. solani*, isolate AS-6 showed maximum colony diameter (71.73mm) followed by AS-2 (67.41mm), AS-1(67.00mm), AS-5(61.64mm) and minimum colony diameter of 43.06 mm was noted in isolate AS-7. Interaction between isolates and media was found significant. Maximum colony diameter was observed on PDA medium in isolate AS-6 (90.00mm) which was at par with PDA in AS-1 (89.35mm), AS-2 (88.50mm), AS-4 (86.17mm) and OMA in AS-1 (86.67mm), AS-2 (87.33mm) and AS-6 (89.00mm) and significantly higher over rest of the combination (Table 4.10 & Plate 4.5a,b,c,d).

The results obtained in the present investigation are confirmed with finding of several workers. Koley and Mahapatra (2015) reported potato dextrose agar and oat meal agar medium better than other media for growth of tomato early blight causing fungi. Potato dextrose agar medium was found a good medium for growth of *Alternaria solani* (Neergaard, 1945; Barksdale, 1968). Arunakumara (2006) reported maximum growth of *Alternaria* on PDA followed by corn meal agar after 9 days of inoculation. Hubballi *et al*. (2010) tested different culture media against the growth of *A. alternata*. Host leaf extract medium supported significantly which exhibited maximum growth of all the fifteen isolates of *A. alternata* followed by potato dextrose agar. Chohan *et al.* (2015) postulated that the maximum growth of *Alternaria solani* was obtained at 25°C on PDA medium.

Table 4.9: Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 192 hrs of incubation

Media	Colony diameter after 192 hrs incubation (mm)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Agar	79.57	78.57	74.40	78.57	76.57	82.17	55.33	**75.03**
Oat Meal Agar	77.67	77.33	73.67	68.33	74.10	81.12	54.57	**72.40**
Asthana & Hawker's	73.67	70.83	65.83	64.83	58.00	70.00	45.67	**64.12**
Corn Meal Agar Media	74.17	71.17	69.67	65.17	61.00	70.33	48.73	**65.75**
PDA + $CaCo_3$	73.00	67.00	48.50	64.33	56.50	61.67	35.50	**58.07**
Tomato Leaf Agar	75.83	77.33	70.67	67.67	71.17	77.83	51.33	**70.26**
Czapek Dox Agar	62.83	42.67	40.67	34.23	39.83	45.00	25.50	**41.53**
Waksman's Agar	59.33	62.83	47.83	47.83	46.67	57.83	30.63	**50.42**
Malt Extract Agar	55.50	57.17	65.00	64.33	55.67	58.17	38.33	**56.31**
Mineral Agar	46.50	39.50	34.50	38.17	40.50	44.00	24.17	**38.19**
YPSS	45.50	37.67	41.83	28.00	39.00	54.00	25.00	**38.71**
Martin's Agar	35.00	37.67	44.67	40.17	46.00	57.33	29.00	**41.41**
Glucose Peptone Yeast Agar	34.33	53.50	61.17	48.67	50.67	69.00	25.77	**49.02**
Water Agar	31.83	35.83	29.33	27.17	36.67	36.67	25.17	**31.81**
Mean	**58.91**	**57.79**	**54.84**	**52.68**	**53.74**	**61.79**	**36.76**	

	CV (%)	**SEm±**	**CD at 5%**
Factor(A)		0.48	1.35
Factor(B)	4.12	0.34	0.95
Factor(A X B)		1.28	3.57

Table 4.10: Effect of culture media on colony diameter of different isolates of *Alternaria solani* after 240 hrs of incubation

Media	Colony diameter after 240 hrs incubation (mm)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Agar	89.35	88.50	83.43	86.17	83.83	90.00	65.50	**83.83**
Oat Meal Agar	86.67	87.33	80.17	78.33	83.50	89.00	64.00	**81.29**
Asthana & Hawker's	79.67	84.83	72.83	70.83	69.00	81.17	55.00	**73.33**
Corn Meal Agar Media	80.17	86.17	77.67	72.17	71.00	81.33	58.33	**75.26**
PDA + $CaCo_3$	77.50	79.00	55.50	70.33	65.50	76.50	40.50	**66.40**
Tomato Leaf Agar	80.83	87.03	78.67	77.67	81.17	87.83	60.00	**79.03**
Czapek Dox Agar	70.83	47.67	45.67	38.00	44.83	53.67	29.00	**47.10**
Waksman's Agar	78.33	75.83	54.83	57.83	54.67	67.50	36.67	**60.81**
Malt Extract Agar	63.50	70.17	71.00	69.33	64.67	70.00	43.33	**64.57**
Mineral Agar	55.50	43.50	37.50	42.17	46.50	49.17	28.50	**43.26**
YPSS	54.50	43.67	46.83	32.00	44.00	64.33	28.83	**44.88**
Martin's Agar	41.00	43.67	45.67	47.17	54.00	67.67	34.83	**47.72**
Glucose Peptone Yeast Agar	40.33	63.50	66.17	58.67	58.67	81.00	29.00	**56.76**
Water Agar	39.83	42.83	32.33	31.17	41.67	45.00	29.33	**37.45**
Mean	**67.00**	**67.41**	**60.59**	**59.42**	**61.64**	**71.73**	**43.06**	
	CV (%)			**SEm±**			**CD at 5%**	
Factor(A)				0.69			1.93	
Factor(B)	5.13			0.49			1.36	
Factor(A X B)				1.83			5.09	

4.6.2 Effect of culture media on colony colour of different isolates of *Alternaria solani*

Data have been presented in table 4.11 indicated that the colony colour of different isolates of *Alternaria solani* exhibited variation on different culture media. In case of isolates, isolate AS-1, AS-2, AS-3, AS-4, AS-5, AS-6 and AS-7 produced whitish brown to dark brown, whitish gray to greenish black, olive green to dark green, whitish pink to greenish black, whitish gray to greenish black, light brown to greenish black and whitish gray to greenish black, respectively on different culture media. In case of media, Waksman's and Asthana & Hawker's medium showed grayish brown with white border and Olive green colony, respectively. While OMA produce gray colony colour in isolate AS-1, dark gray colony in AS-2, AS-4, AS-5 and AS-7, greenish black in AS-3 and greenish brown in AS-6. In case of isolate AS-6, PDA, host leaf extracts and cornmeal agar medium produced gray, whitish gray and grayish gray colour colony, respectively. However, greenish black colony was observed on water agar medium (Plate 4.5a,b,c,d).

The result obtained in the present study agreed with the findings of Kaul and Saxena (1988). They observed differences in cultural characters like growth rate, type of growth, colony colour of the substrate and sporulation of the isolates. Similar result was also reported by Hubballi *et al*. (2010) reveal that isolates showed variation in colony colour on different culture media. Similar type result were also noticed by Koley and Mahapatra (2015), Arunakumara (2006) Babu *et al.* (2000c) and Anand and Bhaskaran (2009).

Table 4.11: Effect of culture media on colony colour of different isolates of *Alternaria solani*

Isolates	Colony colour of *Alternaria solani* isolates on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Greenish brown	Whitish brown	Grayish brown	Grayish brown with white border	Whitish gray	Whitish brown	Grayish brown	Gray	Olive green with white dots	Greenish brown	Greenish brown	Greenish brown	Yellowish brown	Dark brown
AS-2	Grayish brown	Whitish gray	Olive green	Brown	Brown	Grayish brown to dark brown	Grayish brown to dark brown	Dark gray	Olive brown	Grayish black	Light brown to brown	Whitish brown	Grayish brown	Greenish black
AS-3	Grayish black	Dark brown	Grayish black	Grayish black	Whitish black	Dark green	Olive green	Greenish black	Olive green	Grayish black	Greenish brown	Greenish brown	Grayish black	Brown
AS-4	Grayish brown	Whitish pink	Grayish brown	Light brown	Whitish gray	Grayish to dark brown	Grayish brown	Dark gray	Olive green	Dark brown	Light brown	Grayish brown	Grayish brown	Greenish black
AS-5	Grayish brown	Whitish gray	Gray	Light brown	Grayish brown	Grayish brown	Gray	Dark gray	Olive green	Grayish brown	Light brown	Olive	Whitish gray	Greenish black
AS-6	Gray	Yellowish brown	Greenish brown	Light brown	Grayish brown	Whitish gray	Grayish gray	Greenish brown	Olive green	Greenish brown	brown	Grayish brown	Whitish brown	Greenish black
AS-7	Dark brown	Brown	Greenish brown	Greenish brown	Whitish gray	Whitish gray	Dark gray	Dark gray	Dark brown	Greenish gray	Dark brown	Whitish gray	brown	Greenish black

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

4.6.3 Effect of culture media on substrate colour of different isolates of *Alternaria solani*

Data pertaining to substrate colour in different isolates of *Alternaria solani* on different culture media have been presented in table 4.12 reveal that the substrate colour of different isolates of *Alternaria solani* exhibited variation from whitish brown to black on different culture media. Among the isolates, isolates AS-1 and AS-7 produced black colour substance, AS-2 and AS-3 brown to black and AS-4 grayish black, while AS-6 showed dark brown colour pigments on PDA medium. Other media also showed variation in substrate colour among the isolates and the details are furnished in the table 4.8. In case of media, isolate AS-1 produced black pigment on PDA and PDA+$CaCo_3$, grayish black on YPSS, reddish black on Czapek Dox, Martin's and GPYA, brownish black on Waksman's, MEA, CMA, OMA, dark brown on WA, brown on TLEA, while olive green pigment produced on Asthana & Hawker,s medium. However, bown to black pigment on PDA, Czapek Dox, PDA+$CaCo_3$, grayish black on YPSS, whitish brown on Waksman's and MEA, dark brown to black on TLEA, light brown on CMA, MA and GPYA, grayish on OMA, olive on Asthana & Hawker,s, olive green on WA were observed in isolate AS-2. Isolate AS-3 showed various colour of substance on different culture media *viz.* brown to black on PDA, light brown to dark brown on Czapek dox, grayish on YPSS and Waksman's, brown on MEA, dark brown on TLEA, light brown on CMA, MA, Martin's, GPYA and WA, greenish on OMA and greenish brown on Asthana & Hawker,s. While, isolate AS-4 produced grayish black colour substance on PDA, light brown on Czapek dox and PDA+$CaCo_3$, dark brown to black on YPSS and MA, brown to dark brown on Waksman's, dark brown on MEA and TLEA, Brown to black on CMA and OMA, grayish brown on Asthana & Hawker,s, whitish brown on Martin's, raddish brown in GPYA and WA. Isolate AS-5 showed dark brown to black colour substance on PDA and MA, raddish black on Czapek Dox, light gray on YPSS, dark brown on Waksman's, MEA, TLEA, light brown on CMA and OMA, blackish brown on Asthana & Hawker's, olive on PDA+$CaCo_3$, light brown on Martin's, reddish

brown on GPYA and WA. Dark brown colour substance was observed on PDA and TLEA, reddish brown on Czapek dox, MEA and WA, Greenish gray on YPSS, brown on Waksman's and GPYA, light brown on CMA, OMA and Martin's, greenish brown on Asthana & Hawker's, olive green on PDA+$CaCo_3$ media in isolate AS-6. However, isolate AS-7 produce black colour substance on PDA, PDA+$CaCo_3$, MA and Martin's, dark brown on Czapek dox and Waksman's, brown on YPSS, CMA,OMA, TLEA and Asthana & Hawker's reddish brown on MEA and GPYA and greenish black on WA medium (Table 4.12 and Plate 4.5a,b,c,d).

4.6.4: Effect culture media on colony growth pattern of different isolates of *Alternaria solani*

The result presented in Table 4.13 on colony growth pattern of different isolates of *Alternaria solani* on different culture media exhibited more variation among the isolates and culture media. Isolate AS-1 showed circular smooth growth pattern on Czapek Dox, YPSS, TLEA, OMA, PDA+ $CaCo_3$ and WA media, while on PDA and GPYA it was showed irregular rough growth pattern. Irregular smooth growth pattern was observed on Waksman's, MEA and Martin's media, circular rough on CMA, Asthan & Hawker's and MA media. In case of isolate AS-2, circular rough colony growth was observed on PDA, TLEA, CMA, OMA and PDA+$CaCo_3$, circular smooth on Czapek Dox, Martin's and WA, irregular smooth on YPSS, irregular rough on MEA and GPYA, circular rough with concentric ring on Waksman's, circular with whitish dots on Asthan & Hawker's and irregular smooth with concentric ring on MA media. In case of isolate AS-3, circular smooth colony growth pattern was noticed on PDA, MEA, TLEA, CMA, OMA and WA media, while irregular smooth colony growth pattern was observed on Czapek Dox, YPSS, Waksman's, PDA+$CaCo_3$, MA, Martin's and GPYA and Asthan & Hawker's medium showed circular rough with whitish dot colony. Isolate AS-4 showed irregular rough colony on PDA, YPSS and MA, irregular smooth on

Martin's and GPYA, circular rough on MEA, TLEA, CMA and OMA, circular smooth on Czapek Dox, Waksman's, PDA+$CaCo_3$ and WA, whereas, circular rough with whitish dot colony was observed on Asthan & Hawker's medium. In case of isolate AS-5, irregular rough colony was noticed on PDA and Czapek Dox, circular rough on MEA, TLEA, CMA, OMA, Asthan & Hawker's medium, PDA+$CaCo_3$, MA and Martin's, circular smooth on YPSS, Waksman's and WA. However, irregular smooth colony was noticed on GPYA medium. Whereas, isolate AS-6 showed circular rough colony on PDA, TLEA, OMA and PDA+$CaCo_3$, circular smooth on Czapek Dox, Waksman's, Asthan & Hawker's, GPYA and WA, irregular rough on Martin's, irregular smooth on YPSS and irregular smooth with concentric rings on MA media. Isolate AS-7 produced irregular smooth colony on PDA, Czapek Dox, YPSS, Waksman's, Asthan & Hawker's, MA and GPYA, circular smooth on MEA, TLEA an WA, irregular rough on PDA+$CaCo_3$, Martin's, CMA and OMA medium (Plate 4.5a,b,c,d)..

Among the isolates, AS-1, AS-4 and AS-5 isolates showed irregular rough growth pattern, AS-2 and AS-6 produced as circular rough colony, AS-3 produced circular smooth colony on PDA medium. However, isolate AS-7 produced irregular smooth colony on PDA medium. Variations in colony growth pattern on different media were observed among the isolates and the details are furnished in the table 4.13 (Plate 4.6).

Table 4.12: Effect of culture media on substrate colour of different isolates of *Alternaria solani*

Isolates	Substrate colour of different isolates of *Alternaria solani* on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana & Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Black	Reddish black	Grayish black	Brownish black	Brownish black	Brown	Brownish black	Brownish black	Olive green	Black	Whitish brown	Reddish black	Reddish black	Dark brown
AS-2	Brown to black	Brown to black	Grayish black	Whitish brown	Whitish brown	Dark brown to black	Light brown	Grayish	Olive	Brown to black	Light brown	Brown	Light brown	Olive green
AS-3	Brown to black	Light brown to dark brown	Grayish	Grayish	Brown	Dark brown	Light brown	Greenish	Greenish brown	Dark green	Light brown	Light brown	Light brown	Light brown
AS-4	Grayish black	Light brown	Dark brown to black	Brown to dark brown	Dark brown	Dark brown	Brown to black	Brown to black	Grayish brown	Light brown	Dark brown to black	Whitish brown	Reddish brown	Reddish brown
AS-5	Dark brown to black	Reddish black	Light gray	Dark brown	Dark brown	Dark brown	Light brown	Light brown	Blackish brown	Olive	Dark brown to black	Light brown	Reddish brown	Reddish brown
AS-6	Dark brown	Reddish brown	Greenish gray	Brown	Reddish brown	Dark brown	Light brown	Light brown	Greenish brown	Olive green	Dark brown to black	Light brown	Brown	Reddish brown
AS-7	Black	Dark brown	Brown	Dark brown	Reddish brown	Brown	Brown	Brown	Brown	Black	Black	Black	Reddish brown	Greenish black

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

Table 4.13: Effect of culture media on growth pattern of different isolates of *Alternaria solani*

Isolates	Growth Pattern of different isolates of *Alternaria solani* on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Irregular rough	Circular smooth	Circular smooth	Irregular smooth	Irregular smooth	Circular smooth	Circular rough	Circular smooth	Circular rough	Circular smooth	Circular rough	Irregular smooth	Irregular rough	Circular smooth
AS-2	Circular rough	Circular smooth	Irregular smooth	Circular rough with concentric ring	Irregular rough	Circular rough	Circular rough	Circular rough	Circular rough and whitish dots	Circular rough	Irregular smooth with concentric ring	Circular smooth	Irregular rough	Circular smooth
AS-3	Circular smooth	Irregular smooth	Irregular smooth	Irregular smooth	Circular smooth	Circular smooth	Circular smooth	Circular smooth	Circular rough and whitish dots	Irregular smooth	Irregular smooth	Irregular smooth	Irregular smooth	Circular smooth
AS-4	Irregular rough	Circular smooth	Irregular rough	Circular smooth	Circular rough	Circular rough	Circular rough	Circular rough	Circular rough with whitish dots	Circular smooth	Irregular rough	Irregular smooth	Irregular smooth	Circular smooth
AS-5	Irregular rough	Irregular rough	Circular smooth	Circular smooth	Circular rough	Circular rough	Circular rough	Circular rough	Circular rough	Circular rough	Circular rough	Circular rough	Irregular smooth	Circular smooth
AS-6	Circular rough	Circular smooth	Irregular smooth	Circular smooth	Irregular smooth	Circular rough	Circular rough	Circular rough	Circular smooth	Circular rough	Irregular smooth with concentric ring	Irregular rough	Circular smooth	Circular smooth
AS-7	Irregular smooth	Irregular smooth	Irregular smooth	Irregular smooth	Circular smooth	Circular smooth	Irregular rough	Irregular rough	Irregular smooth	Irregular rough	Irregular smooth	Irregular rough	Irregular smooth	Circular smooth

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

4.6.5: Effect of culture media on colony margin colour of different isolates of *Alternaria solani*:

Data regarding effect of culture media on colony margin colour of different isolates of *Alternaria solani* have been deputed in table 4.14 indicated that the margin colour of the colony varied from isolate to isolate on different media. In case of media, isolate AS-1 produced whitish margin on PDA, Czapek Dox, Waksman's and TLEA, olive green on YPSS and Asthana & Hawker's, grayish on MEA, whitish brown on CMA, MA and Martin's, brown on OMA and WA, olive on PDA+$CaCo_3$ and white on GPYA media. In case of isolate AS-2, whitish gray margin was observed on PDA, CMA, OMA, whitish brown on Czapek Dox, MEA, MA, Martin's, GPYA, gary on YPSS, brown on Waksman's, TLEA, olive on Asthans & Haker's, grayish brown on PDA+$CaCo_3$ and olive green on WA. However, isolate AS-3 showed whitish brown margin on PDA, MEA, MA, whitish gary on Czapek Dox, YPSS, Waksman's, TLEA, CMA, Martin's, GPYA, grayish on OMA, PDA+$CaCo_3$, olive on Asthana & Hawker's and light brown in WA. In isolate AS-4, whitish gray margin was noticed on PDA, TLEA, CMA, OMA, Martin's, whitish on Czapek Dox, brown on YPSS, PDA+$CaCo_3$, WA, light brown on Waksman's, MA, gray on MEA, GPYA and olive green in Asthana & Hawker's. Isolate AS-5 showed gray margin colour on PDA, reddish brown on Czapek Dox, whitish gray on YPSS, MEA, TLEA, CMA, OMA, PDA+$CaCo_3$, Martin's, GPYA, light brown in Waksman's, olive in Asthana & Hawker's, whitish brown in MA and greenish brown in WA. In case of isolate AS-6, whitish gray margin was noticed on PDA, YPSS, CMA, GPYA, whitish brown on Czapek Dox, MA, Martin's, light brown on in Waksman's, brown on MEA, gray on TLEA, greenish brown on OMA, PDA+$CaCo_3$, WA and olive on Asthan & Hawker's. However, isolate As-7 produced brown margin on PDA, Asthan & Hawker's, whitish brown on Czapek Dox, YPSS, MEA, MA, GPRA, whitish gray on Waksman's, TLEA, CMA, Martins, gray on OMA, greenish gray on PDA+$CaCo_3$ and greenish brown in WA. Among the isolates, AS-1 gives whitish margin, while AS-2, AS-4 and AS-6 give whitish gray margin on PDA medium. Isolate AS-3 produced whitish brown margin, AS-5 gray margin and AS-7 brown margin on PDA medium. Other media also showed variation in margin colour of colony among the isolates details are furnished in table 4.14 (Plate 4.5 a,b,c,d)

Table 4.14: Effect of culture media on margin colour of different isolates of *Alternaria solani*

Isolates	Margin Colour of different isolates of *Alternaria solani* on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Whitish	Whitish	Olive green	Whitish	Grayish	Whitish	Whitish brown	Brown	Olive green	Olive	Whitish brown	Whitish brown	White	Brown
AS-2	Whitish gray	Whitish brown	Gray	Brown	Whitish brown	Brown	Whitish gray	Whitish gray	Olive	Grayish brown	Whitish brown	Whitish brown	Whitish brown	Olive green
AS-3	Whitish brown	Whitish gray	Whitish gray	Whitish gray	Whitish brown	Whitish gray	Whitish gray	Grayish	Olive	Grayish	Whitish brown	Whitish gray	Whitish gray	Light brown
AS-4	Whitish gray	Whitish	Brown	Light brown	Gray	Whitish gray	Whitish gray	Whitish gray	Olive green	Brown	Light brown	Whitish gray	Gray	Brown
AS-5	Gray	Reddish brown	Whitish gray	Light brown	Whitish gray	Whitish gray	Whitish gray	Whitish gray	Olive	Whitish gray	Whitish brown	Whitish gray	Whitish gray	Greenish brown
AS-6	Whitish gray	Whitish brown	Whitish gray	Light brown	Brown	Gray	Whitish gray	Greenish brown	Olive	Greenish brown	Whitish brown	Whitish brown	Whitish gray	Greenish brown
AS-7	Brown	Whitish brown	Whitish brown	Whitish gray	Whitish brown	Whitish gray	Whitish gray	Gray	Brown	Greenish gray	Whitish brown	Whitish gray	Whitish brown	Greenish brown

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

4.6.6: Effect of culture media on topography of different isolates of *Alternaria solani*

The result obtained are present in Table 4.15 indicated that the topography of different isolates of *Alternaria solani* exhibited variation on different culture media. In case of media, AS-1 produced merged topography on Czapek Dox, Waksman's, Asthan & Hawker's, PDA+ $CaCo_3$ and WA. While, on YPSS, CMA, OMA, MA, and GPYA media were showed sub-merged topography. Areal topography was observed on PDA, MEA, Martin's and TLEA medium in isolate AS-1. Other isolates also showed variation in topography on different culture media which are furnished in table 4.15. Among the media PDA gives aerial topography in all the isolates except AS-7 which produced merged topography. The other isolates were showed aerial to submerged topography on other culture media. Variations in the topography on different media in seven isolates of *A. solani* are furnished in the table 4.15 (Plate 4.5 a,b,c,d).

4.4.7: Effect of culture media on thickness of mycelium mat of different isolates of *Alternaria solani*

Experimental data on thickness of mycelium mat of different isolates of *Alternaria solani* on different culture media have been presented in Table 4.16 revealed that the mycelium thickness of different isolates of *Alternaria solani* showed variation on different culture media. Isolate AS-1 produced very thick mycelium on PDA, CMA and OMA and thick mycelium mat was observed on MEA, TLEA, Martin's, $CaCo_3$ and GPYA medium. While, Czapek Dox, YPSS, Waksman's, MA showed thin mycelium growth and Asthana & Hawker's and WA media very thin mycelium mat. In case of isolate AS-2 very thick mycelium

mat was observed on PDA, TLEA, CMA, OMA and PDA+$CaCo_3$, thick on Waksman's, MEA and GPYA, thin on Czapek Dox, YPSS, Asthana & Hawker's, MA and Martin's. However, very thin mycelium mat was observed on WA medium. Isolate AS-3 showed thick mycelium mat on PDA, TLEA, CMA, OMA, and PDA+$CaCo_3$, thin on Czapek Dox, YPSS, Waksman's, MEA, Asthana & Hawker's, MA, GPYA and Martin's, while very thin mycelium was noticed on WA media. Very thick mycelium growth was observed on PDA, TLEA and OMA in isolate AS-4, whereas thick mycelium growth was observed on Waksman's, MEA, CMA, PDA+$CaCo_3$, MA, Martin's and GPYA medium and thin on YPSS, Czapek Dox and Asthana & Hawker's medium. Only WA medium showed very thin mycelium in isolate AS-4. However, isolate AS-5 showed very thick mycelium mat on all the culture media. Isolate AS-6 was produced very thick mycelium mat on PDA, TLEA and PDA+$CaCo_3$, thick on MEA, CMA, OMA, Martin's and GPYA and thin on Czapek Dox, YPSS, Waksman's, Asthana & Hawker's and MA medium. However, very thin mycelium mat was observed on WA medium. In case of isolate AS-7, very thick mycelium growth was noticed on CMA, Asthana & Hawker's and OMA medium, thick on PDA, YPSS, Waksman's, MEA, PDA+ $CaCo_3$ and Martin's, thin on Czapek Dox, TLEA, MA and GPYA medium, while very thin mycelium was observed on WA medium. Among the isolates, isolate AS-1, AS-2, AS-4, AS-5 and AS-6 produced very thick mycelium on PDA medium, whereas isolate AS-3 and AS-7 produced thick mycelium. The other media also showed variations among the isolates in the thickness of mycelium mat are furnished in table 4.16 (Plate 4.5 a,b,c,d).

4.6.8: Effect of culture media on sporulation of different isolates of *Alternaria solani*

Data pertaining to sporulation of different *Alternaria solani* isolates on different culture media have been presented in Table 4.17 indicated that the sporulation of different isolates of *Alternaria solani* showed highly variation on different media. Isolate AS-3, AS-5, AS-6 and AS-7 had excellent sporulation on PDA, TLEA, $CaCo_3$, MA, GPYA and WA media except in isolate AS-5 with good sporulation on GPYA medium, However, isolate AS-1, AS-2 and AS-4 had moderate sporulation on the same media except AS-2 isolate with poor sporulation on TLEA medium. In the isolate AS-1 no sporulation was recorded on YPSS, CMA and OMA medium. The PDA, TLEA, $CaCo_3$, MA, GPYA and WA media were found better for sporulation which exhibited excellent sporulation of *Alternaria solani* isolates. Isolate AS-3, AS-5, AS-6 and AS-7 were found virulent as compare to isolate AS-1, AS-2 and AS-4 regarding sporulation.

The result obtained in the present study at par with the finding of Arunakumar (2006). They reported variation in colony characters among the *A. solani* isolates on PDA medium. Similar result was also obtained by Koley and Mahapatra (2015), Hubballi *et al*. (2010), Kaul and Saxena (1988). Kumar *et al.* (2015) conducted an experiment on effect of culture media on various cultural characters of *Alternaria solani*. *Alternaria solani* grow well on Czapek dox agar medium and Jenson medium but maximum average radial growth of 54.7 mm was recorded on PDA at 25 ^{0}C temperature with pH 7.5. Rahmatzai *et al*. (2016) reveal that maximum mycelial growth of AS1 was noted with Sabouraud's Agar medium (9 cm) followed by host agar medium (8.7cm) and PDA (7.9cm). While, the maximum linear growth of AS2 was recorded with Richard's Agar (9cm) followed by Czapeck's Agar (8.6cm) and Sabouraud's Agar (8.5cm).Isolates of *A. solani* showed highly variation in pigmentation, sporulation and feature of mycelial growth such as colony surface, growth margin and zonation. (Plate 4.5 a,b,c,d).

Table 4.15: Effect of culture media on topography of different isolates of *Alternaria solani*

Isolates	Topography of different isolates of *Alternaria solani* on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Aerial	Merged	Sub-merged	Merged	Aerial	Aerial	Sub-merged	Sub-merged	Merged	Merged	Sub-merged	Aerial	Sub-merged	Merged
AS-2	Aerial	Merged	Merged	Sub-merged	Aerial	Aerial	Aerial	Aerial	Merged	Aerial	Merged	Merged	Sub-merged	Merged
AS-3	Aerial	Merged	Merged	Merged	Merged	Sub-merged	Sub-merged	Sub-merged	Sub-merged	Sub-merged	Merged	Merged	Merged	Merged
AS-4	Aerial	Merged	Sub-merged	Sub-merged	Aerial	Aerial with white dots	Sub-merged	Sub-merged	Merged	Merged	Sub-merged	Sub-merged	Sub-merged	Merged
AS-5	Aerial	Sub-merged	Submerged	Sub-merged	Sub-merged	Sub-merged	Aerial	Aerial	Merged	Aerial	Sub-merged	Sub-merged	Aerial	Merged
AS-6	Aerial	Merged	Merged	Merged	Aerial	Aerial	Sub-merged	Sub-merged	Merged	Sub-merged	Merged	Sub-merged	Aerial	Merged
AS-7	Merged	Merged	Sub-merged	Merged	Sub-merged	Aerial	Sub-merged	Sub-merged	Merged	Aerial	Merged	Aerial	Merged	Merged

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

Table 4.16: Effect of culture media on thickness of mycelium growth of different isolates of *Alternaria solani*

Isolates	Thickness of mycelium growth of different *Alternaria solani* isolates on different culture media													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	Very thick	Thin	Thin	Thin	Thick	Thick	Very thick	Very thick	Very thin	Thick	Thin	Thick	Thick	Very thin
AS-2	Very thick	Thin	Thin	Thick	Thick	Very thick	Very thick	Very thick	Thin	Very thick	Thin	Thin	Thick	Very thin
AS-3	Thick	Thin	Thin	Thin	Thin	Thick	Thick	Thick	Thin	Thick	Thin	Thin	Thin	Very thin
AS-4	Very thick	Thin	Thin	Thick	Thick	Very thick	Thick	Very thick	Thin	Thick	Thick	Thick	Thick	Very thin
AS-5	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick	Very thick
AS-6	Very thick	Thin	Thin	Thin	Thick	Very thick	Thick	Thick	Thin	Very thick	Thin	Thick	Thick	Very thin
AS-7	Thick	Thin	Thick	Thick	Thick	Thin	Very thick	Very thick	Very thick	Thick	Thin	Thick	Thin	Very thin

Note: YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

Table 4.17: Effect of different culture media on sporulation of different isolates of *Alternaria solani*

Isolates	Sporulation of different isolates of *Alternaria solani*													
	PDA	**Czapek Dox**	**YPSS**	**Waksman's**	**MEA**	**TLEA**	**CMA**	**OMA**	**Asthana& Hawker's**	**PDA + $Caco_3$**	**MA**	**Martin's**	**GPYA**	**WA**
AS-1	++	+	-	+	+	++	-	-	+	++	++	+	+	++
AS-2	++	+	++	++	++	+	++	+	++	++	++	+	++	++
AS-3	++++	+	+++	+++	+++	++++	++	+++	+++	++++	++++	++	++++	++++
AS-4	++	++	+	++	++	++	+	+	++	++	++	+	++	++
AS-5	++++	+	++	+++	++	++++	+	+++	+++	++++	+++	++	+++	++++
AS-6	++++	++	+++	+++	+++	++++	++	++++	++++	++++	++++	+++	++++	++++
AS-7	++++	+	+++	+++	+++	++++	+	+++	+++	++++	++++	+++	++++	++++

Note:

1. YPSS – Yeast Powder Soluble Starch, MEA – Malt Extract Agar, TLEA – Tomato Leaf Extract Agar, CMA – Corn Meal Agar, OMA – Oatmeal Agar, PDA + $CaCo_3$ – Potato Dextrose Agar + $CaCo_3$, MA – Mineral Agar, GPYA – Glucose Peptone Yeast Extract Agar, WA – Water Agar

2. - = (0 spore) No sporulation, + = (1-10 spore per microscopic field) Poor sporulation, ++ = (11-20 spore per microscopic field) moderate sporulation, +++ = (21-30 spore per microscopic field) good sporulation, ++++ = (more than 30 spore per microscopic field) excellent sporulation

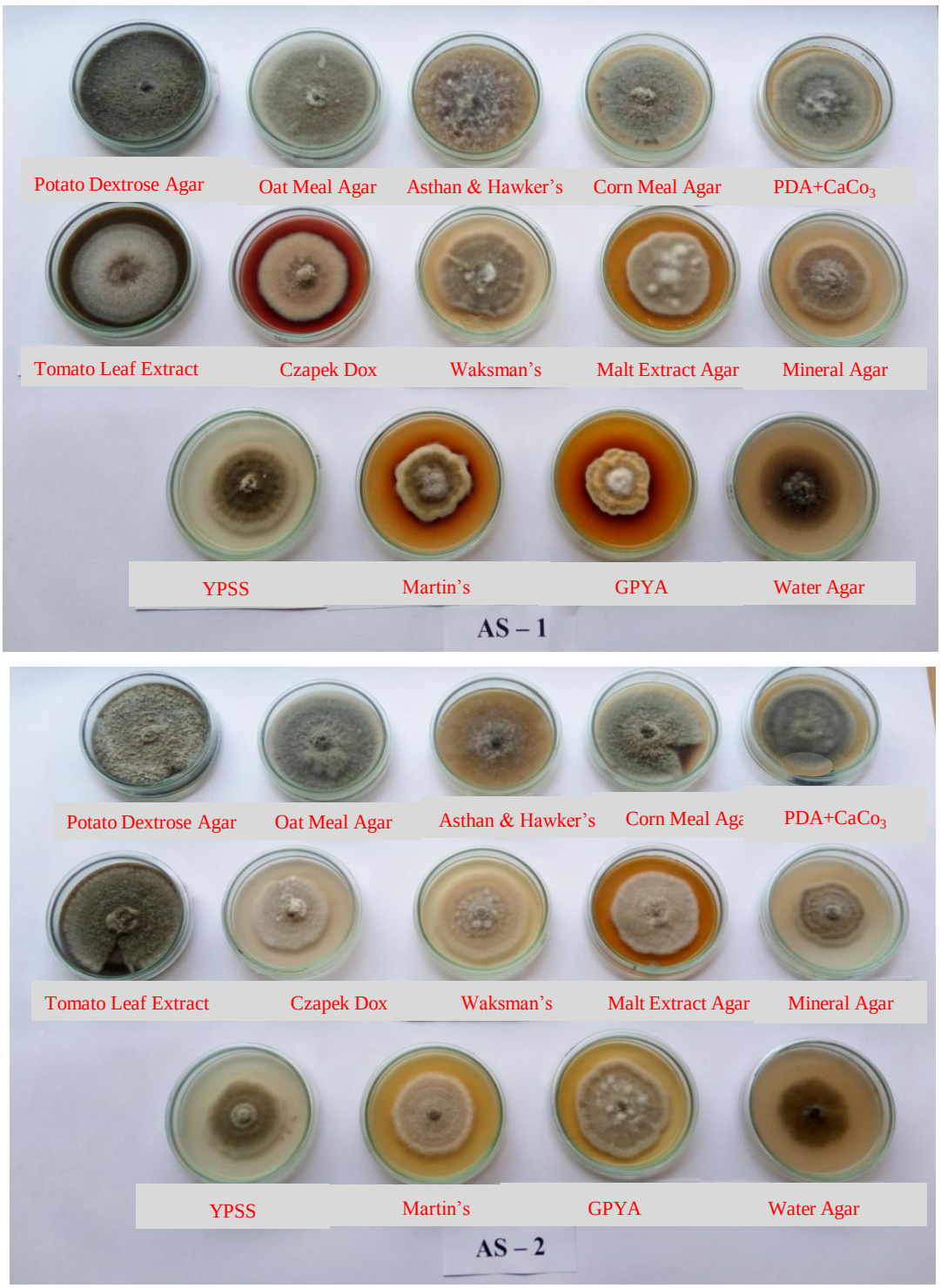


Plate 4.5a: Effect of culture media on colony characters of *Alternaria solani* isolate AS-1 and AS-2

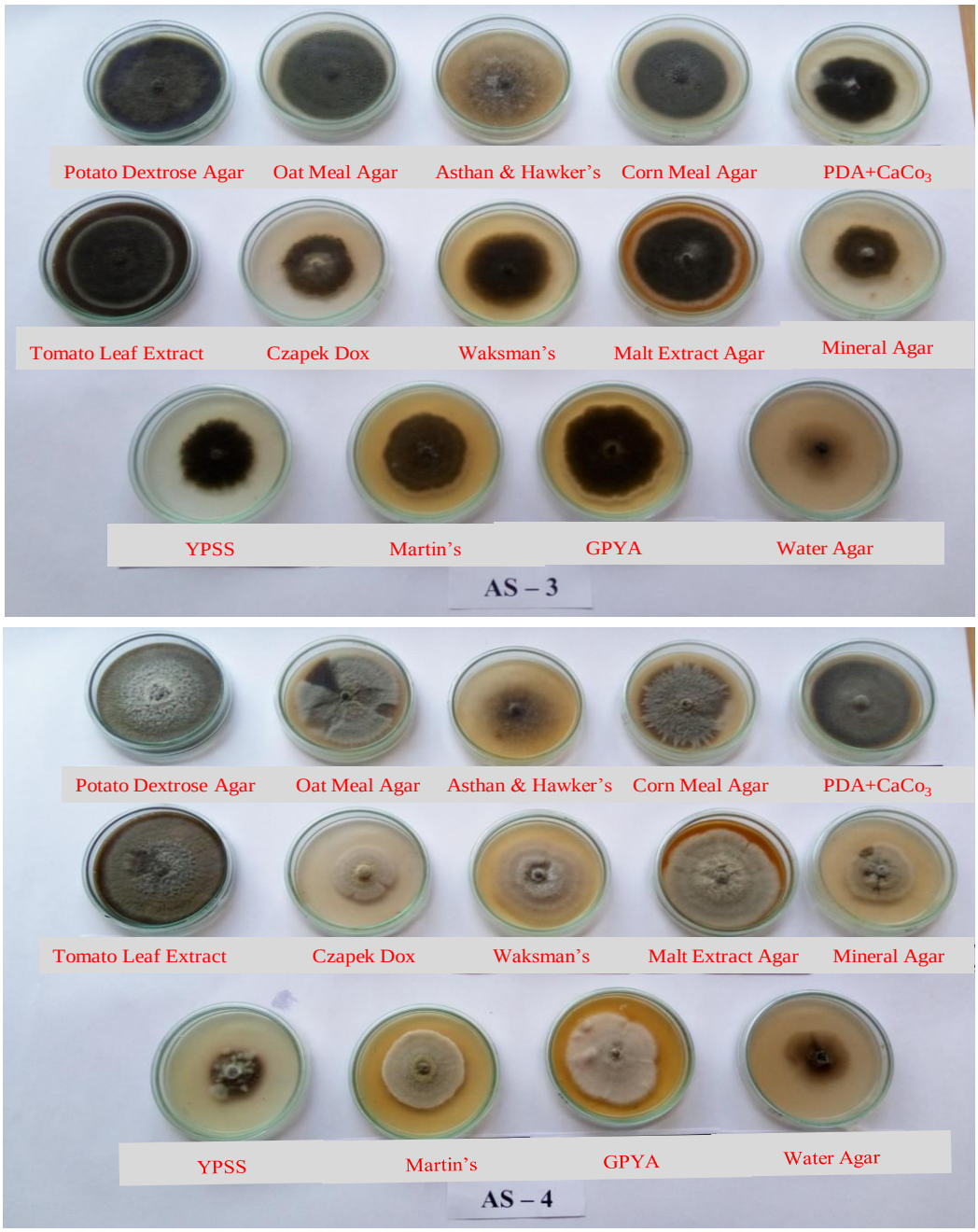


Plate 4.5b: Effect of culture media on colony characters of *Alternaria solani* isolate AS-3 and AS-4

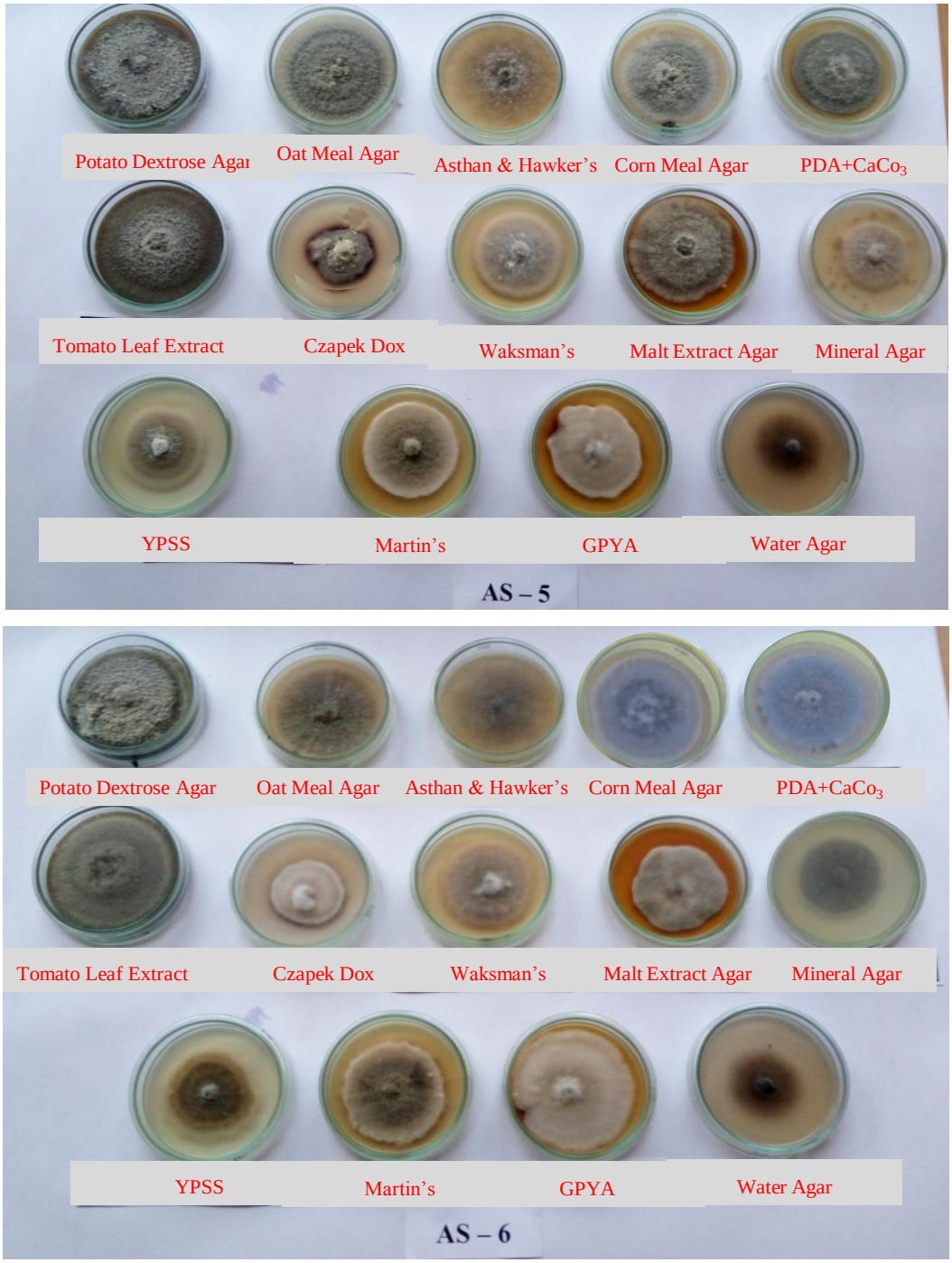


Plate 4.5c: Effect of culture media on colony characters of *Alternaria solani* isolate AS-5 and AS-6

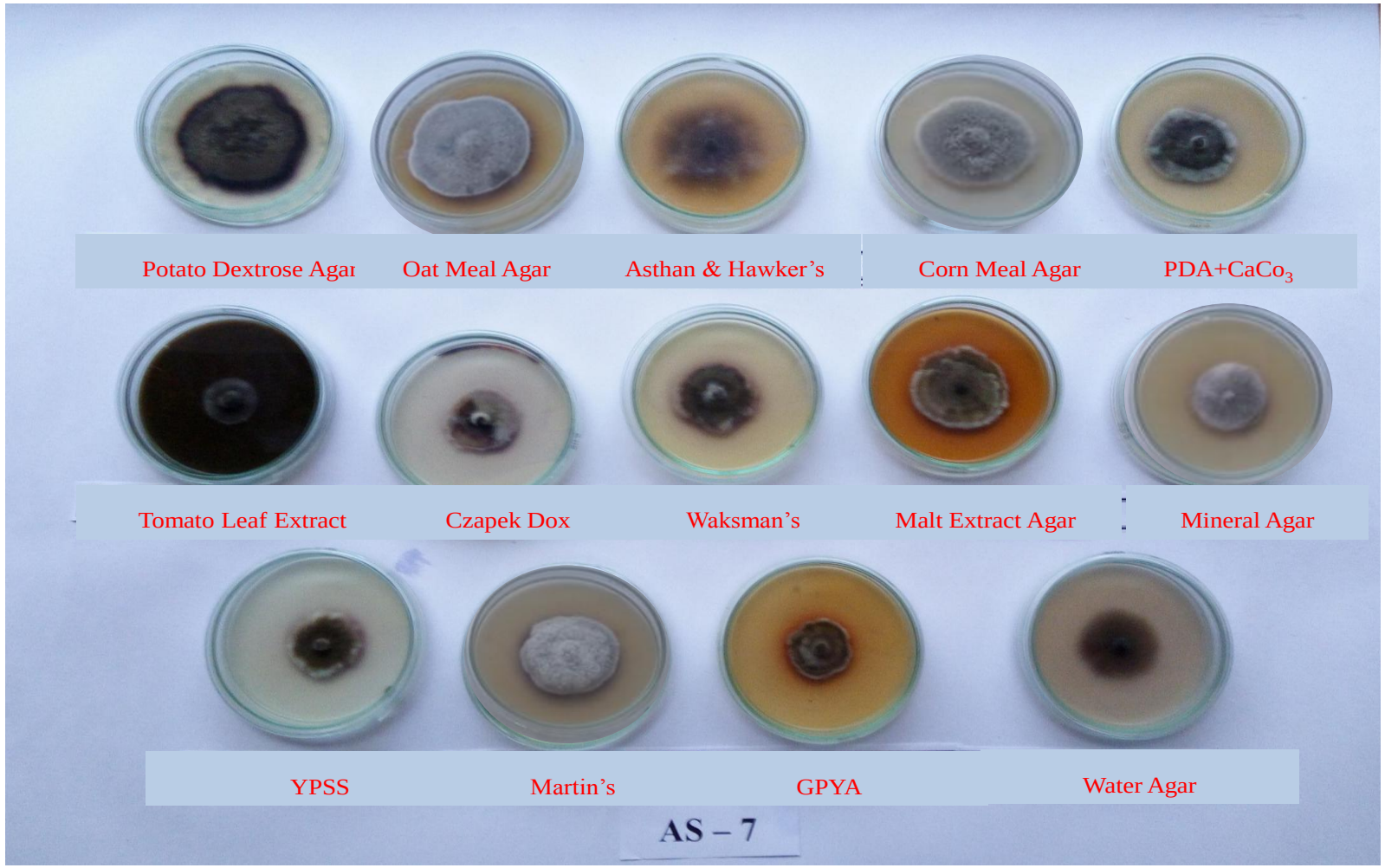


Plate 4.5 d: Effect of culture media on colony characters of *Alternaria solani* isolate AS-7

4.7. Effect of liquid media on Mycelium dry weight (biomass) production of different isolate of *A. solani*

The biomass of *Alternaria solani* was recorded on different liquid media after 10 days of inoculum have been presented in table 4.18 revealed that the mycelia dry weight of *Alternaria solani* was showed significant difference on among the isolate on all the liquid media (Plate 4.6 a,b). Maximum mycelium dry weight (300.54 mg) was obtained on oatmeal broth followed by potato dextrose broth (297.21mg), tomato leaf extract broth (292.55mg) and cornmeal broth with (286.63mg), while minimum mycelium dry weight (73.29mg) was observed on mineral broth. Among the isolates, isolate AS-6 was showed maximum mycelium dry weight (224.62mg) followed by AS-5 (216.77mg) and AS-4 (215.69mg). However, least mycelium dry weight (129.06mg) was obtained in isolate AS-7. Interaction was found significant. Maximum mycelium dry weight was obtained (334.00mg) on isolates AS-1in oatmeal broth and minimum mycelium dry weight (45.45mg) was observed in Isolate AS-7 on Mineral broth.

The present findings are similar with the result of Koley and Mahapatra (2015) who reported that Richard's broth and Sabouraud's broth among liquid media appeared be better than other media for growth of tomato early blight causing fungi. Similar results were also obtained by Somappa *et al*. (2013).

Table 4.18: Mycelium dry weight of different isolates on different broth after 10 days after incubation

Media	Mycelium dry weight after 10 days after incubation (mg)							
	AS-1	**AS-2**	**AS-3**	**AS-4**	**AS-5**	**AS-6**	**AS-7**	**Mean**
Potato Dextrose Broth	326.00	321.00	238.50	321.00	318.00	328.00	228.00	297.21
Oat Meal Broth	334.00	331.00	240.75	326.00	322.00	328.00	222.01	300.54
PDA + Caco3 Broth	270.00	263.00	186.00	253.00	274.00	276.00	157.20	239.89
Tomato Leaf Broth	310.00	318.00	239.26	325.00	320.00	324.00	211.58	292.55
Corn Meal Broth	317.00	324.00	247.50	318.00	286.00	315.00	198.92	286.63
GPY Broth	265.00	241.00	195.00	258.00	276.00	268.00	177.31	240.04
Malt Extract Broth	195.00	252.00	150.75	282.00	286.00	297.00	113.24	225.14
YPSS Broth	161.00	145.00	109.50	208.00	204.00	187.00	77.48	156.00
Czapek Dox Broth	105.00	114.00	94.50	130.00	135.00	149.00	73.01	114.36
Martin's Broth	87.00	89.00	64.50	94.00	101.00	138.00	58.86	90.34
Mineral Broth	74.00	82.00	54.00	78.00	91.00	93.00	45.45	73.92
Waksman's Broth	78.00	98.00	72.00	100.00	108.00	114.00	61.09	90.16
Asthana & Hawkar's Broth	81.00	106.00	81.75	111.00	97.00	103.00	53.64	90.48
Mean	**200.23**	**206.46**	**151.85**	**215.69**	**216.77**	**224.62**	**129.06**	

	CV (%)	**SEm±**	**CD at 5%**
Factor(A)		2.66	7.44
Factor(B)	6.35	1.95	5.46
Factor(A X B)		7.04	19.67

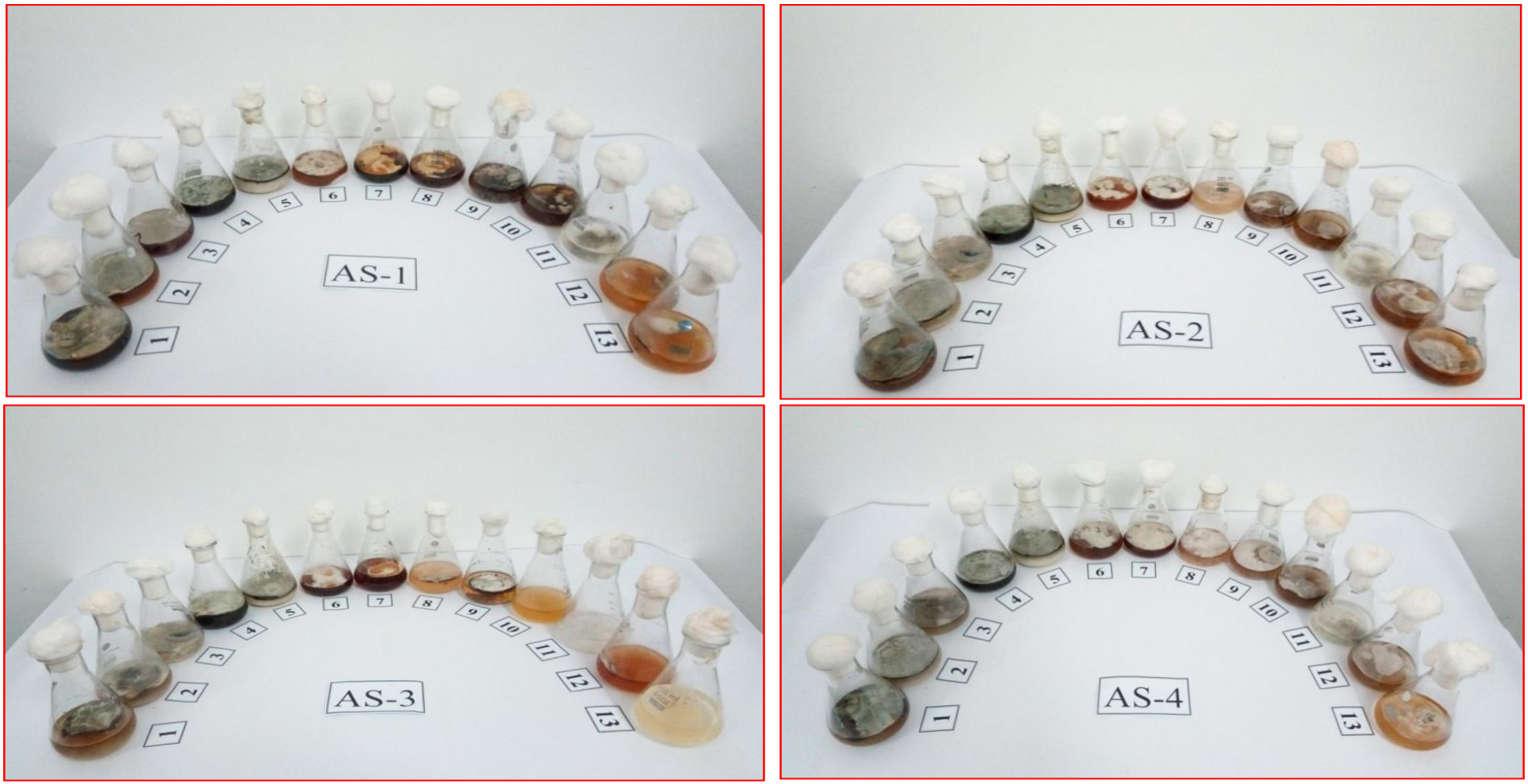


Plate 4.6a: Mycelium growth and pigmentation of different isolates of *Alternaria solani* on liquid media

Note: 1= Potato dextrose broth
2= Oatmeal Broth
3= Potato+$CaCO_3$ Broth
4= Tomato leaf Extract Broth
5= Cornmeal Broth
6= Glucose Peptone Yeast Broth
7= Malt Extract Broth
8=YPSS Broth
9= Czepak Dox Broth
10= Martin's Broth
11= Minerals Broth
12= Waksman's Broth
13= Asthana & Hawkar's Broth

Plate 4.6b: Mycelium growth and pigmentation of different isolates of *Alternaria solani* on liquid media

4.8 Effect of pH on colony diameter, sporulation and mycelium dry weight of *Alternaria solani*

Effect of different pH level on colony diameter, sporulation and mycelium dry weight of *Alternaria solani* Isolate AS-6 on PDA medium have been presented in table 4.19. Maximum colony diameter (89.16mm) was observed at 6.5 pH level followed by 6.0 pH level (78.04mm), while minimum colony diameter (35.32mm) was observed at 8.5 pH. However, mycelium dry weight was observed maximum at pH 6.5 (330.03 mg). It was found at par with pH 6.0 (316.06mg) and significant higher over rest of pH levels. Minimum mycelium dry weight (109.32 mg) was obtained at 8.5 pH level (Plate 4.7).

In case sporulation, pH level influenced the sporulation of *A. solani*. Excellent sporulation was observed at pH level 6.0 to 7.0, good sporulation at 5.0, 5.5 and 7.5 pH levels and moderate sporulation was observed at 4.5pH, while poor sporulation was recorded at 4.0, 8.0 and 8.5 pH level (Table 4.19).

The results of the experiment indicated that *Alternaria solani* prefers a pH range of 6.0 to 7.0 for growth, sporulation and biomass production. The present finding confirming with the results obtained by Alhussaen (2012) they observed that the optimum pH level of *Alternaria solani* grow *in vitro* were 6-7. Similar types of result were also obtained by several workers. Samuel and Govindaswamy (1972) demonstrated that good mycelial growth and sporulation of *A. solani* between pH 4.0 to 8.0 and pH 5.0 was the best for mycelia growth and pH 7.0 for sporulation. Gemawat and Ghosh (1980) observed that the *A. solani* was capable to grow on wide range of pH (4.0 to 9.5) and maximum growth and sporulation were observed at 6.3 pH.

Table 4.19: Effect of pH on colony growth, sporulation and mycelium dry weight of *Alternaria solani* isolate AS-6

pH	Colony diameter (mm)	Sporulation	Mycelium dry weight (mg)
4.0	42.25	+	132.44
4.5	47.77	++	177.57
5.0	57.84	+++	223.20
5.5	64.83	+++	269.91
6.0	78.04	++++	316.06
6.5	89.16	++++	330.03
7.0	75.32	++++	303.50
7.5	62.33	+++	252.89
8.0	45.20	+	143.93
8.5	35.32	+	109.32
CV (%)	**5.50**		**5.59**
SEm±	**1.90**		**7.29**
CD at 5%	**5.64**		**21.65**

- = (0 spore) No sporulation, + = (1-10 spore per microscopic field) Poor sporulation, ++ = (11-20 spore per microscopic field) moderate sporulation, +++ = (21-30 spore per microscopic field) good sporulation, ++++ = (more than 30 spore per microscopic field) excellent sporulation.

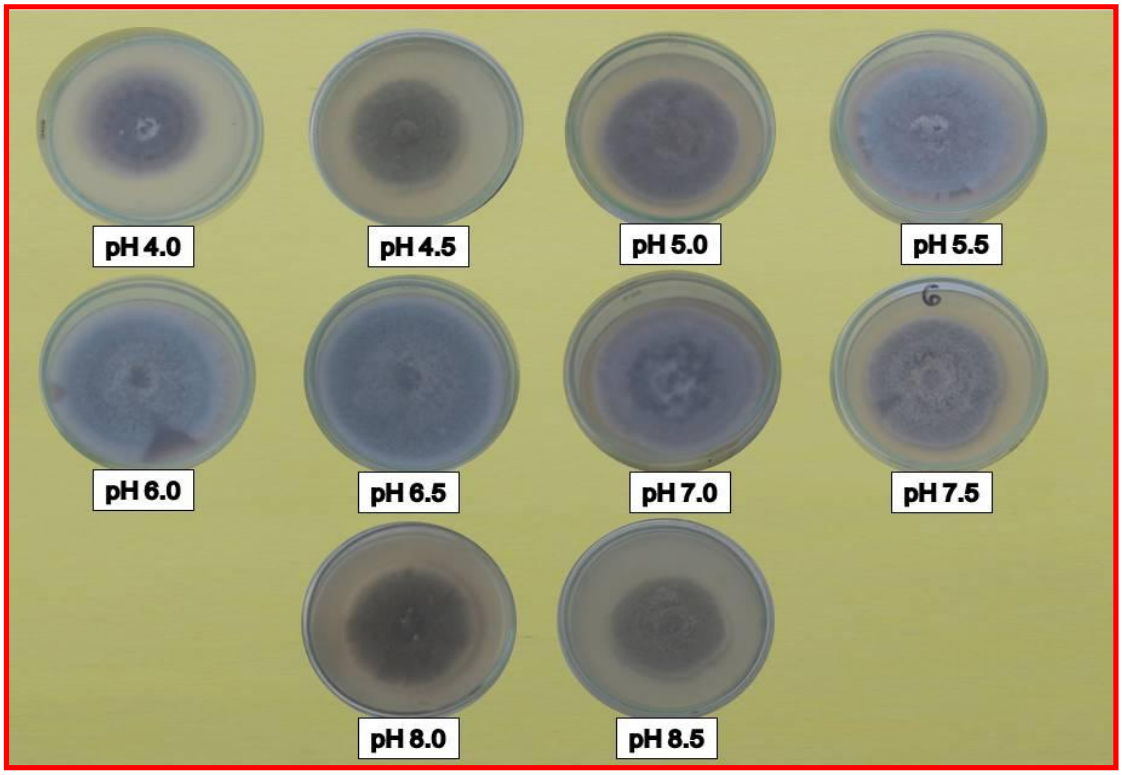


Plate 4.7: Effect of pH on *Alternaria solani* isolate AS-6 on PDA medium

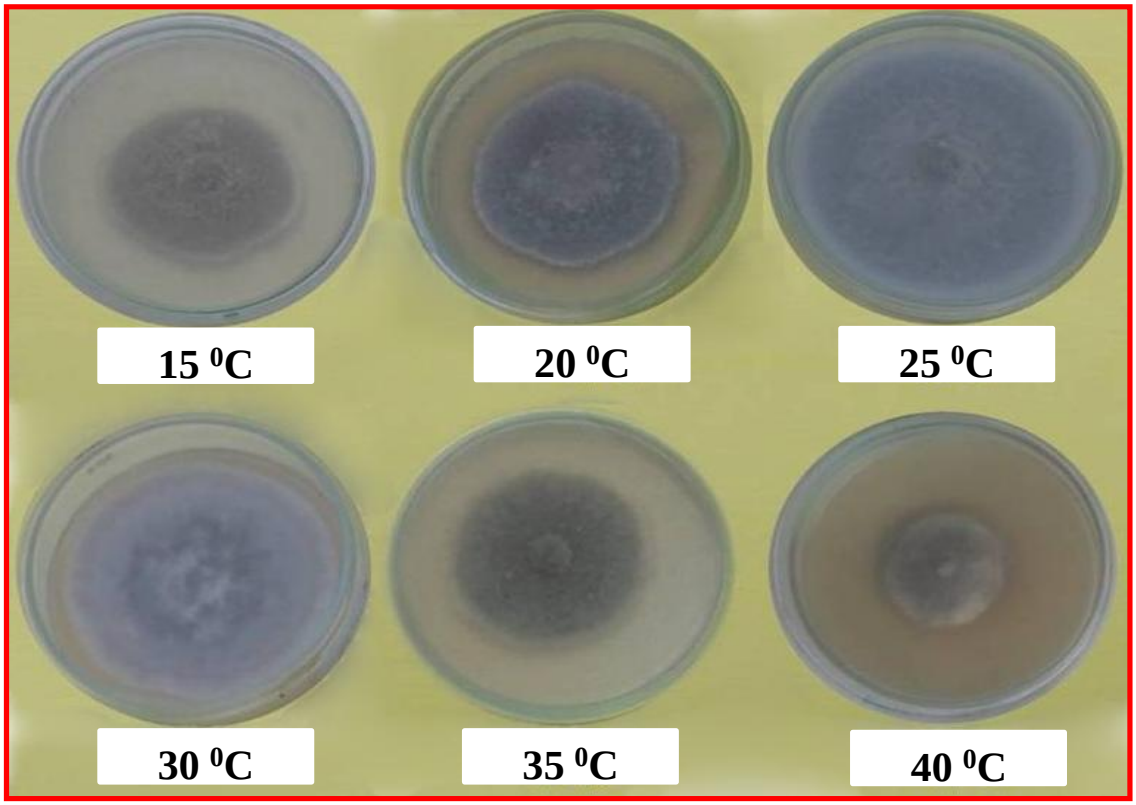


Plate 10: Effect of temperature on colony growth of *Alternaria solani* isolate AS-6 on PDA medium

4.9: Effect of temperature on colony growth and sporulation of *Alternaria solani* isolate AS-6

The results obtained are presented in table 4.20 reveal that the mycelium growth of *Alternaria solani* was significantly influenced with temperature (Plate 4.8). Maximum colony growth of 89.78 mm was recorded at 25^{o}C followed by 30^{o}C (78.10mm), 20^{o}C (58.64mm), 35^{o}C (30.50mm), 15^{o}C (24.32mm) and minimum colony growth (18.36 mm) was recorded at 40^{o}C. Temperature also influenced the sporulation of *Alternaria solani* on PDA medium. Excellent sporulation was noticed at 20^{o}C and 25^{o}C temperature, good sporulation at 30^{o}C and poor sporulation was observed at 15 and 35^{o}C. However, no sporulation was found at 40^{o}C temperature.

Table 4.20: Effect of temperature on colony growth and sporulation of *Alternaria solani* isolate AS-6

Temperature (^{0}C)	Colony diameter (mm)	Sporulation
15	24.32	+
20	58.64	++++
25	89.78	++++
30	78.10	+++
35	30.50	+
40	18.36	-
CV (%)	**3.77**	
SEm±	**0.94**	
CD at 5%	**2.82**	

- = (0 spore) No sporulation, + = (1-10 spore per microscopic field) Poor sporulation, ++ = (11-20 spore per microscopic field) moderate sporulation, +++ = (21-30 spore per microscopic field) good sporulation, ++++ = (more than 30 spore per microscopic field) excellent sporulation.

The result of experiment indicated that *Alternaria solani* preferred temperature range of 25°C to 30°C for its growth and sporulation. The present findings are similar with the result of Kaul and Saxena (1988) who reported that the maximum growth of *A. solani* was obtained at 25°C followed by 20, 15, 10 and 5°C and least growth at 35°C temperature. Similar types of results were also obtained by Arunakumara (2006). he reported *A. solani* produced maximum growth at 25 to 30°C temperature. Similar results were also reported by several workers (Kemmitt, 2002; Rodrigues *et al.,* 2010; Hubballi *et al.,* 2010).

4.10 *In vitro* evaluation of plant extracts, fungicides and bio-control agents against *Alternaria solani*

4.10.1 Evaluation of plant extracts against *Alternaria solani*

Data regarding on *in vitro* efficacy of plant extracts against *Alternaria solani* have been presented in table 4.21 indicated that significant difference on mycelium growth was recorded in different plant extract in at all the concentrations. Among the six plant extract tested, most effective plant extract was found *Allium sativum* which exhibited minimum mycelium growth (53.87mm). It was significantly lower of over rest of the plant extracts. However, maximum mycelium growth (84.67mm) was observed in *Euphorbia hirta*. In case of concentrations, minimum growth of the mycelium was observed in higher concentration (5%) in all the plant extracts. It's indicated that the mycelium growth was reduced with gradually increased in concentration of plant extract (Plate 4.9). Interaction was found significant. In case of interaction between plant extract and concentration, minimum mycelium growth (49.33mm) was found in *Allium sativum* @ 5% which was at par with *Crotalaria juncea* @ 5%(50.00mm), *Allium sativum* @ 4% (50.67mm), *Azadirachta indica* @ 5% (52.00mm), *Allium sativum* @ 3% (52.33mm), *Eucalyptus spp.* @ 5% (52.33mm), *Crotalaria juncea* @4% (53.00 mm), *Crotalaria juncea* @3% (54.33 mm) and *Azadirachta indica* @ 5% (55.33mm) and significantly lower over rest of concentrations of plant extracts.

Table 4.21: *In vitro* efficacy of botanicals against *Alternaria solani*

Botanicals	Mycelium diameter (mm) at different concentrations					
	1%	**2%**	**3%**	**4%**	**5%**	**Mean**
P_1–*Datura stramonium*	76.00	74.00	71.00	71.67	67.67	**72.07**
P_2–*Azadirachta indica*	70.33	66.33	63.67	55.33	52.00	**61.53**
P_3 – *Allium sativum*	60.33	56.00	52.33	50.67	49.33	**53.87**
P_4 – *Eucalyptus spp.*	73.67	66.00	59.67	57.00	52.33	**61.73**
P_5–*Crotalaria juncea*	61.33	59.67	54.33	53.00	50.00	**55.53**
P_6 – *Euphorbia hirta*	87.33	86.00	84.67	83.33	82.00	**84.67**
Control	89.93	89.93	89.93	89.93	89.93	**89.93**
Mean	**74.13**	**71.13**	**67.94**	**65.85**	**63.32**	

	CV (%)	**SEm±**	**CD at 5%**
Botanicals	5.37	0.95	2.68
Concentration		0.80	2.27
Botanicals X Concentration		2.12	5.99

On the other hand, maximum inhibition in mycelium growth was noticed in *Allium sativum* @ 5% (45.15%) followed by *Crotalaria juncea* @ 5% (44.40%), *Allium sativum* @ 4% (43.66%), *Azadirachta indica* @ 5% (42.18%), *Allium sativum* @ 3% (41.81%), *Eucalyptus spp.* @ 5% (41.81), while minimum inhibition in mycelium growth of 2.89% was recorded in *Euphorbia hirta* @ 1% (Fig. 4.4).

The present findings are confirmed with the results of Nashwa *et al.* (2012) they reported leaf extracts of *D. stramonium, A. indica*, and *A. sativum* @ 5% inhibited highest mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively). Dalpati *et al*. (2010) tested ten botanicals *viz.* Neem, Custard apple, Lantana,

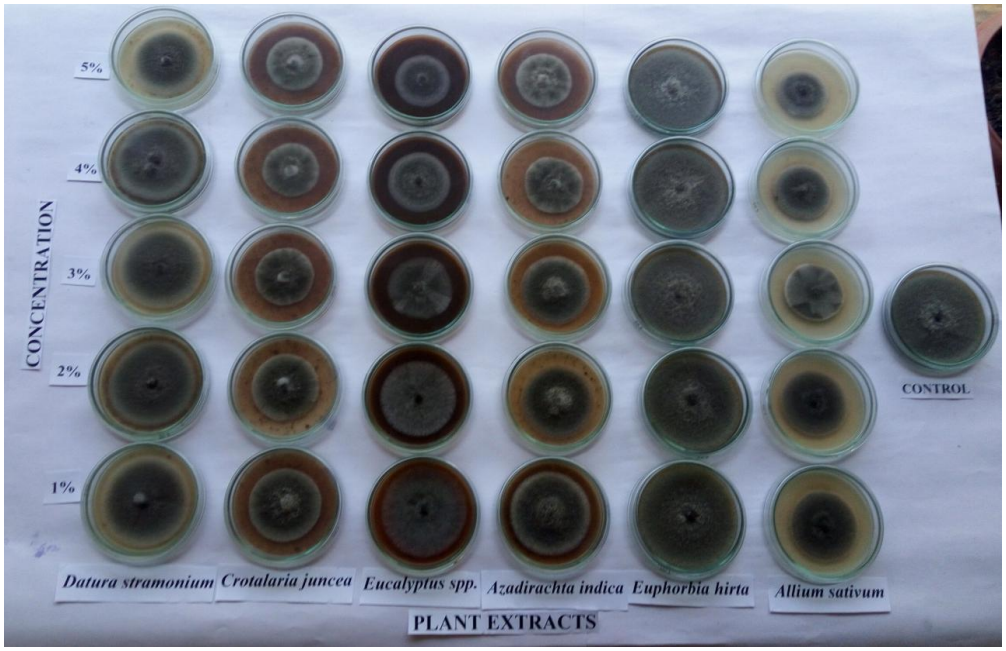


Plate 4.9: *In vitro* efficacy of plant extracts against *Alternaria solani*

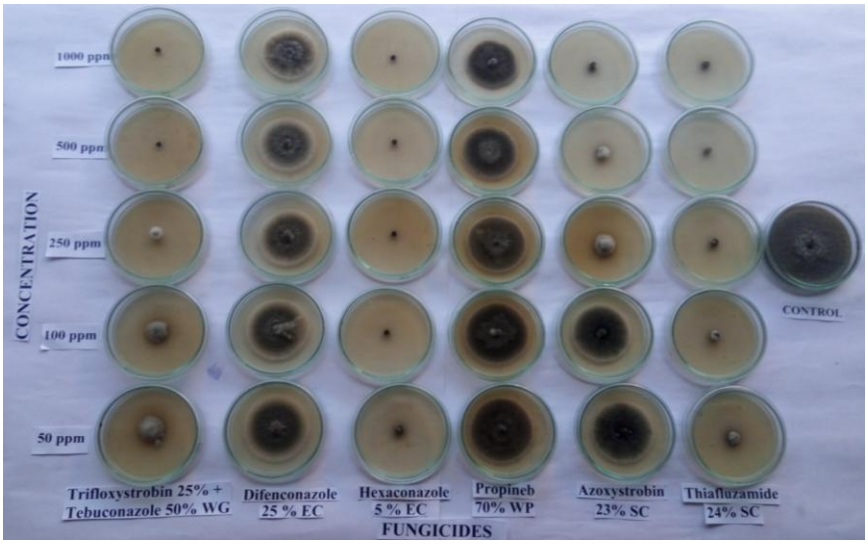


Plate 4.10: *In vitro* efficacy of fungicides against *Alternaria solani*

Eucalyptus, Marigold, Tamarind, Kanher, Garlic, Datura and Congress grass against the *Alternaria macrospora* causing leaf spot of cotton. The percent inhibition of botanicals ranged from 44.59 to 8.25 percent. Similar results were also reported by Sadana, and Didwania (2015) he reveal that the fresh aqueous extract of *Eucalyptus obliqua* @ 15% was most effective which exhibited 88 percent inhibition of mycelial growth of *A. solani* strain A1 followed by *Datura stamonium, Azadirachta indica, Calotropis procera* and *Polyalthia longifolia*.

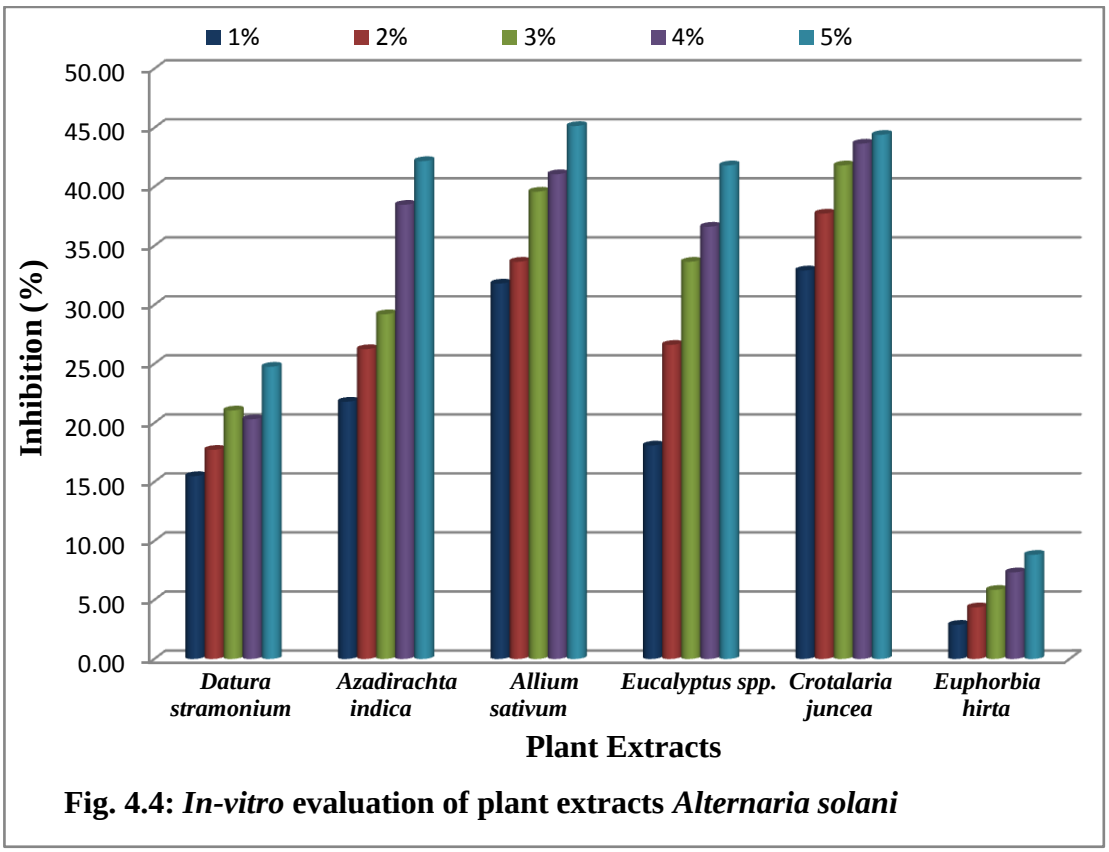


Fig. 4.4: *In-vitro* evaluation of plant extracts *Alternaria solani*

4.10.2 Evaluation of fungicides against *Alternaria solani*

Six fungicides were assessed *in vitro* to find out the most effective fungicide against *A. solani* at different concentrations *viz.* 50, 100. 250, 500, and 1000ppm using poison food technique. The results are presented in table 4.22 reveal that the significant difference among fungicides against *A. solani* was observed. Fungus growth was checked in Hexaconazole 5% EC at 100ppm, while in Trifloxystrobin 25% w/w + Tebuconazole 50%WG at 500ppm and Thiafluzamide 24% SC at 500ppm concentration (Plate 4.10).

Table 4.22: *In vitro* efficacy of fungicides against *Alternaria solani*

Fungicides	Colony diameter (mm) at different concentration					
	50ppm	**100ppm**	**250ppm**	**500ppm**	**1000ppm**	**Mean**
Trifloxystrobin 25% w/w + Tebuconazole 50%WG	26.33	20.67	13.00	0.00	0.00	**12.00**
Difenconazole 25 EC	67.00	64.33	62.00	61.00	59.00	**62.67**
Hexaconazole 5 EC	12.33	0.00	0.00	0.00	0.00	**2.47**
Propineb 70 WP	65.67	65.00	62.67	61.33	55.33	**62.00**
Azoxystrobin 23%SC	62.67	60.67	26.33	13.33	6.33	**33.87**
Thiafluzamide 24%SC	13.67	11.33	7.33	0.00	0.00	**6.47**
Control	89.27	89.27	89.27	89.27	89.27	**89.27**
Mean	**48.10**	**44.47**	**37.23**	**32.13**	**29.99**	

	CV (%)	**SEm±**	**CD at 5%**
Fungicides	9.51	0.94	2.65
Concentration		0.79	2.24
Fungicides X Concentration		2.10	5.93

Among the six fungicides, most effective fungicides was found Hexaconazole 5% EC which exhibited 100.00 percent inhibition in mycelium growth at 100 ppm followed by Thiafluzamide 24% SC and Trifloxystrobin 25% w/w + Tebuconazole 50%WG at 500 ppm. However, other fungicides were unable to check 100.00 percent mycelium growth up to 1000 ppm concentration (Fig. 4.5).

The present findings are similar with the result of Singh and Singh (2006) reported that the Hexaconazole 5% EC was very effective as it caused 100% growth inhibition of *A. alternata*. Similar type of result were also obtained by Mesta *et al.* (2009) who reported that the maximum inhibition of mean fungal growth of *Alternaria helianthi* in hexaconazole (72.87%) followed by difenoconazole (72.61%), mancozeb (58.29%), chlorothalonil (51.54%) and captan (50.43%).

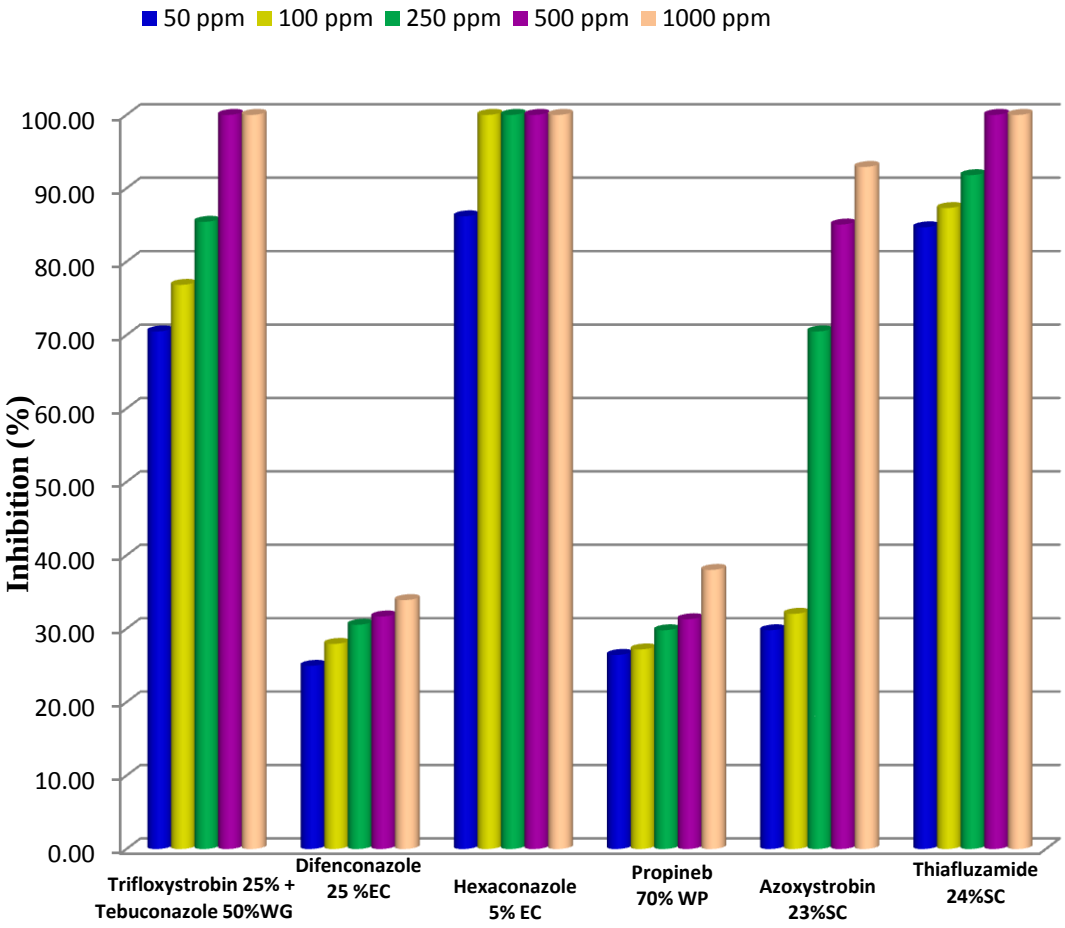


Fig. 4.5: *In-vitro* evaluation of fungicides against *Alternaria solani*

4.10.3 Efficacy of bio - control agents against *Alternaria solani*

Experimental data pertaining to *in-vitro* efficacy of bio-control agents against *A. solani* have been presented in table 4.23 and illustrated in figure 4.6 indicated that the significance different were observed among the all bio-control agents. Among these bio-control agents, *P. fluorescens* showed maximum inhibition in mycelium growth (60.34%) followed by *Trichoderma harzianum* (55.89%), while minimum mycelium growth inhibition was observed in *Trichoderma viride* (52.93%). On the other hand, maximum colony growth of *A. solani* was observed in *Trichoderma viride* (42.33mm) followed by *T. harzianum* (39.67mm) and *P. fluorescens* (35.67mm) (Plate 4.11).

Table 4.23: *In vitro* efficacy of bio-control agents against *Alternaria solani*

Bi-control agents	Mycelium diameter (mm)	Percent inhibition
P. fluorescens	35.67	60.34
Trichoderma viride	42.33	52.93
T. harzianum	39.67	55.89
Control	89.93	0.00
CV (%)	8.35	-
SEm±	1.94	-
CD at 5%	5.85	-

The result of present findings are supported with the finding of Babu *et al.* (2000c) they reported that all the six isolates of *P. fluorescens* used, were significantly inhibited the growth of *A. solani* compared to control. Casida and Lukezie (1992) reported that *Pseudomonas* strain 679-2 was able to reduce the severity of the leaf spot disease caused by *A. solani*. Similar type of results was reported also by Leifort *et al. (*1992), Koley *et al.* (2015).

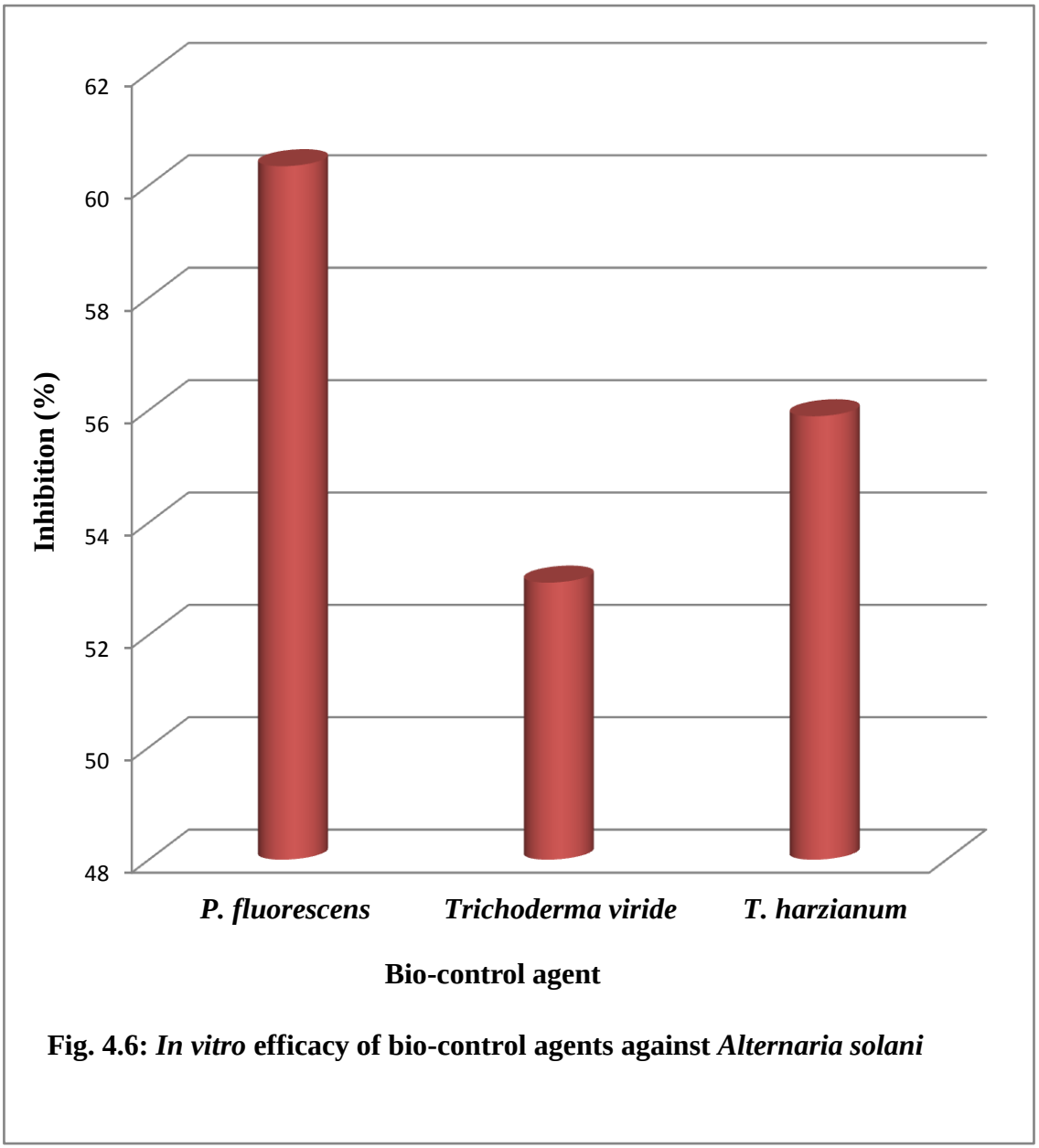


Fig. 4.6: *In vitro* efficacy of bio-control agents against *Alternaria solani*

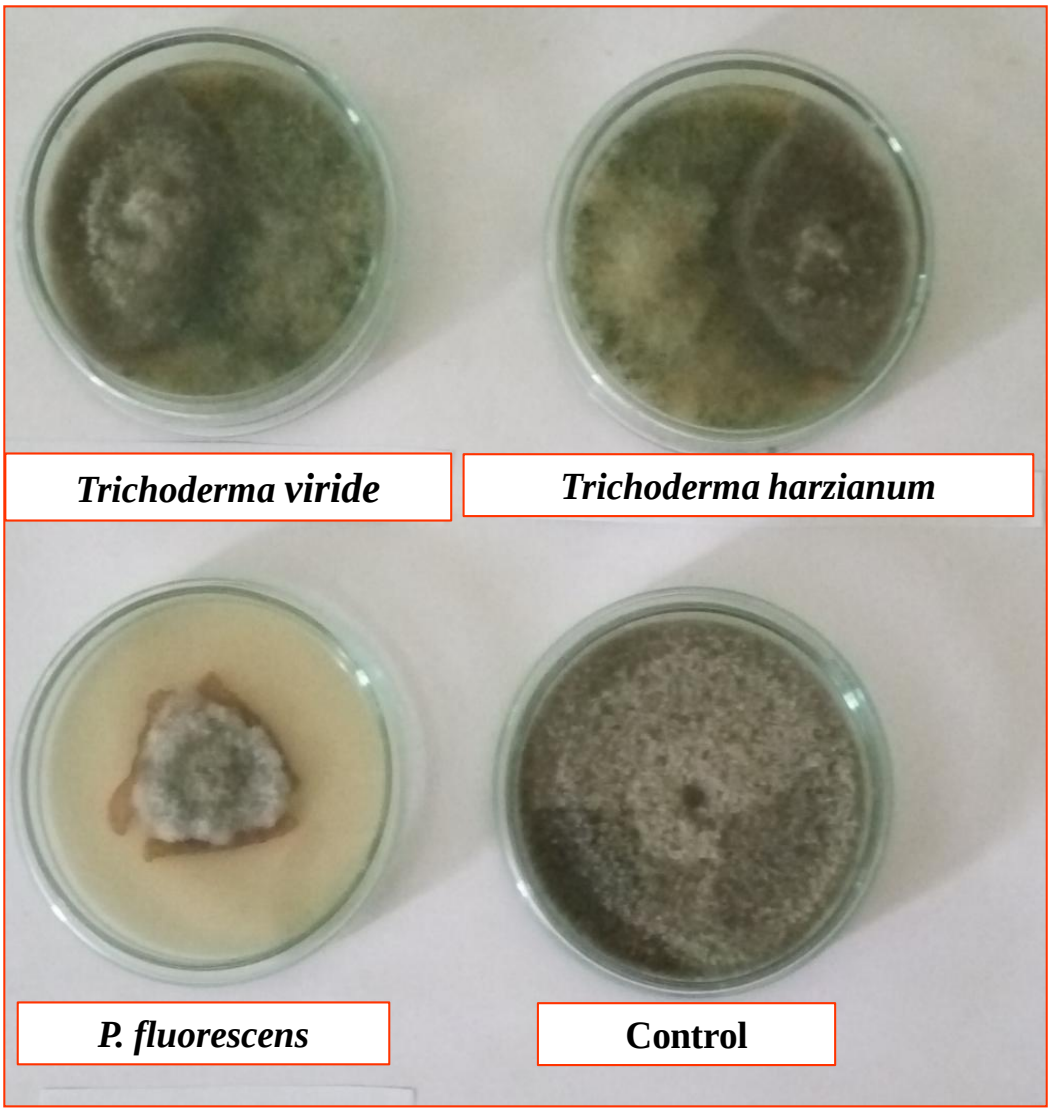


Plate 4.11 : *In vitro* efficacy of bio-control agents against *Alternaria solani*

4.11 Integrated effect of fungicides, bio-control agent and botanicals on early blight of tomato under field condition

4.11.1 Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on early blight intensity on tomato

Integrated effect of fungicides, *P. fluorescens* and NSKE was studied against early blight disease severity on Pusa Ruby variety of tomato and results are presented in table 4.24 and illustrated in Fig. 4.7. The data on PDI of early blight was recorded periodically intervals of 15 days after spray. After 15 days of spray minimum disease intensity (2.0%) was recorded in treatment T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) which was at par with T_3, T_4 and T_6 and significantly lower over T_1, T_2, T_7 and T_8 = Control. Whereas, 30 days after spray minimum disease intensity (4.00%) was recorded in T_5 which was at par with T_4 and T_6 and significantly lower over rest of the treatments. After 45 days of spray, minimum disease intensity (8%) was noticed in treatment T_5. It was at par with treatment T_6 which exhibited 10.67 percent disease intensity. Same trend was found 60 days after spray. Whereas, 75 days after spray treatment T_5 was found significantly superior over rest of the treatments which exhibited minimum disease intensity of 33.33 percent. In case of 90 days of spray, minimum disease intensity (43.33%) was recorded in treatment T_5 which was at par with T_6 and significantly lowers over rest of treatments. While, maximum disease intensity of 83.33 percent was recorded in control plot (Plate 4.12a,b).

On the other hand, maximum reduction in disease intensity of 81.26, 76.00, 79.31, 73.08, 54.55 and 48.00 was recorded after 15, 30, 45, 60, 75 and 90 days of spray, respectively in treatment **T_5 =** Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) followed by T6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) and **T_4 =** Pr opineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) (Fig. 4.7).

Table 4.24: Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on intensity of early blight in tomato under field condition

Treatments	Disease Intensity (%)					
	15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
T_1=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS)	7.33 (15.32)	12.00 (20.08)	22.00 (27.91)	38.00 (38.03)	62.67 (52.41)	72.00 (58.16)
T_2=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS)	6.00 (14.04)	10.67 (18.94)	19.33 (26.05)	34.67 (36.04)	58.00 (49.63)	68.67 (56.03)
T_3=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)	4.67 (12.03)	8.67 (16.95)	15.33 (22.97)	28.67 (32.34)	50.67 (43.37)	60.00 (50.83)
T_4=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)	4.00 (11.28)	7.33 (15.46)	13.33 (21.32)	24.67 (29.70)	46.00 (43.68)	55.33 (48.09)
T_5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)	2.00 (8.13)	4.00 (11.28)	8.00 (16.34)	14.00 (21.83)	33.33 (33.18)	43.33 (41.14)
T_6=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)	3.33 (10.40)	6.67 (14.79)	10.67 (18.98)	19.33 (26.00)	43.33 (41.14)	50.67 (45.37)
T_7=Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas fluorescens* @ 1x 10^9	8.67 (16.95)	14.67 (22.32)	31.33 (33.94)	46.00 (42.68)	70.00 (56.90)	77.33 (61.96)
T_8=Control (water only)	10.67 (18.98)	16.67 (24.03)	38.67 (28.39)	52.00 (46.13)	73.33 (59.06)	83.33 (66.67)
CV (%)	17.29	13.34	10.61	9.18	9.74	11.55
SEm±	1.32	1.28	1.73	1.97	2.91	3.57
CD at 5%	3.72	3.36	4.83	5.54	8.23	9.92

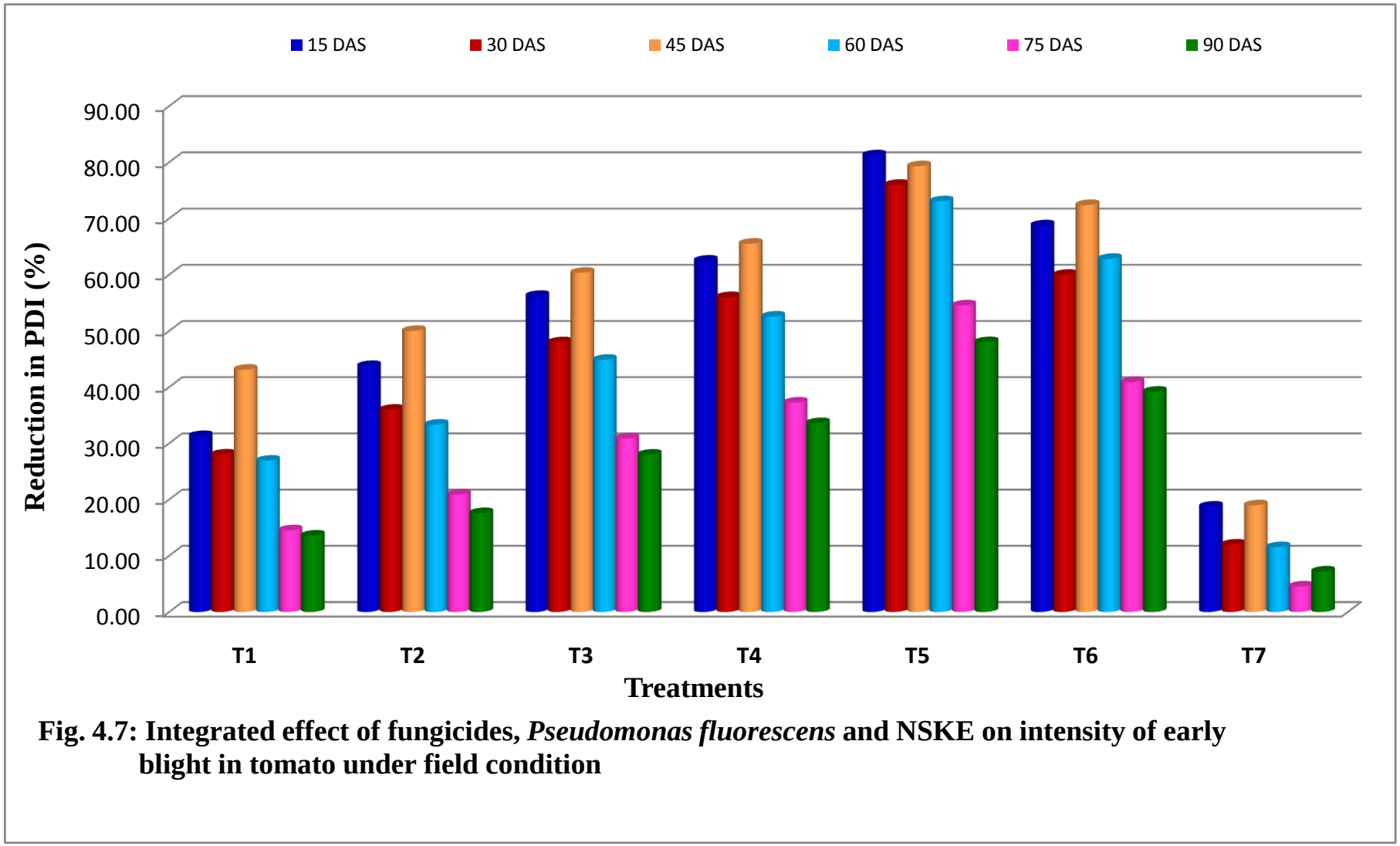


Fig. 4.7: Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on intensity of early blight in tomato under field condition

4.11.2 Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on AUCPC of early blight in tomato

Data pertaining to AUDPC in different treatments have been illustrated in figure 4.8 indicated that the minimum AUDPC (1275) was recorded with the application of treatment T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) followed by $\mathbf{T_6}$ = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) (1680), $\mathbf{T_4}$ = Pr opineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) (1905), $\mathbf{T_3}$ = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) (2140), $\mathbf{T_2}$ = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + Tebuconazole 50% WG@ 0. 05% (FS) (2535), $\mathbf{T_1}$ = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) (2780) and $\mathbf{T_7}$ = Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas fluorescens* @ 1x 10^9, whereas maximum AUDPC (3655) was recorded in control plot.

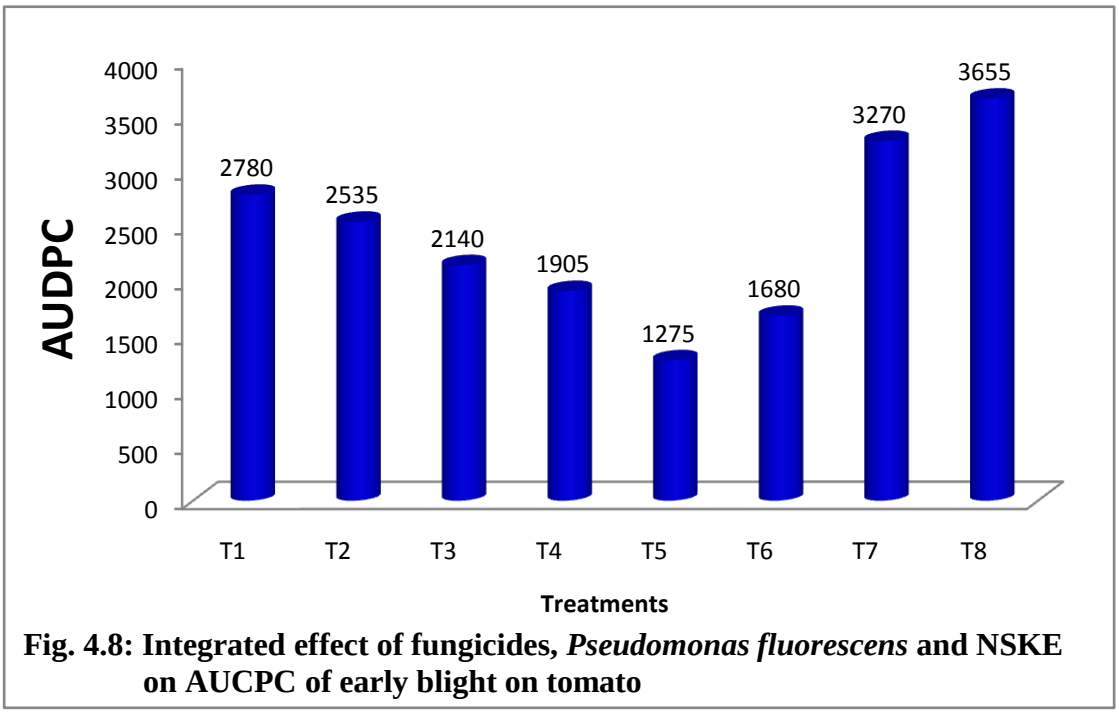


Fig. 4.8: Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on AUCPC of early blight on tomato

The result of present findings are partially to agreement with the results obtained by Ganie *et al.* (2013) they reported that the seed treatment with mancozeb 75WP (0.3 %) + foliar spray with hexaconazole 5 EC (0.1%) + foliar spray with Dhatura (5.0%) + foliar spray with *Trichoderma harzianum* (1×10^7 spore/ml) was highly effective in controlling the disease severity of early blight of tomato. Similar type result also was obtained by Horsfield *et al.* (2010) and Kavyashree *et al. (*2016). Sallam (2011) studied the effect of six plant extracts and some fungicides against *Alternaria solani in vivo*. The greatest reduction of disease severity was achieved by Redomil Plus 74.2% followed by *A. sativum* @ 5% and the smallest reduction was obtained when tomato plant was treated with *O. basilicum* @1 and 5% (46.1 and 45.2%, respectively). Fungicide, *D. stramonium* and *A. sativum* at 5% increased in fruit yield 85.7, 76.2 and 66.7% compared to infected control. Soni *et al.* (2015) evaluated bionanoformulation (Cu-chitosan) in integration with fungicide and botanicals to develop effective management strategies against early blight of tomato caused by *Alternaria solani.* Under pot study the integration of three component; Cu- chitosan 0.1% as seed treatment with spray of Mancozeb 0.25% and neem oil 2% was found best that gave maximum efficacy of disease control (43.01 and 50.81%) with minimum PDI mean (27.50 and 30.38%), respectively, at first and second spray of the treatment as compare to inoculated control. Rani *et al*. (2017) developed integrated disease management module for early blight of tomato fungicides, plant extracts and bio agents were integrated in different treatments and applied in field with varying spray schedules consecutively for two seasons. It was observed that treatment comprising of Mancozeb (0.25%), Datura (50%) and *T. harzianum* S.T (1x107 spores ml^{-1}) reduced disease intensity up to 84.00% followed by treatment comprising of Mancozeb (0.25%) and *T. harzianum* S.T (1x107 spores ml^{-1}) which reduced disease intensity to 82.33%.

Over view of experiments

T_3 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas florescence* @ 1x 10^9 (FS)

T_1 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS)

T_4 = Pr opineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas florescence* @ 1x 10^9 (FS)

T_2 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS)

T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)

Plate 4.12a: Effect of different treatments on early blight of tomato under field condition

T_6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)

T_7 = Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *P. florescence* @ 1x 10^9

T_8 = Control (water only)

Plate 4.12b: Effect of different treatments on early blight of tomato under field condition

4.11.3 Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on number of fruits and fruit yield of tomato under field condition

4.11.3.1 Number of fruits plant^{-1}

Highest number of fruits (45.93 plant^{-1}) was recorded in the treatment T_5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS)) which was at par with T6=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) (41.53 plant^{-1}) and T4=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) (40.00 plant^{-1}) and significantly higher over rest of treatments. In control plot it was recorded lowest (22.27plant^{-1}) (Table 4.25). In case of avoidable losses in number of fruit, highest losses in number of fruit can be avoided (51.51%) with the application of treatment T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)) followed by T_6 (46.38%), T_4 (44.33%), T_3 (38.48%), T_2 (33.06%), T_1 (26.26%) and T_7 (11.63%) (Fig. 4.9).

4.11.3.2 Fruit yield plant^{-1}

Data pertaining to fruit yield plant^{-1} have been presented in table 4.25 reveal that maximum fruit yield (1.301 Kg plant^{-1}) was recorded in treatment T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS) followed by T_6 = Propineb @ 3g kg^{-1} (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) (1.198 Kg plant^{-1}) and T_4 = Propineb @ 3g kg^{-1} (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS) (1.147 Kg plant^{-1}). While, least fruit yield per plant was recorded in control plot (0.716 Kg plant^{-1}). On the other hand maximum losses in fruit yield per plant can be avoided (44.97 %) with the application of treatment T_5 followed by T_6 (40.23%) and T_4 (37.58%) (Fig. 4.9).

4.11.3.3 Fruit yield plot^{-1}

Maximum fruit yield per plot (84.574 Kg) was recorded in treatment T_5 = Propineb @ 3g/kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS) which was at par with T6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) (75.934 Kg) and significantly higher over rest all the treatments. While, least Fruit yield per plot was recorded in control plot (49.171 Kg) (Table 4.25).

4.11.3.4 Fruit yield (ha^{-1})

Total fruit yield was significant higher in all the treatments over control. However, maximum fruit yield (487.5 qha^{-1}) was obtained in Treatment T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0.05% (FS) + NSKE @5% (FS). It was significantly higher over rest of the treatments. While, least fruit yield (283.85 q ha^{-1}) was recorded in control plot (Table 4.25). Data of avoidable yield losses have been illustrated in figure 4.9 indicated that the maximum avoidable yield losses (41.77%) was recorded in treatment T5 followed by T_6 (35.38%), T_4 (31.65%), T_3 (29.61%), T_2 (24.10%), T_1 (19.72%) and T_7 (18.45%).

Sallam (2011) reported greatest reduction of disease severity by Redomil Plus 74.2% followed by *A. sativum* @ 5% and the smallest reduction was obtained when tomato plant was treated with *O. basilicum* @1 and 5% (46.1 and 45.2%, respectively). Fungicide, *D. stramonium* and *A. sativum* at 5% were increased in fruit yield 85.7, 76.2 and 66.7% compared to infected control. Tewari and Vishunavat (2012) evaluated fungicides along and with cultural practices to develop an effective management strategy for early blight of tomato. Cultural practices (inter cropping with marigold, mulching and stacking) when integrated with fungicides reduced the percent disease index and increased the yield.

Table 4.25: Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on number of fruits and fruit yield of tomato under field condition

Treatments	No. of fruits per plant	Fruit yield per plant	Fruit yield (kg) per plot	Fruit yield (q/ha)
T_1=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS)	30.20	0.942	61.378	353.59
T_2=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS)	33.27	0.996	64.400	373.96
T_3=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)	36.20	1.097	69.505	403.24
T_4=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)	40.00	1.147	70.278	415.27
T_5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)	45.93	1.301	84.574	487.5
T_6=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)	41.53	1.198	75.934	439.24
T_7=Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas fluorescens* @ 1x 10^9	25.20	0.862	59.246	348.09
T_8=Control (water only)	22.27	0.716	49.171	283.85
CV (%)	14.34	9.538	12.312	12.31
SEm±	2.84	0.057	4.749	2.75
CD at 5%	8.70	0.174	14.544	8.42

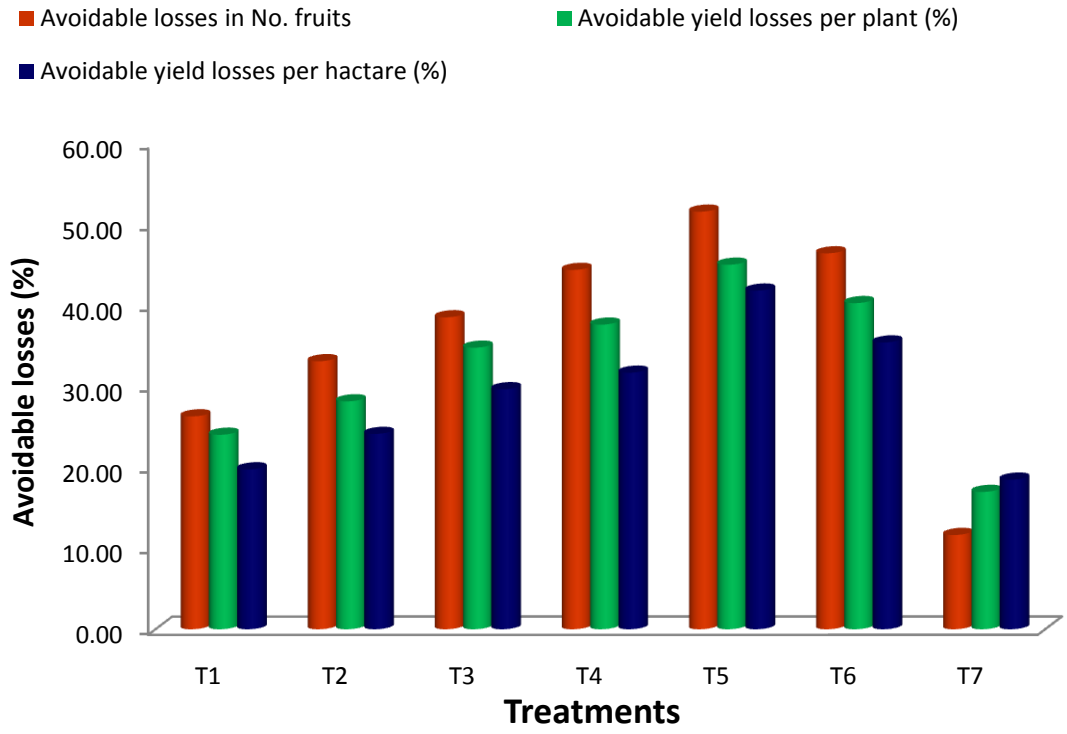


Fig. 4.9: Integrated effect of fungicides, *Pseudomonas fluorescens* and NSKE on number of fruits and fruit yield of tomato under field condition

4.11.4 Economics of different management practices

Economics of different treatments for the control of early blight of tomato have been presented in Table 4.26 indicated that the cost of treatment per hectare was Rs. 3468, 1340, 4596, 2468, 3375, 5503 and 3163 of treatment T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. Although higher return (Rs. 203700 ha^{-1}) was recorded in treatment T_5 followed by T_6 (Rs. 155400 ha^{-1}), T_4 (Rs. 131400 ha^{-1}) T_3 (Rs. 119400 ha^{-1}), T_2 (Rs. 90100 ha^{-1}), T_1 (Rs. 69800 ha^{-1}) and T_7 (Rs. 64200 ha^{-1}). However, highest cost benefit ratio (C: B) was obtained (1:67.24) in the treatment T_2 = Propineb @ 3g kg^{-1} (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS) followed by T_5=Propineb @ 3g kg^{-1} (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS) (1:60.36), T_4=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) +*Pseudomonas fluorescens* @ 1x 10^9 (FS) (1:53.24) and T_6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS) (1:28.24). While, least cost benefit ratio (C: B) was obtained (1:20.13) in treatment T_1 = Propineb @ 3g kg^{-1} (ST) + Azoxystrobin 23%SC @ 0.1% (FS).

Table 4.26: Economics of different fungicides in management practices

Treatment	Cost of fungicide		Cost botanical		Cost of bioagent)		Labour cost @ 207/ day	Total Cost of treatment (Rs.)	Yield (t ha^{-1})	Price (Rs/tonnes)	Total income (Rs)	Income from treatment (Rs.)	C B Ratio
	Quantity	Cost (Rs)	Quantity	Cost (Rs)	Quantity	Cost (Rs)							
1	2	3	4	5	6	7	8	9	10	11	12	13	14
T_1 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS)	400 ml	2640	-	-	-	-	828	3468	35.36	10000	353600	69800	1:20.13
T_2 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS)	200g	512	-	-	-	-	828	1340	37.39	10000	373900	90100	1:67.24
T_3 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS) + *Pseudomonas fluorescens* @ 1x 10^9 (FS)	400ml	2640	-	-	2 lit	300	1656	4596	40.32	10000	403200	119400	1:25.98
T_4 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) +*Pseudomonas fluorescens* @ 1x 10^9 (FS)	200g	512	-	-	2 lit	300	1656	2468	41.52	10000	415200	131400	1:53.24
T_5 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)	200g	512	20Kg	1000	-	-	1863	3375	48.75	10000	487500	203700	1:60.36
T_6 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)	400 ml	2640	20Kg	1000	-	-	1863	5503	43.92	10000	439200	155400	1:28.24
T_7 = Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas fluorescens* @ 1x 10^9	-	-	20Kg	1000	2 lit	300	1863	3163	34.80	10000	348000	64200	1:20.30
T_8 = Control (water only)	-	-	-	-	-	-	-	0.00	28.38	10000	283800	-	-

Ganeshan and Chethana (2009) also previously documented that pyraclostrobin gave higher cost benefit ratio in comparison to other treatments. However, Prasad and

Naik (2003) reported that mancozeb gave the highest cost-benefit ratio (1:11.4) in addition to reducing the disease incidence. This clearly indicated that foliar spray of Pristine (1.0 g/litre) was most effective for disease management and it was also a cost effective treatment and gave higher benefits thus can be recommended for the management early blight of tomato followed by Maccani (3.0 g/litre), and Boscalid (1.0 g/litre). Hence, spraying of Pristine (1.0 g/litre) could be considered as an effective management practice to manage early blight of tomato.

* * * * * * *

CHAPTER–V

SUMMARY AND CONCLUSION

Investigation on **"**Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon Esculentum* Mill.)" was carried out at S.K. College of Agriculture and Research Station (Indira Gandhi Agriculture University, Raipur), Kabirdham (C.G.) during *Rabi* 2016-17. The investigation mainly consists of four objectives:

1. Survey on severity of early blight of tomato in different districts of Chhattisgarh.
2. Cultural and pathogenic variability of *Alternaria solani* isolates.
3. *In vitro* evaluation of fungicides, bio-control agents and plant extracts against *Alternaria solani.*
4. Development of integrated management strategies for the control of Early Blight in tomato.

The summary and conclusion of findings of the present investigation are given below:

Survey carried out during *Rabi* 2016-17 revealed that severity of early blight of tomato was more in Mungeli district followed by Rajnandgaon and Kabirdham district. In case of village the highest PDI was noticed in Kalegondi village of Rajnandgaon district, while the lowest PDI was recorded in Arasnara village of Durg district. Maximum AUDPC was recorded in Rajnandgaon district (6063) followed by Mungeli (6046), Kabirdham (5776), Bemetara (5646). However, least AUDPC (4845) was found in Durg district.

The pathogen was identified as *A. solani* on the bases of morphological characters. The conidiophores were straight and flexuous, brown to olivaceous brown in colour, formed singly and in groups. The conidia were observed solitary straight and slightly flexuous oblong or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 38-80µm and 15-20µm thick in the broadest part with 5- 11 transverse and 0-3 longitudinal septa.

The symptoms of early blight of tomato were observed under natural condition on leaf, stem and fruit. The first symptom of the early blight disease was observed as small brown water soaked lesions on the older leaf. Yellow hallo was found surrounding the spot. Concentric rings were formed in the center of the spots. Symptoms also reported on stem and petioles as brown to dark brown elongated target board type spots. The symptoms on fruits was appeared first at stem end as black or brown sunken spots both on green and ripe fruits which enlarged within eight days involving most of the fruits, finally the fruits were rotted.

Cultural variability among the isolates was observed. Maximum colony diameter was recorded in isolate AS-6 (Kabirdham isolate) followed by AS-1 (Durg isolate), AS-2 (Bemetara isolate) and minimum colony diameter was recorded in AS-7 (Raipur isolate). Variability in colony colour, substrate colour, growth pattern, margin colour, topography, thickness of mycelium mat and sporulation were observed among the isolates of *Alternaria solani*. Greenish brown colour colony was observed in isolates AS-1, grayish brown in isolate AS-2, AS-4 and AS-5, gray colour in AS-6, grayish black in AS-3, while dark brown colony colour was observed in isolate AS-7. Black colour substrate was observed in isolate AS-1 and AS-7 and grayish black in isolate AS-4. Growth pattern were also varied among the isolates from circular smooth to irregular rough. Margin colour of colony was varied from whitish to brown among the isolates. Arial topography was observed in isolate AS-1, AS-2, AS-3, AS-4, AS-5 and AS-6. While, merged topography was observed in isolate AS-7. Very thick mycelium mat was observed in all the isolates, while isolate AS-7 produced thick mycelium mat. Excellent sporulation was noticed in isolate AS-3, AS-5, AS-6 and AS-7 and poor sporulation in isolate AS-1, AS-2 and AS-4.

The microscopic examination was done on septation in conidia of different isolates of *Alternaria solani*. Highest horizontal septa (2-11) was recorded in isolate AS-7, while least horizontal septa (3-6) was observed in isolate AS-1 and AS-5. However, maximum vertical septa (1-3) were observed in isolate AS-3, AS-4 and AS-6 and minimum was found in isolate AS-1, AS-2, AS-5 and AS-7. Conidia length with beak was varies from 39.17-74.35µm in isolate AS-6, while in

isolate AS-3 it was varied from 28.12 -58.35µm. Maximum beak length (7.64 - 14.78µm) was recorded in isolate AS-4, while minimum length of beak (4.18 - 7.62µm) was recorded in isolate AS-3. Width of conidia was maximum (8.82–24.23µm) in isolate AS-3 and minimum width of conidia (8.13–19.48 µm) was found in isolate AS-7.

Pathogenic variability regarding to appearance of symptoms, PDI and AUDPC among the isolates of *Alternaria solani* were recorded on variety Pusa Ruby. Symptoms were early noticed in isolate AS-6 (3-4 DAI), while delay symptoms was appeared in isolate AS-1(6-8 DAI). Highest PDI was observed in isolate AS-6, while least PDI was observed in isolate AS-1. Isolate AS-6 showed maximum AUDPC and minimum AUDPC was observed in isolate AS-1.

Fourteen solid media were used to study the influence of culture media on culture characters of *Alternaria solani* isolates. Among the different media potato dextrose agar media supporting good fungal growth followed by oatmeal agar medium. Minimum growth was observed in water agar medium. Culture media also influenced the colony colour, substrate colour, growth pattern, margin colour, topography, thickness of mycelium. Fungus produced grayish, dark brown, grayish black to olive green colony colour in different isolate on different medium. Sporulation of different isolates of *Alternaria solani* showed highly variation on different media. Excellent sporulation was noticed on PDA, TLEA, $CaCo_3$, MA, GPYA and WA medium in isolates AS-3, AS-5, AS-6 and AS-7, while poor sporulation was observed on Czapek Dox and CMA medium in same isolates.

Maximum mycelium dry weight (300.54 mg) was obtained on oatmeal broth followed by potato dextrose broth (297.21gm) and minimum mycelium dry weight (73.29gm) was observed on mineral broth. Among the isolates, isolate AS-6 was showed maximum mycelium dry weight. However, minimum mycelium dry weight (129.06mg) was obtained in isolate AS-7.

Effect of different pH level on colony diameter, sporulation and mycelium dry weight of *Alternaria solani* Isolate AS-6 on PDA medium reveal that the maximum colony diameter, dry weight were observed at 6.5 pH level followed by 6.0 pH level, while minimum colony diameter and dry weight were observed at 8.5

pH. Excellent sporulation was observed at pH level of 6.0 to 7.0, while poor sporulation was recorded at 4.0, 8.0 and 8.5 pH level.

Mycelium growth and sporulation of *Alternaria solani* was significantly influenced with temperature. Maximum colony growth was recorded at 25^{o}C followed by 30^{o}C and minimum colony growth was recorded at 40^{o}C. Excellent sporulation was noticed at 20^{o}C and 25^{o}C temperature. However, no sporulation was found at 40^{o}C temperature.

Out of six plant extracts tested against *A. solani in vitro,* most effective plant extract was found *Allium sativum* @ 5% which exhibited maximum inhibition in mycelium growth (45.15%) followed by *Crotalaria juncea* @ 5% (44.40%), while minimum inhibition in mycelium growth was recorded in *Euphorbia hirta*. Among the six fungicides, most effective fungicides was found Hexaconazole 5% EC which exhibited 100.00 percent inhibition in mycelium growth at 100ppm followed by followed by Thiafluzamide 24%SC and Trifloxystrobin 25%w/w + Tebuconazole 50%WG at 500ppm. Out of the different antagonists tested *in vitro* against *A. solani, P. fluorescens* showed maximum inhibition in mycelium growth followed by *Trichoderma harzianum*, while minimum mycelium growth inhibition was observed in *Trichoderma viride*.

Under the development of integrated management strategies for the control of early blight in tomato, most effective treatment was found T5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50%WG @ 0. 05% (FS) + NSKE @5% (FS) which exhibited minimum diseases intensity, AUDPC and maximum number of fruits and fruit yield followed by T6=Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% + NSKE @5% (FS)). While, treatment T_7=Propineb @ 3g /kg (ST) + NSKE @ 5% (FS) + *Pseudomonas florescence* @ 1x 10^9) was observed less effective against early blight. Highest return was obtained from treatment T_5 followed by T_6. However, highest C: B ratio (1:67.24) was obtained in the treatment T_2 = Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG@ 0. 05% (FS) followed by T_5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS)

(1:60.36). While, lowest C: B ratio was obtained (1:20.13) in treatment T_1 = Propineb @ 3g /kg (ST) + Azoxystrobin 23%SC @ 0.1% (FS).

CONCLUSION

In the light of finding of present investigation, some conclusions are drawn here:

- Early blight disease of tomato caused by *Alternaria solani* was more prevalent on Mungali district followed by Rajnandgaon.
- The pathogen was isolated from the different district of Chhattisgarh as *Alternaria solani*.
- Variability in colony diameter, colony colour, substrate colour, growth pattern, margin colour, topography, thickness of mycelium mat and sporulation were observed among the isolates of *Alternaria solani*. Pathogenic variability was also observed among the isolates of *Alternaria solani*. On the basis of cultural and pathogenic variability it is concluded that the isolate AS–6 (Kabirdham isolate) was found more virulent as compare to other isolates.
- Out of fourteen solid culture media, PDA was found most suitable for growth and sporulation of *Alternaria solani* followed by OMA medium.
- Out of thirteen liquid media, oatmeal broth was found better in biomass production of *Alternaria solani* followed by potato dextrose broth.
- Most suitable pH was found 6 to 7 for growth and sporulation of *Alternaria solani*
- Mycelium growth and sporulation of *Alternaria solani* was significantly influenced with temperature. Temperature 25 to 30^{o}C was found better for growth and sporulation of *Alternaria solani.*
- Out of six plant extracts, most effective was found *Allium sativum* followed by *Crotalaria juncea.*
- Among the six fungicides, most effective fungicides was found Hexaconazole 5% EC followed by followed by Thiafluzamide 24% SC and Trifloxystrobin 25% w/w + Tebuconazole 50%WG.

- Out of the three bio-control agents, *P. fluorescens* was found most effective under *in vitro* condition.
- Under development of integrated management strategies for the control of Early Blight in tomato, most effective treatment was found T5=Propineb @ 3g /kg (ST) + Trifloxystrobin 25% + tebuconazole 50% WG @ 0. 05% (FS) + NSKE @5% (FS).

SUGGESTIONS FOR FUTURE RESEARCH WORK

1. Mapping of the disease intensity of early blight caused by *Alternaria solani* in the field over the year has to be carried out throughout the Chhattisgarh.
2. Cultural, morphological, pathogenic and molecular variability have to be employed for characterization of *Alternaria solani.*
3. Detailed in influence of culture media, pH level and temperature have to be carried out.
4. Testing of newer fungicides, medicinal plant extracts and native isolates of bio-control agents has to be done.
5. Integration of cultural, biological and chemical measures for the control of early blight in tomato has to be carried out.

* * * * * * *

REFERENCES

Abeysinghe S. 2009. Effect of combined use of *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 on biological control of *Rhizoctonia solani* on *Solanum melongena* and *Capsicum annum*. Plant Pathol. J., 8: 9-16.

Afroz, M., Ashrafuzzaman, M., Ahmed, M.N., Ali, M.E. and Azim, M.R. 2008. Integrated management of major fungal diseases of tomato. Int. J. Sustain. Crop Prod., 3(2): 54-59.

Agrios, G.N. 2005. Plant Pathology. 5th edition, Alsevier Academic Press, New York, 665pp.

Ahmad, A., Khaliq, I.U. and Zaman M. 2014. Prevalence of early blight of tomato and differences among isolates of *Alternaria Solani* in Peshawar Division. Asian Journal of Agriculture Biology, 2(4): 263-267.

Ainsworth, G. C. 1961. Ainsworth & Bisby's Dictionary of the Fungi. 5th edn. Commonwealth Mycological Institute, Kew.

Alhussaen, K.M. 2012. Morphological and phiysiological characterization of *Alternaria solanai* isolated from Tomato in Jordan Valley. Research Journal of Biology Science, 7(8):316-319.

Anonymous 2016. Horticultural statistics at a glance. Horticultural statistics Division, Department of Agriculture, Cooperation & Farmers Welfare, Ministry of agriculture &Farmers Welfare, Government of India. pp. 1-437.

Arunakumar, K.T. 2006. Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India).

Arunakumara, K.T. Satyanarayana, C. and Srinivas, N. 2015. Impact of abiotic and nutritional factors on the growth of *Alternaria solani* causing early blight of potato. Pest Management in Horticultural Ecosystems, Vol. 21(2): 190-193.

Arunkumar, K. T. 2006. Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato, *M. Sc. (Agri) Thesis*. Unvi. Agril. Sci., (India).

Atherton, J.G. and J. Rudich. 1986. In: Tomato crop. *Chapman and Hall, London*, New York. pp. 661.

Babu, S., Seetaraman, K., Nandkumar, R. and Johnson, I. 2000. Variability in cultural characteristics of tomato leaf blight pathogen. Plant Dis. Res., 15(1): 121.

Babu, S., Seetharaman, K., Nandakumar, R. and Johnson, I. 2000. Effect of selected plant extracts/oils against tomato leaf blight. International Journal of Tropical Agriculture, 18:153-157.

Babu, S., Seetharaman, K., Nandakumar, R. and Johnson, I. 2000a. Biocontrol efficacy of *Pseudomonas fluorescens* against *Alternaria solani* an tomato leaf blight disease, Ann. Plant Protec. Sci., 8(2): 252-254.

Babu, S., Seetharaman, K., Nandakumar, R. and Johnson, I. 2000b. Efficacy of fungal antagonists against leaf blight of tomato caused by *Alternaria solani* (Ell. and Mart.), J. Biol. Control, 14(2): 79-81.

Barksdale, T.H. 1968. Resistance of tomato seedling to early bright. Phytopathology, 58: 443-446.

Basu, P.K. 1971. Existence of chlamydospores of *Alternaria porrii* f. sp. *solani* as over wintering propagules in soil. Phytopathology, 61: 1347-1350.

Bell, A.A. and Wheeler, M.H. 1986. Biosynthesis and functions of fungal melanins. Annual Review of Phytopathology, 24: 411-451.

Bellishree, K.,Girija,G.,Ramachandra,Y.L., Archana, S. R. and Chethana, B.S. 2015. Mitigation of early blight of tomato by the intervention of fungal and bacterial bioagents. International Journal of Current Science and Technology, 3(5): 14-19.

Berkeley, M.J. 1836. *Fungi. (Ed. Smith, J.E.),* The English Flora, Vol. V, pt. II. Pp. 339.

Bernat, E. 2004. New possibilities of chemical protection against early and late blights in potato crops using a fungicide Pyton Consento 450SC. Biuletyn Instytutu Hodowli Aklimatyzacji Roslin, 233: 303-308.

Blancard D, Laterrot H, Marchoux G, Candresse T (2012) A colour Handbook - Tomato Diseases: identification, biology and control. Manson Publishing Manson Publishing Limited, London UK: pp. 688.

Bonde, R.1929. Physiological strains of *Alternaria solani*. Phytopathology, 19: 533-548.

Bose, T.K. and Som, M.G. 1986. Vegetable crops in India. Nayaprakash Publishing, Calcutta. pp.773.

Butler E.J. 2003. Potato disease of India. Agric.Ledger, Crop Disease And Pest Series, 7: 87.

Castro, M.E.A., Zambolim, L., Chaves, G.M., Cruz, C.D. and Matsuoka, K. 2000. Pathogenic variability of *Alternaria solani*, the causal agent of tomato early blight. Summa Phytopathologica, 26: 24-28.

Cerkauskas, R., 2005. Early Blight. AVRDC, the world vegetable centre, www.avrdc.org

Chaerani, R., Voorrips, R.E. and Roeland, E. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics and breeding for resistance. J. Gen. Plant Pathol., 13: 335- 347.

Chaurasia, A.K., Chaurasia, S., Chaurasia, S. and Chaurasia, S. 2013. Studies on the development of fruit rot of tomato caused by *Alternaria solani* (Ellis & Mart.). Jones & Grout. International Journal of Pharmacy & Life Sciences, 4(6): 215-221.

Chohan, S., Perveen, R., Mehmood, M.A., Naz S. and Akram, N. 2015. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the Causal agent of tomato early blight. International Journal of Agriculture & Biology, 17(1): 111–118.

Choulwar, A.B. and Datar, V.V.1994. Tolerance of *Alternaria solani* to fungicides, J. Maharashtra Agril. Unvi., 19(1): 133-134.

Coffey M.D., Whitbread, R. and Marshall C. 1975. The effect of early blight disease caused by *Alternaria solani* on shoot growth of young tomato plants. Ann. Appl. Biol., 80: 17–26.

Dalpati, N.N.S., Parate, R.L. and Ingle, S.T. 2010. Efficacy of some bioagents and botanicals against *Alternaria macrospora* causing leaf spot of cotton. J. Plant Dis. Sci., 5(1): 95.

Datar, V.V. and Mayee, C.D., 1981. Assessment of loss in tomato yield due to early blight. Indian Phytopathol., 34: 191-195.

Datar, V.V. and Mayee, C.D., 1986, Chemical management of early blight of tomato. J. Maharashtra Agric. Univ., 10: 278-280.

Dubey, S.C., Patel, B. and Jha, D.K. 2000. Chemical management of Alternaria blight of broad bean. Indian Phytopath., 53(2): 213-215.

Ellis, J.B. and Martin G B. 1882. *Macrosporium solani* E&M. American Naturalist, 16: 1003.

Ellis, M.B. 1971. Dematiaceous hyphomycetes. Commonwelth Mycological Institute, Kew, Surry, England, pp 608.

Enikuomehin, O.A., and Peters, O.T. 2002. Evaluation of crude extracts from some Nigerian plants for the control of field diseases of sesame (*Sesamum indicum* L.). Tropical Oilseeds Journal., 7: 84-93.

Foolad, M.R., Merk, H.L. and Ashrafi, H. 2008. Genetics, genomics and breeding of late blight and early blight resistance in tomato. Critical Reviews in Plant Sciences, 27: 75–107.

Ganeshan, G. and Chethana, B.S. 2009. Bioefficacy of Pyraclostrobin 25%EC against early blight of tomato. World Applied Sciences Journal, 7(2): 227-229.

Ganie, S.A., Ghani, M.Y., Nissar, Q. and Rehman, S.U. 2013. Bioefficacy of plant extracts and biocontrol agents against *Alternaria solani,* African Journal of Microbiology Research, 7(23): 4397-4402.

Gemawat, P. D. and Gosh, S. K. 1979. Studies on the physiology of growth of *Alternaria solani*. India Journal of Mycology and Plant Pathology, (9): 138-139.

Gemawat, P.D. and Ghosh, S.K. 1980. Studies on the physiology and growth of *Alternaria solani*. Indian Journal of Mycology Plant Pathology, 9: 138-139.

Gwary, D.M. and Nahunnaro, H. 1998. Epiphytotics of early blight of tomatoes in North-eastern Nigeria. Crop Prot., 17: 619-624.

Hassan, M., Farooq, M. and Gul, F. 2014. *In vitro* evaluation of some fungicides against common fungal pathogen of early blight and fruit rot of tomatoes. J. Appl. Environ. Biol. Sci., 4(9S): 424-429.

Hassanein, N.M., AbouZeid, M.A., Youssef, K.A. and Mahmoud, D.A. 2010. Control of tomato early blight and wilt using aqueous extract of neem leaves. Phytopathol. Mediterr., 49: 143–150.

Horsfield, A., Wicks, T., Davies, K., Wilson, D. and Scott, P. 2010. Effect of fungicide use strategies on the control of early blight and potato yield. Australas. Plant Pathol., 39(4): 368-375.

Hubballi, M., Nakkeeran, S., Raguchander,T., Anand, T. and Samiyappan, R. 2010. Effect of environmental conditions on growth of *Alternaria alternata* causing leaf blight of noni. World Journal of Agricultural Sciences, 6(2): 171-177.

Jegathambigai, V., Wijeratnam, R.S.W. and Wijesundera, R.L.C. 2010. Effect of *Trichoderma* sp. on *Sclerotium rolfsii*, the causative agent of collar rot on *Zamioculcas zamiifolia* and an on farm method to mass produce *Trichoderma* species. Plant Pathol. J., 9: 47-55.

Johnson , A., and Booth, C. 1983. In Plant Pathologist's Pocket Book, Oxford and IBH Pub. Co. Calcutta: 136 pp.

Joly, P. 1959. Morphological variations and the idea of species in the genus *Alternaria* . Bull. Soc. Mycol. Fr., 75: 149-158.

Jones, J.B., Jones, J.P, Stall, R.E. and Zitter,T.A .1991. Infectious antifungal. Plant Physiology, 108: 17-27.

Jones, L. R. and Grout, A.J. 1897. Notes on two species of Alternaria. Bulletin of the Torrey Botanical Society, 24: 254–258.

Jones, L.R. 1893. The new potato disease or early blight. Vermont Agricultural Experimental Station Bulletin, 6: 66–70.

Jones, L.R. and Grout, 1986. *Alternaria solani* (Ellis & G. Martin). Annual Report of the Vermont Agricultural Experimental Station, 9: 86.

Kagale, S., Marimuthu, T., Nandakumar, R. and Samiyappan, R. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. oryzae. Physiol. Mol. Plant Pathol., 65: 91-100.

Kamble, S.B., Sankeshwari, S.B. and Arekar, J.S. 2009. Survey on early blight of tomato caused by *Alternaria solani.* International journal of Agriculture Science, 5(1): 317-319.

Kaul, A.K. and Saxena, H.K. 1988. Physiologic specialization in *Alternaria solani* causing early blight of tomato. Indian J. Mycol. Pl. Pathol., 18: 128-132.

Kavyashree, M.C., Yadahalli, K.B. and Jahagirdar, S. 2016. Integrated management of foliar fungal diseases of green gram (*Vigna radiata L.*) Journal of Eco-friendly Agriculture, 12(1): 71-74.

Kemmitt, G. 2002. Early blight of potato and tomato. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-0809-01.

Khan, A.A. 2002. Resistance of two tomato species to five isolates of *A. solani.* Asian J. Plant Sci., 1(6): 703-704.

Koley, S., Mahapatra, S.S. and Kole, P.C. 2015. *In vitro* efficacy of bio-control agents and botanicals on the growth inhibition of *Alternaria Solani* causing early leaf blight of tomato. International Journal of Bio-Resource, Environment and Agricultural Sciences, 1(3): 114-118.

Kota, V. 2003. Biological management of post harvest fungal diseases of major fruits. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci. Dharwad, India.

Kulkarni, N. K. 1998. Studies on early blight of potato (*Solanum tuberosum* L.) caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. M.Sc. (Agri) Thesis, University of Agricultural Sciences, Bangalore, 88pp.

Kumar,A., Gour, H.N., Sharma, P. and Shah, R. 2012. Investigations on variability and eco-friendly management of *Alternaria alternata* causing tomato blight. Society of Plant Research, 25(1): 52-58.

Leifort, C., Sigee, D.C., Epton, H.A.S., Stanley, R. and Knight, C. 1992. Isolation of bacterial antagonistic to post harvest fungal disease of cold stored *Brassica* spp. Phytoparastica, 20: 158-163.

Locke, S.B. 1949. Resistance to early blight and septoria leaf spot in the genus *Lycopersicon*. Phytopathology, 89: 829-836.

Madden, L., Pennypacker, S.P. and MacNab, A.A. 1978. FAST, a forecast system for *Alternaria solani* on tomato. Phytopathololgy, 68: 1354-1358.

Mahabaleshwarappa, K.B. 1981. Studies on leaf spot of safflower (*Carthamus tinctorius* Linn) caused by *Alternaria carthami* Choudhary. *M.Sc. (Agri) Thesis*. University of Agricultural Sciences, Bangalore, P.54.

Maiero, M., Ng, T.J. and Barksdale, T.H. 1990. Genetic resistance to early blight in tomato breeding lines. Hort Science, 25: 344-346.

Mallikarjun, G. 1996. Studies on *Alternaria alternata* (Fr.) Keissler a causal agent of leaf blight of turmeric (*Curcuma longa* L.). *M. Sc. (Agri.) Thesis*, Univ. Agril. Sci., Dharwad, India.

Marak, T.R., Ambesh, B.S., Das, S. 2014. Cultural, morphological and biochemical variations of *Alternaria Solani* causing diseases on solanaceous crops. The Bioscan, 9(3): 1295-1300.

Mark, L. Gleason and Brooke, A. Edmunds. 2006. Tomato diseases and disorders. Physiological disorder, 12pp.

Mathur, K. and Shekhawat, K.S. 1986. Chemical control of early blight in *Kharif* sown tomato. Indian Journal of Mycology Plant Pathology, 16: 235-238.

Maurehofer, M., Hase, C., Menwly, P., Metraux, J.P. and Defago, G. 1994. Induction of systemic resistance of tobacco to Tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO. Phytopathology, 84: 139-146.

Maya, C. and Thippanna, M. 2015. *In vitro* antifungal evaluation of various botanical extracts against early blight disease *(Alternaria Solani)* of tomato. International journal of Science and Nature, 6(2): 264-267

Meitei, K.M., Bora, G.C. and Borah, P.K. 2012. Screening of tomato genotypes for resistance to early blight (*Alternaria Solani*). International Journal of Science and Research, 3: 351-358.

Mesta, R.K., Benagi, V.I., Shankergroud, I. and Megeri, S.N. 2009. Effect of dates of sowing and correlation of weather parameters on the incidence of *Alternaria* blight of sunflower. Karnataka J. Agric. Sci., 22(2): 441-443.

Moges, M.M., Selvaraj, T.V. and Jebessa, M.T. 2012. Influance of some antagonistic bacteria against early blight (*Alternaria solani* (Ell. & Mart.) Jones & Grout.) of tomato (Lycopersicon esculentum Mill.). The African Journal of plant Science and Biotechnology.

Momel, T.M. and Pemezny, K.L. 2006. Florida plant disease management guide: Tomato. Florida Cooperation Extensive Service, Institute of Food and Agriculture Sciences, Gaine ville, 32611 (http\\edis.infas.ufl.edu).

Moore, W.D. And Thomas, H.R. 1942. Some cultural practices that influence the development of *Alternaria solani* on tomato seedlings. Phytopathology, 32: 1176-1184.

Morris, P.F., Connolly, M.S. and Clair, C.A. 2000. Genetic diversity of *Alternaria alternata* isolated from tomato in California using RAPD. Mycol. Res., 104: 286-292.

Munde, V.G., Diwakar, M.P., Thombre, B.B. And Dey, U. 2013. Survey and surveillance of early blight of tomato caused by *Alternaria solani* in Konkan region. International Journal of Plant Protection, 6(2): 476-477.

Naik, M.K., Prasad, Y., Bhat, K.V and Rani, D. 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. Indian Phytopathol., 63(2): 168-173.

Nashwa S.M.A. and Abo-Elyousr K.A.M. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Protect. Sci., 48: 74–79.

Neergaard, P. 1945. Danish species of *Alternaria* and *Stemphylium*: taxonomy, parasitism, economic significance. *Oxford University Press*, London, Pp. 260- 287.

Nene, Y. L. and Thapaya, P. N. 1993. Evalution of fungicides. In; fungicide in plant disease control. Oxford and IBH publishing company, New Delhi, P-531.

Nikam, P.S., Suryawanshi, A. P. and Chavan, A. A. 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria solani*, causing early blight of tomato. African Journal of Biotechnology, 14(10): 872-877.

Pachori A., and Sharma, O.P. 2016. Status of early blight of tomato in Morena, Bhind and Gwalior Districts of Madhya Pradesh. The Bioscan, 11(2): 791-793.

Pachori, A., Sharma, O., Sasode, R. and Sharma, R.N. 2016. Collection of different isolates of *Alternaria solani* in Bhind, Morena and Gwalior districts of Madhya Pradesh. International Journal of Applied Research, 2(6): 217-219.

Pandey, · K.K., Pandey, P.K., Kalloo, G. and Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. J. Gen. Plant Pathol., 69: 364–371

Pandey, K.K., Pandey, P.K., Kallo, G. Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. J. Gen. Plant Pathol., 69: 364-371.

Patel, R.L. and Choudhary, R.F. 2010. Management of *Alternaria solani* causing early blight of tomato with fungicides. J. Plant Dis. Sci., 5(1): 65-67.

Pathak,V.N. 1972. Essentials of Plant Pathology. Prakesh Publishers Jaipur, India, P. 448.

Peralta, I.E., Knapp, S. and Spooner, D.M. 2005. New species of wild tomatoes (*Solanum* section *Lycopersicon*: Solanaceae) from Northern Peru. Sys Bot., 30: 424-434.

Perez, S. and Martinez, B. 1995. Selection and characterization of *Alternaria solani* isolates of tomato. Revista de Protect. Vegetal, 10: 163-167.

Rahman, S.M.M., Maniruzzaman, S.M., Nusrat, S., Khair, A. 2015. *In vitro* evaluation of botanical extract, bioagents and fungicides against purple blotch diseases of bunch onion in Bangladesh. Advances in Zoology and Botany, 3(4): 179-183.

Rahmatzai, N. Zaitoun, A.A., Madkour, M.H., Ahmady, A., Hazim, Z. and Mousa, M.A.A. 2016. Morphological, pathogenic, cultural and physiological variability of the isolates of *Alternaria solani* causing early blight of tomato. International Journal of Advanced Research, 4(11): 808-817.

Rahmatzai1, N., Zaitoun1, A.A. Madkour, M.H., Ahmady, A., Hazim, Z. and Mousa, M.A.A. 2016. Morphological, pathogenic, cultural and

physiological variability of the isolates of *Alternaria solani* causing early blight of tomato. Int. J. Adv. Res., 4(11): 808-817.

Ramakrishnan, B., Kamalnathan and Krishnamurthy, C.S. 1971. Studies on *Alternaria* leaf spot of tomato. Madras Agric. J., 58: 275-280.

Rani, S., Singh R., Gupta S., Dubey, S. and Razdan, V.K. 2015. Identification of resistant sources and epidemiology of early blight (*Alternaria solani*) of tomato (*Lycopersicum esculentum*) in Jammu and Kashmir. Indian Phytopathology, 68(1): 87-92.

Rani, S., Singh, R. and Gupta, S. 2017. Development of integrated disease management module for early blight of tomato in Jammu. Journal of Pharmacognosy and Phytochemistry, 6(2): 268-273

Rodrigues, T.T.M.S. Maffia, L.A., Dhingra, O.D. and Mizubuti, E.S.G. 2010. *In vitro* production of conidia of *Alternaria solani*. Tropical Plant Pathology, 35(4): 203-212.

Roopa, R. S, Yadahalli, K. B. and Kavyashree, K. B. 2014. Evaluation of natural plant extracts, antagonists and fungicides against early blight caused by *A. solani in vitro*. The Bioscan, 9(3): 1309-1312.

Rotem, J. 1966. Variability in *Alternaria porri f. sp. solani*. Isr. J. Bot., 15: 47-57.

Rotem, J. 1998. The genus *Alternaria*; biology, epidemiology and pathogencity, 1st ed. The American Phytopathological Society Press St. Paul. Minnesota.

Sabriye Yazici, Yusuf Yanar and Isa Karaman 2011. Evaluation of bacteria for biological control of early blight disease of tomato. African J. Biotechnol., 10: 1573-1577.

Sadana, D. and Didwania, N. 2015. Bioefficacy of fungicides and plant extracts against *Alternaria solani* causing early blight of tomato. *International Conference on Plant, Marine and Environmental Sciences* (PMES-2015) Kuala Lumpur (Malaysia). Pp. 38-42.

Saha, P. and Das, S. 2012. Assessment of yield loss due to early blight (*Alternaria solani*) of tomato. Indian Journal of Plant Protection., 40(3): 195-198.

Sahu, D.K., Khare, C.P. and Patel, R. 2013. Seasonal occurrence of tomato diseases and survey of early blight in major tomato-growing regions of Raipur District. The Ecoscan an International Quarterly Journal of Environmental Sciences, IV: 153-157.

Sahu, D.K., Khare, C.P., Patel, R. 2014. Eco friendly management of early blight of tomato using botanical plant extracts. Journal of Industrial Pollution Control, 30(2): 215-218.

Sahu, D.K., Khare, C.P., Singh,H.K. and Thakur, M.P. 2013. Evaluation of newer fungicide for management of early blight of tomato in Chhattisgarh. The Bioscan, 8(4): 1255-1259.

Sallam, N.M.A. 2011. Control of tomato early blight disease by certain aqueous plant extracts. Asian Network for Scientific Information, 10(4): 187-191.

Samuel, G.S. and Govindaswamy, C.V. 1972. Effect of vitamins and levels of pH on the growth and sporualtion of *Alternaria solani*, the causal agent of the leaf blight disease of seasame (*Sesamum indicum*). Indian Journal of Mycological Plant Pathology, 2: 185-186.

Samuel, G.S. and Govindaswamy, C.V. 1972. Effect of vitamins and levels of pH on the growth and sporualtion of Alternaria solani, the causal agent of the leaf blight disease of seasame (Sesamum indicum). Indian J Mycol Pl Path., 2: 185-186.

Sharma, R.N. and Gaur, R.B. 2009. Management of post-harvest core rot, *Alternaria alternata* in Kinnow, *Citrus deliciosa* fruits. Indian J. Plant Protect., 37(1/2): 207-210.

Sherf, A.F., and MacNab, A.A. 1986. Vegetable diseases and their control. John Wiley and Sons, New York, 634-640.

Simmons E.G. 2000. Alternaria themes and variations (244- 286) species on Solanaceae. Mycotaxon, 75: 1-115.

Singh, A., Singh, V. and Yadav, S.M. 2014. Cultural, Morphological and Pathogenic Variability of *Alternaria solani* Causing Early Blight in Tomato. Plant Pathology Journal, 11(3): 167-172.

Singh, P.C. and Singh, D. 2006. *In vitro* evaluation of fungicides against *Alternaria alternata.* Annals of Plant Protection Sciences, 14(2): 500-502.

Singh, R.S. 1987. Diseases of Vegetable Crops. Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, Bombay, Calcutta. pp. 419.

Smith L.J. and Kotcon 2002. Intercropping with tomato resistant variety 'Juliet' reduces early blight on susceptible variety 'brandywine'. Phytopathology, 92: 77.

Somappa, J., Srivastava, K., Sarma, B.K., Pal, C. and Kumar, R. 2013. Studies on growth conditions of the tomato *Alternaria* leaf spot causing *Alternaria Solani* L. The Bioscan, 8(1): 101-104.

Soni, R., Bunker, R.N., Dhakad, U.K., and Yadav A. 2015. Efficacy of fungicide, botanical and bio-nanoformulation for suppression of early blight of tomato causing by *Alternaria Solani.* The Bioscan, 8: 337-340.

Stammler, G., Bohme, F., Philippi, J., Miessner, S. and Tegge, V. 2014. Pathogenicity of *Alternaria species* on potatoes and tomatoes. PPO – Special Report, 16: 85-96.

Stevenson, R.E. and Packer, S.P.1988. Effect of radiation temperature and moisture on conidial germination of *Alternaria solani*. *Phytopathology*, 78: 926-930.

Sundaramoorthy, S. and Balabaskar, P. 2012. Consortial effect of endophytic and plant growth promoting rhizobacteria for the management of early blight of tomato incited by *Alternaria solani*. J Plant Pathol. Microb., 3: 7.

Tewari, R. and Vishunawat, K. 2012. Management of early blight (*Alternaria solani*) in tomato by integration of fungicides and cultural practices. International Journal of Plant Protection, 5(2): 201-206.

Tippeswamy, B., Sowmya, H.V. and Krishnappa, M. 2010. Pathogenicity and management of early blight and wilt in tomato caused by *Alternaria solani* and *Fusarium oxysporum.* Asian J. Microbiol. Biotech. Env. Sci., 12(4): 739-744.

Verma, K.P., Singh, S. and Gandhi, S.K. 2007. Variability among Alternaria solani isolates associated with early blight of tomato. Indian Phytopathology, 60(2): 180-186.

Verma, N. and Verma, S. 2010. Alternaria disease of vegetable crops and new approach for its control. Asian J. Exp. Biol. Sci., 1(3): 681- 692.

Verma, V. 1970. Effect of temperature and hydrogen-ion concentration of three pathogenic fungi. Sydowia, 23: 164-168.

Vincent, J. M. 1927. Distortion of fungal hyphae in presence of certain inhibitors. Nature, 159: 850.

Vloutoglou, I. 1994. Epidemiology of *Alternaria linicola* on linseed (*Linum usitatissimum* L.). *Ph.D. Thesis*, University of Nottingham, UK, 378pp.

Vloutoglou, I., Kalogerakis, S.N. and Darras, A. 2000. Effects of isolate virulence and host susceptibility on development of early blight (*Alternaria solani*) on tomato. Bulletin- OEPP., 30(2): 263- 267.

Walker, J.C. 1952. Diseases of Vegetable Crops, 1st ed. MacGraw-Hill Book Company, Inc. New York. Pp. 471-474.

Wheeler, B.E.J. 1969. *An Introduction of Plant Diseases*. John Willey and Sons Ltd. London. pp. 301.

Yadav, S.M., Singh, S.M. and Chand, R. 2015. Mass sporulation of *Alternaria Solani* causing early blight of tomato. Indian Phytopathology, 68(1): 83-86.

Zafar, H., Shaukat, S. and Rao, T.A. 2013. Antagonistic activity of cultural filtrates of five *Trichoderma* species against pathogenic fungus *Alternaria Solani*. Int. J. Biol. Biotch., 10(4): 547-551.

*** * * * * * ***

VITA

Name	:	Pankaj Kumar
Date of Birth	:	21/08/1982
Nationality	:	Indian
Present Address	:	Quarter No. 13, New Collector Colony , Kawardha, disst.- Kabirdham(C.G.) Pin- 491559
	:	Phone No. - 9926245304
		Email ID - pankajkd21@gmail.com
Permanent Address	:	Vill.-Funda, Post- Bori, Tah. & Block- Dhamdha, Distt- Durg, (C.G.), Pin- 491001

Academic qualifications:

Examination	Year of passing	Board /University
Higher Secondary Certificate	2000	M.P. Board, Bhopal
B. Sc. (Agriculture)	2005	IGKV, Raipur (C.G.)
M.Sc. (Agriculture)	Course work completed, thesis is being submitted	I.G.K.V. Raipur (C.G.)

Professional experience	:	Work as Rural Agriculture Extension Officer
Membership of Professional Societies	:	No
Awards/Recognitions	:	No
Publications	:	No

Signature