"STUDIES ON BANDED LEAF AND SHEATH BLIGHT (*Rhizoctonia solani* kühn) DISEASE OF MAIZE"

M.Sc. (Ag.) Thesis

By

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
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By

Krishna Kumar Maravi

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CERTIFICATE - 1

This is to certify that the thesis entitled **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of maize"** submitted in partial fulfilment of the requirements for the degree of **"Master of Science in Agriculture (Plant Pathology)"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a recorded of the bonafide research work carried out by **Krishna Kumar Maravi** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date: 21.07.2016

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This is to certify that the thesis entitled **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of maize"** submitted by **Krishna Kumar Maravi** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur in partial fulfilment of the requirements for the degree of **M.Sc. (Ag.)** in the **Department of Plant Pathology** has been approved by the external examiner & Student's Advisory Committee after oral examination.

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Krishna

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Date: 21/07/16
Department of Plant Pathology
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LIST OF ABBREVIATION

ABBREVIATION	DESCRIPTION
%	Percent
@	At the rate of
°C	Degree Celsius
CD	Critical difference
Sem	Standard error of mean
DAI	Days after inoculation
et al.	And other
Fig.	Figure
Ha	Hectare
i.e.	That is
Gm	Gram
m ha	Million hectare
Mt	Metric tone
Cm	Centimeter
No.	Number
Wt	Weight
viz.	Namely
V	Variety
T	Treatment
BOD	Biological Oxygen Demand
PDA	Potato Dextrose Agar

THESIS ABSTRACT

a) Title of the Thesis:	**"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"**
b) Full Name of the Student:	Krishna Kumar Maravi
c) Major Subject:	Plant Pathology
d) Name and Address of the: Major Advisor	Dr. R. K. Dantre (Principal Scientist) Department of Plant Pathology, College of Agriculture, Raipur (Chhattisgarh)
e) Degree to be awarded:	M.Sc. (Ag.) in Plant Pathology

Signature of the Student

Signature of Major Advisor

Date: 21.07.2016

Signature of Head of the Department

ABSTRACT

The present investigation entitled **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"** was carried out in the Department of Plant Pathology, College of Agriculture, IGKV, Raipur (C.G.).

In symptomatological studies, the initial symptoms were observed on the first and second leaf sheath above the ground and eventually spreaded on the ear causing ear rot. Infected leaves and sheaths were water soaked concentric bands and discoloured, brown in color. Whole plant was blighted within a weeks. White mycelial fungal growth was seen under surface of infected leaves and sheaths and young branches. Sclerotia were observed on severely blighted leaves, sheaths and the ears.

The infected plants showed typical banded leaf and sheath blight disease symptoms were collected from the field for isolation of the pathogen in the

laboratory. The pathogen was isolated on PDA from infected plant parts. The culture were purified by single hyphal tip method and were maintained on PDA at 27 ± 1^{o} C in BOD incubator. The isolated fungi were identified on the basis of morphological characteristics.

Pathogenicity was proved in maize under laboratory condition by deteched method and under field condition by attached method.

In host range studies, 6 plant species were inoculated with pathogen. All the 5 host crop (bean, soyabean, wheat, chilli, brinjal) not showed host susceptibility against *Rhizoctonia solani* but rice crop showed host susceptibility against *Rhizoctonia solani.*

Twenty one entries of maize were evaluated against *Rhizoctonia solani* under natural field conditions. All the entries showed highly resistant and resistant reactions against banded leaf and sheath blight disease.

Antagonistic efficacy of *Pseudomonas fluorescens* were studied against isolates of *Rhizoctonia solani* by bacterial funnel technique, where the 78.66 % highest growth inhibition percent was found in P72 followed to 72.43% in P201 and 66.25 % in P5. The least 0.00% growth inhibition was found in P205, P126, P124, P99, P143, P151, P179, P161, P247, P248 and P216.

Seven fungicides (Captan 70% + Hexaconazole 5% WP, Propiconazole 25% EC, Carbendazim 50%WP, Thifluzamide 24% SC, Hexaconazole 5% SC, Metalaxyl 72%WP, Carbendazim 75%WP + Mancozeb) were evaluated *in vitro* by poisoned food techniques at three concentrations i.e. 10, 20 and 30 ppm. All the fungicides significantly effective in reducing the mycelia growth at all the three concentrations. Carbendazim 50%WP, Hexaconazole 5% SC, Propiconazole 25% EC, Thifluzamide 24% SC proved to be the best fungicides giving best mycelial growth inhibition of the test fungus (100.00%) after 9 days of inoculation followed by Captan 70% + Hexaconazole 5% WP (77.78 %), Carbendazim 75%WP + Mancozeb (84.82%) and Metalaxyl 72%WP (88.15%).

Five fungicides were evaluated under *in vivo* conditions for banded leaf and sheath blight of maize clearly revealed that commercially available fungicides like Taqat (Captan 70% + Hexaconazole 5% WP) (24.60%), Tilt (Propiconazole 25% EC) (22.11%), Bavistin (Carbendazim 50%WP) (23.33%), Pulsor

(Thifluzamide 24% SC) (20.29%), Contaf Plus (Hexaconazole 5% SC) (22.10 %) were significantly reduced the banded leaf and sheath blight disease severity over control (95.92%).

शोधग्रंथ सारांश

1. शोधग्रंथ का शीर्षक:	"मक्का की बंधी पत्ती और म्यान तुषार (राइजोक्टोनिया सोलानाई कुहन) रोग का अध्ययन"
2. छात्र का पूरा नाम	कृष्ण कुमार मरावी
3. प्रमुख विषय	पादप रोग विभाग
4. प्रमुख परामर्शदाता का नाम एवं पता	डॉ. आर. के. दाँतरे (प्रमुख वैज्ञानिक) पादप रोग विभाग कृषि महाविद्यालय, रायपुर, छत्तीसगढ़
5. उपाधि	एम. एस सी. (कृषि) पादप रोग विज्ञान

छात्र का हस्ताक्षर

प्रमुख परामर्शदाता का हस्ताक्षर

दिनांक 21.07.2016

विभागाध्यक्ष का हस्ताक्षर

सारांश

वर्तमान शोध सारांश **"मक्का की बंधी पत्ती और म्यान तुषार (राइजोक्टोनिया सोलानाई कुहन) रोग का अध्ययन"** पर अन्वेषण इंदिरा गांधी कृषि विश्वविद्यालय रायपुर, कृषि महाविद्यालय के पौध रोग विभाग में किया गया।

इसके लक्षणों के अध्ययन में, प्रांरभिक लक्षण जमीन के ऊपर पहली और दूसरी पत्ती म्यान पर देखा गया एवं अन्ततः इसका फैलाव बालीयों पर बाली सड़न के रूप में देखा गया। संक्रमित पत्तियाँ एवं शीथ पानी रहित गाढ़ा बैंड एवं भूरें रंग की हो गई थी। पूरा पौधा एक सप्ताह के भीतर अभिशप्त हो गया था। सफेद कवक का विकास संक्रमित पत्तियों एवं शीथ एवं युवा शाखाओं की सतह के नीचे देखा गया था।

संक्रमित पौधे जो कि ठेठ बंदी पत्ती और म्यान तुषार रोग के लक्षण हैं, प्रयोगशाला में रोगजनक के अलगाव के लिए खेत से एकत्र किए गए थे। संक्रमित पौधे के भागों से रोगजनक का अलगाव पीडीए पर किया गया। कल्चर को एकल हाइपल टिप विधि द्वारा शुद्ध किया गया था और उसे बीओडी इनक्यूवेटर में, 27$\pm$1 डिग्री सेल्सियस पर पीडीए पर बनाए रखा गया। इस अलग किए गए कवक की पहचान, इसके लक्षणों के आधार पर की गई।

मक्का मे ंरोगजनकता, प्रयोगशाला में अलगाव विधि द्वारा एवं खेत में जुडाव विधि द्वारा सिद्ध किया गया था।

मेजबान रैंज अध्ययन में, 6 पौधों की प्रजातियाँ, रोगजनक के साथ टीका किए गए थें। सभी पाँच मेजबान प्रजातियाँ (सेम, सोयाबीन, गेंहूँ, मिर्च, बैंगन) रोग के प्रति संवेदनशील नहीं दिखाई दिए परन्तु धान की प्रजाति राइजोक्टोनिया सोलानाई के प्रति संवेदनशील दिखाई दिया।

मक्का की इक्कीस प्रविष्टियों को, प्राकृतिक क्षेत्र की स्थिति के तहत राइजोक्टोनिया सोलानाई के खिलाफ मूल्याकंन किया गया। सभी प्रतिष्टियाँ, बंधी पत्ती एवं म्यान तुषार रोग के खिलाफ प्रति रक्षा और प्रतिरोधी प्रतिक्रिया व्यक्त करती हैं।

स्यूडोमोनास फ्लूरेंसेंस के विरोधी बैक्टीरियल प्रभावकारिता कीप तकनीक, जहाँ 78.66.% उच्चतम विकास निषेध प्रतिशत पी 72 राइजोक्टोनिया सोलानाई के खिलाफ अध्ययन किया गया। वहीं कम विकास निषेध प्रतिशत (0.00%) पी205, पी124, पी126, पी99, पी143, पी151, पी171, पी247 पी248 और पी216, राइजोक्टोनिया सोलानाई के खिलाफ मिला था।

सात फफूंदनाशी (केप्टान 70% + हेक्साकोनाजोल 5% डब्ल्यू.पी., प्रोपीकोनाजोल 25% ई.सी., कार्बेन्डाजिम 50% डब्ल्यू.पी., थाइफ्लूजामाइड 24% एस.सी., हेक्साकोनाजोल 5% एस.सी., मेटलेक्जाइल 72% डब्ल्यू.पी., कार्बेन्डाजिम 75% डब्ल्यू.पी. + मेन्कोजेब) तीन सांद्रता अर्थात् 10. 20 एवं 30 पी पी एम पर जहर खाद्य तकनीक द्वारा ''इनविट्रो'' में मूल्याकंन किया गया। सभी फफूंदनाशी सभी तीन सांद्रता में माइसेलिया वृद्वि को कम करने में काफी प्रभावी हैं। केप्टान 70% + हेक्साकोनाजोल 5% डब्ल्यू.पी., कार्बेन्डाजिम 75% डब्ल्यू.पी. + मेन्कोजेब एवं मेटलेक्जाइल 72% डब्ल्यू.पी. की तुलना में कार्बेन्डाजिम 50% डब्ल्यू.पी., हेक्साकोनाजोल 5% एस.सी., प्रोपीकोनाजोल 25% ई.सी., थाइफ्लूजामाइड 24% एस.सी. टीका के 9 दिनों बाद सबसे अच्छा माईसेलियस विकासनिषेध फफूंदनाशी साबित हुई।

पाँच फफूंदनाशीयो मक्का के बंधी पत्ती और म्यान तुषार के लिए 'इनविवो' की शर्तो के तहत मूल्यांकन किया गया। इसमे ंस्पष्ट रूप से पता चला है कि केप्टान 70% + हेक्साकोनाजोल 5% डब्ल्यू.पी. (24.60%), प्रोपीकोनाजोल 25% ई.सी. (22.11%), कार्बेन्डाजिम 50% डब्ल्यू.पी. (23.33%), थाइफ्लूजामाइड 24% एस.सी. (20.29%), हेक्साकोनाजोल 5% एस.सी. (22.10%) के द्वारा बंधी पत्ती एवं म्यान तुषार रोग की गंभीरता कम हो गई थी।

CHAPTER-I
INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in the world agricultural economy as food, feed and industrial products. It is a miracle C4 crop having a very high yield potential, known as queen of cereals. Maize is predominantly a kharif crop with 85 per cent of the area under cultivation in the season in India. It is one of the important cereal crops of the world in terms of area (177 M ha) production (967 M tonne) and productivity (5.5 t/ha) (FICCI,2013-14).

In India, maize is the third most important cereal crop after rice and wheat. It accounts for 9 per cent of total food grain production in the country. In India, maize is cultivated in an area of about 9.4M ha with the production of 23 M tonne and productivity of 2.5 t/ha (FICCI, 2013-14).The maize cultivated area of Chhattisgarh is estimated to be 192.30 thousand ha and productivity is 1820 Kg. /ha (Anonymous,2012-13).

Chhattisgarh state is most congenial for maize cultivation as well as for diseases also. Several diseases were reported on maize and among them seed rot and seedling blight, banded leaf and sheath blight, maydis and turcicum leaf blights are the most common for the state causing considerable economic yield losses.

One of the important diseases that became serious in recent years is banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* Kühn. The disease causes severe loss in several countries of Asia (Sharma *et al.* 2002). In India, disease mainly occurs in hot humid foothill region in Himalayas and in plains covering states of Jammu & Kashmir, Himachal Pradesh, Almora, Sikkim, Meghalaya, Assam, Nagaland Punjab, Haryana, Rajasthan, Madhya Pradesh, Delhi, Uttar Pradesh and Bihar (Payak and Sharma,1981).

The disease appears on leaves and sheaths on 40-50 days old plants and later on spread to the ears. The characteristic lesions appear as concentric bands and ring on lower leaves and sheaths (first and second). The affected plant produces large, discoloured areas alternating with irregular dark bands are typical symptoms of the disease. Severe infection leads to blotching of the leaf sheath as well as leaves. The symptoms under favourable conditions extend upto silk, glumes and

kernels. Disease generally appears at pre-flowering stage. Symptoms also appear on stalk and the internodes break at the point of infection. Sclerotia initially, are white in colour and later turn to dark brown at maturity. They are produced superficially on or near the lesions and are the primary means for the perpetuation of the fungus during off season period. Sclerotia can survive from one to several years in the soil and can also attack several weeds hosts and cause infection.

Lack of desirable and durable field resistant varieties was mainly responsible for the cultivation of high yielding but susceptible varieties to this disease in most of the areas of the country. This disease recurrence is increasing year after year, efforts are targeted for managing this disease. Presently, the disease is considered asa major disease not only in India but also in several countries of tropical Asia wherever maize is grown. The disease causes direct losses resulting in premature death of the plant, stalk breakage and ear rot besides causing indirect losses by reducing the gross yield.

This disease causes a considerable reduction of high yielding varieties. In India this disease reduce the yield from 23.9 to 31.9 %.(Singh *et al.,* 2012). Payak and Sharma (1985) reported that annually around 1% of the total grain yield is reduced by BLSB in India.

Under these circumstances timely sowing, cultivation of resistant maize cultivar, need based spray of commercially available fungicides and their compatibility use of bioagent can play a major role in reducing the losses. Therefore the present objectives of the investigations are undertaken for effective management of the disease in context of present maize cultivation scenario:

1. Collection and isolasion of *Rhizoctonia solani* from naturally infected maize plants.
2. Pathogenicity and host range studies of the *R. solani.*
3. Evaluation of maize genotypes against the *R. solani.*
4. Evaluation of bioagent and fungicides against the *R. solani* under *in vitro* and *in vivo.*

CHAPTER-II
REVIEW OF LITERATURE

The literature pertinent to the present study thesis entitled **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"** are included in this chapter.

2.1 The pathogen (*Rhizoctonia solani*)

Ogoshi (1975) reported that the *R. solani* is generally identified by characteristics of the mycelium and sclerotia as it lacks spores formation. Mycelium often is colorless at young stage, while turns to light brown as it matures. The characteristics of hyphae of *Rhizoctonia* are **a)** branching near distal septum of cells in young vegetative hyphae; **b)** formation of septum in the branch near the point of origin, **c)** construction of branch; **d)** dolipore septum; **e)** no clamp connection; **f)** no conidium; **g)** sclerotium not differentiated in rind and medulla and **h)** no rhizomorph.

Singh *et al.* (2012) found that the diameter of vegetative hyphae is 8-12 µm and is constructed at the point of branching. The mature hyphae branch at right angle and sclerotia are produced abundantly in culture and on infected plant parts. Mostly, sclerotia are 1 to 5 mm in diameter with spherical shape, and dark brown to black colour. *R. solani* survives in the soil and on infected crop debris in form of sclerotia or mycelium. Sclerotium acts as primary source of inoculum. Sclerotia are known to survive for several years in the soil. The fungi spread by irrigation, movement of contaminated soil and infected plant debris. At the onset of the growing season, in response to favourable humidity and temperatures (15 to 35°C), the fungal growth is attracted to newly planted crops by chemical stimulants released by growing plant cells. Secondary spread of this disease occurs by contact of diseased leaves or sheaths with healthy plants. Although horse shoe shaped lesions are caused by the pathogen on kernels, the kernels are not considered as source of inoculum.

Hyphae of Banded leaf and sheath spot are usually colourless when young becoming a light brown when mature. Typical hyphae width is 8 - 12 µm, with a slight constriction at the base of the lateral branch, which is typically almost 90° to

the main hyphae. Hyphae have dolipore septums, which are typically formed a short distance from the branch (Singh and Shahi 2012). The hyphae of this species also lack clamp connections. Sclerotia are grey-black in colour and 1 - 6 mm in diameter (Patra 2007). The rind and medulla of the sclerotia are not differentiated (Singh and Shahi 2012).

2.2 Disease symptoms

Saxena (2002), Patra (2007) and Singh and Shahi (2012) observed the visual symptoms usually appear between 30 and 50 days after germination. The disease causes necrotic lesions (spots and bands) that affect all aerial parts of the plant (except the tassels). Close inspection of lesions may reveal white to light brown mycelia. Small 1 - 6 mm round, black-grey coloured sclerotia may also be visible on infected surfaces.

Ahuja and Payak (1982) found that maximum damage is caused when ears are infected. In addition to ear rots, kernels are often wrinkled, dry, chaffy and light in weight. These symptoms are stalk lesions, stalk breakage, clumping and cracking of silk and horse shoe shaped lesions on caryopsis.

Saxena (1997) observed symptoms of the BLSB on all aerial parts of maize plant except tassel. Under natural conditions, disease appeared at pre flowering stage on 30 to 40 days old plants but infection can also occur on young plants which may subsequently result in severe blighting and death of apical region of growing plants.

Saxena (2002) reported that this disease appears at pre flowering stage in 40-50 days old plant. This pathogen affects maize and causes the formation of lesions and sclerotia on all aerial parts of the plant (except the tassels)

Sharma (2005) reported that high relative humidity and rain fall significantly favors development and spread of this disease. An optimum temperature about 28°C and high relative humidity (88 to 90%) in the first week of infection favor rapid disease progress. If the relative humidity goes below 70%, disease development and spread becomes slow.

Lu *et al.* (2012) observed disease develops on leaves, sheaths, and stalks and can spread to the ears. Typically, disease develops on first and second leaf

sheath above the ground as this disease is soil borne and eventually extends to the ears that ultimately lead to ear rot. When infection reaches ear, light brown cottony mycelial growth and small round mustered seed sized small round black sclerotia are observed. Premature drying and craking of ear sheath is also observed. Crop damage is caused by loss of photosynthetic leaf area due to foliar infection and stalk rot which lead to crop lodging.

Singh and Shahi (2012) reported the pathogen causes damage to the leaves, sheaths, stalks and ears of maize plants. The disease typically affects the lowest leaves first and moves up the plant reaching the sheaths and ears. Typical leaf symptoms include spotting of theleaves, reduction in leaf area, drying/death of leaves, leaf sheaths and husks. Kernels from infected plants are often wrinkled, dry and light weight.

2.2.1 Leaf and sheath symptoms

The disease causes the formation of concentric spots on the leaves and sheaths of infected plants. Over time these grow and cover larger areas of the leaf. Damage occurs in bands as the straw coloured, necrotic area progresses along the leaf. The nacrotic bands are a classic symptom of the disease (Ahuja and Payak 1982).

2.2..2 Stalk symptoms

Ahuja and Payak (1982) and Saxena (2002) observed the dark coloured stalk lesions can develop under the infected leaf sheaths. Lesions can range from 2 - 10 mm by 3 - 15 mm. Occasionally lesions girdle the stem near the nodes causing cankers, which can lead to lodging.

2.2.3 Husk, ear and kernel symptoms

Ahuja and Payak (1982) and Saxena (2002) observed the bottom of the husk is the first area affected. Once infected husks become spotted and banded lesions form. Silk can also be affected (like broken, clumped togeather, etc.).

The lesions can also develop on the ear giving infected ears a blackened appearance. Mycelia can be seen along the silk and sclerotia commonly appear on the husk.

2.3 Establishment and spreading potential of *Rhizoctonia solani*

Sharma (2005) reported that the high relative humidity and rain fall significantly favors development and spread of this disease. An optimum temperature about 28°C and high relative humidity (88 to 90%) in the first week of infection favor rapid disease progress. If the relative humidity goes below 70%, disease development and spread becomes slow. Additionally, high crop densities impact disease severity.

Singh and Shahi (2012) described the optimal environmental conditions for the development of Banded leaf and sheath blight as being 28°C with a relative humidity of 88 - 90 % during the first week of infection, development is slow when the relative humidity drops below 70 %. The requirement for high humidity levels means that the pathogen is more likely to develop in coastal areas or tropical areas of Australia rather than southern maize production areas such as the NSW Riverina.

Banded leaf and sheath blight is spread with the movement of infected soil and plant debris (Singh and Shahi, 2012; Lin *et al.*, 2008), irrigation water (Singh and Shahi, 2012) and seed (Ahuja and Payak, 1982). For shorter distances (i.e. between plants) the pathogen is spread by direct contact between infected and non-infected leaves (Singh and Shahi, 2012).

Singh and Shahi (2012) studied the pathogen is spread with seed (Ahuja and Payak, 1982) irrigation water, contaminated soil and plant material and by contact between infected and non-infected leaves.

2.4 Collection, isolation and pathogenicity of *Rhizoctonia solani.*

Ahuja and Payak (1978) proved the pathogenicity on 40 day old maize plants ofvar. BVM 5 by inserting 2 to 3 grains covered with mycelial growth of isolate, separately, between the rind and the leaf sheath of test plants in triplicate. High humidity was maintained during disease development by frequent watering. The inoculated plants were regularly observed for development of symptoms. Re-isolations were made from infected plant parts and compared with previous cultures for resemblance.

Pathogen causes losses in grain yield ranging from 11.0 to 40.0 percent (Singh and Sharma, 1976). Lal *et al.* (1985) reported that the losses in grain yield to the extent of over 90.0 percent. Although, reports on variability in the pathogen based on anastomosis behaviour, cultural and morphological appearance and pathogenicity are available (Talbot, 1970; Ogoshi 1987; Chen *et al*., 1990; Naiki and Kanoh, 1978; Wang and Hsich, 1993).

Pascual and Raymundo (1989) reported the pathogenicity of 27 maize isolates and one rice isolate of *R. solani,* collected from different geographical areas and tested on rice and bajra using leaf sheath inoculation method.

Sharma *et al.* (2004) observed the pathogen affects all the aerial plant parts except the tassel. The symptoms appeared within 4-5 days after inoculation of the maize plant. The symptoms were irregular, water-soaked, straw-coloured lesions on leaf bases and sheaths. The lesions enlarged rapidly resulting in discoloured areas alternating with dark bands, apparent on lower leaves after 7 to 8 days. Fungal growth on different media was highly variable with regards to colony diameter, colony characteristics and sclerotial production. The best growth resulted at 28± 1^{o}C (room temperature) with maximum sclerotial production followed by 30^{o}C and 25^{o}C. The mycelial growth and sclerotial production was maximum at pH 6.5 followed by 6.0 and 7.0.

Garcia *et al*. (2006) described the members of the form genus *Rhizoctonia* D.C. are considered as a complex mixture of filamentous fungi, having in common the possession of a non-spored imperfect state, usually referred to as the *Rhizoctonia* anamorph. The group includes several of the most devastating crop pathogens like *Thanatephorus cucumeris* (Frank) Donk (anamorph = *Rhizoctonia solani* Kühn), the majority of orchid mycorrhizal symbionts (mainly belonging to genus *Ceratobasidium* D.P. Rogers) and a collection of saprotrophic organisms of different systematic placement. The *Rhizoctonia* anamorph is characterized by several common features present among members of the entire *Rhizoctonia* species complex.

Akhtar *et al*. (2009) studied diversity of *R. solani* among the naturally occurring populations and revealed that banded leaf and sheath blight incited by *Rhizoctonia solani*, is showing wide spread with the disease severity ranging from

30.30 to 80.46 per cent and gaining the economic importance in the state of Jharkhand.

Madhavi *et al.* (2011) reported that a field experiment was conducted during kharif season 2008 and 2009 at the Regional Agricultural Research Station, lam farm, Acharya N G Ranga Agricultural University, Hyderabad to develop a suitable non damaging method for inoculating *Rhizoctonia solani* which causes banded leaf and sheath blight disease in maize. Results revealed that inoculation with paddy straw method is superior to other methods as well as reported techniques and can be adopted on a large scale for evaluating maize germplasm against banded leaf and sheath blight disease.

Fernando *et al*. (2015) studied that the different disease inoculation techniques for banded leaf and sheath blight disease of maize (z*ea mays* L.) in Srilanka. In this techniques all the varieties are showing same response for the inserting sclerotia to the sheath technique giving higher DSI values. When selecting a technique for the varietal screening, it should perform well in all the varieties among the techniques tested. Results revealed that technique of inserting sclerotia into sheath shows disease severities prominently without depending on the varieties. Considering used three varieties, inbred variety Ruwan showed slightly higher DSI value than other local hybrid variety Sampath and exotic hybrid variety Pacific 984 saying susceptibility of open pollinated variety Ruwan for the BLSB than used hybrid varieties. But all the tested varieties were susceptible for the BLSB.

2.5 Economic importance of banded leaf and sheath blight disease.

Singh and Sharma (1976) recorded 10-40% yield loss against BLSB. Lal *et al*. (1985) had suggested that grain yield loss can go up to an extent of 90%. In Guangxi province of China, yield losses of 87.5 and 57.8% were recorded under natural conditions in the hybrids Luyu 13 and Guiding planted at Bao Qiao and Chen Xiang (Sharma, 2005).

Lal *et al.* (1980) estimated in ten cultivars a loss in grain yield ranging from 23.9 to 31.9% in India, and observed a considerable reduction of high yielding varieties.

Buddemeyer *et al*. (2004) conducted studies to estimate the damages, caused by *R. solani* AG2-2IIIB, for different maize cultivars under German growing conditions of sugar beet-maize cropping system. They reported that shoot fresh matter and grain yield of infested plants as compared to healthy plants were reduced up to 37 and 12%, respectively.

Anonymous, (2014) reported that the banded leaf and sheath blight is considered to be a major pathogen of maize overseas, especially in Asia. This disease has been reported to cause yield losses of 23 - 97 % .

2.6 Distribution of banded leaf and sheath blight disease

Payak and Sharma (1981) reported BLSB from Himachal Pradesh, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh and Rajasthan. In recent years BLSB of maize was observed in many districts of North Karnataka. And also reported from different parts of the world (Wiltshire, 1956; Von Eignatten, 1961; Payak, 1988 and Hirel *et al.,* 1988).

Sharma *et al*. (2002) reported that BLSB caused by *R. solani* was serious in recent years. During last two decades or so far the disease had continuous devastating advance, causing epidemic out break in maize growing countries *viz.*, Bhutan, China, India, Indonesia, Philippines, Vietnam and Nepal, as well as in several countries of Africa and Latin America.

González-Vera *et al.* (2010) and Singh and Shahi (2012) observed the banded leaf and sheath blight of maize occurs in Asia, South America, parts of Africa, Europe and North America particularly in areas with warm humid conditions.

Singh *et al.* (2012) reported the disease in Germany, USA, Nigeria, Venezuela, Sierra Leone, Ivory Coast and England. In particular, BLSB is recognized as a serious impediment to maize production in China, South Asia and Southeast Asia (Sri Lanka, Indonesia, Cambodia, Bangladesh Pakistan, Nepal, Myanmar, Thailand, Laos, Vietnam, Philippines, Taiwan, Malaysia, Korea and Japan). Surprisingly, in China, yield losses close to 100% have been attributed to BLSB.

Rani *et al.* (2013) in India, the disease has been reported from states of Himachal Pradesh, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Rajasthan, West Bengal, Meghalaya, Assam and Orissa.

2.7 Host range studies of the *R. solani*.

Kozaka (1961) from Japan recorded 188 species of plants from 32 families that can be infected by this fungus. Two virulent isolates of *Thanatephorus cucumeris* could infect and survive on several weed hosts which are commonly found in rice fields namely *Echinocloa crusgalli, E. colonum, Fimbristylis littoralis, Cyperus rotandus.*

Kozaka (1965) observed that rice fungus *R. solani* infected 20 species which are from 11 families and observed that the sclerotia from diseased tissue of weed hosts produced typical symptoms of sheath blight on paddy plants.

Singh and Saksena (1980) found that aerial strain causing banded blight disease in bajra infected 22 plants species of both crop and wild plants belonging to 6 different families.

Saxena (1997) studied the pathogen has wide host range and infects plant belonging to over 32 families in 188 genera. *H. sasakii* infects by artificial inoculations a number of crop plants belonging to families Graminae, Papilionacae and Solanaceae : *Paspalum scrobiculatum, Pannisetum purpureem, Setaria italica, Panicum miliaceum, Coix lachryma–jobi, Echnochola fromentacea, Pennisetum americanum, Zeamaxicana Zea mays, Oryza sativa, Saccharum officinarum Sorghum bicolor, Arachis hypogea, Glycine max, Pisum sativum, Vigna radiate* and *Lycopersicum esculentum*. Rice and maize isolates are, however, in distinguishable on the basis of cross inoculation tests, host range, virulence, number of nuclei per hyphal cell, and other morphological characters including pathogenicity. Comparison studies of rice maize, sugarcane and sorghum isolates revealed that maize and rice are similar than those isolates of sugarcane and sorghum.

Lenka *et al.* (2014) described the host range of sheath blight disease in rice caused by *Rhizoctonia solani* Kuhn was studied in different non-paddy hosts viz. maize, wheat, jowar, bajra, ragi, sugarcane and weed hosts namely *Digitaria*

ciliaris, Dactyloctenium aegyptium, Eclipta alba, Euphorbia hirta, Scoparia dulcis, Echinochloa colonum, Aegeratum conyzoides, Cyperus rotundus, Paspalum scrobiculatum, Cynodon dactylon and Commelina benghalensis, which served as collateral hosts for the survival of the pathogen. Production of the highest lesion length was recorded in the weed host *Dactyloctenium aegyptium* while the lowest lesion length was observed in *Euphorbia hirta.*

Srinivas *et al.* (2014) described the survival of sclerotia stored under different condition. Out of fourteen plant species belonging to three families tested, the *R. solani* produced disease symptoms on all the tested plants and stating pathogen has wide host range.

Debbarma and Dutta (2015*)* studied the *Rhizoctonia solani* (teleomorph: *Thanatephorus spp.*) is a plant pathogenic fungus with a wide host range. It is best known to cause various plant diseases such as collar rot, root rot, damping- off, sheath blight, stem canker, web blight, and wire stem throughout the world. Morphological variability was studied in 6 isolates of *R. solani* having different hosts from Assam. Colony size, colony growth, colour and sclerotia formation (ring at periphery, peripheral or scattered), location (surface) and texture (smooth or rough) varied in these isolates.

2.8 Evaluation of maize genotypes against the *R. solani*.

Buddemeyer, J. *et al* (2004) screened 55 maize genotype or breeding lines for resistance to *R. solani.*

Ming, bo *et al*. (2007) studied on advances of research on the disease symptom, pathogens, occurrence rule, penetration paths, damage, preventive treatment and inheritance of resistance to Banded Leaf and Sheath Blight in maize. Works on Marker-assisted Selection (MAS) for Banded Leaf and Sheath Blight in maize were also summarized.

Li *et al.* (2009) observed the two inbred lines R15 and Ye 478 were grown in field plots in 2007 in Ya'an, Sichuan, China, to investigate the functional effects of different defense enzymes on the banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* of maize. The leaves and sheaths at joining stage were inoculated and un-inoculated (as the control) with a *R. solani* strain AG1-IA, and

the inoculated and un-inoculated leaves and sheathes were sampled 12, 24, 36, 48 and 60 h after inoculation, to determine the activities of some defense enzymes, i.e., peroxidase (POD), phenylalanine ammonia-lyase (PAL), ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT). It is concluded that the activities of POD and CAT in the leaves and sheaths of maize were positively correlated with the disease resistance of maize, and these defense enzymes functioning in the different parts and different growth stages of plant coordinate with each other, thus jointly accomplishing the defense reaction of plant to disease.

Akhtar *et al.* (2011) found the possible role of the phenolics involved in resistance against banded leaf and sheath blight of maize. The phenolic content in all cultivars of maize increased after infection. This increase was more pronounced in resistant cultivars as compared to susceptible cultivars. Further analysis of the data revealed that the disease severity was negatively correlated with the accumulation of phenol having coefficient of correlation r=-0.83.

Bhavana *et al*. (2011) screened for resistance to banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* under artificial epiphytotic condition. Found resistant to BLSB, indicating paucity of resistant genotypes or higher virulence of the pathogen or both. Lines showing intermediate disease reaction of 2.5-3.0 score remained healthy till maturity.

Madhavi *et al*. (2012) studied in sixteen maize genotypes including five popular varieties and twelve inbred lines at Maize research centre. Out of 12 inbred lines, BH11 showed moderate resistance to the disease.

Chen *et al.* (2013) studied on the *Rhizoctonia solani* causal agent of banded leaf and sheath blight (BLSB), is widely distributed in the South China and Southeast Asian in maize causing severe yield losses. In this study, 282 maize inbred lines were identified as resistance to BLSB. The results showed that no immune and highly resistant germplasm was found and four moderately resistant inbred lines were identified. These four moderately resistant inbreds had good performance in grain yield, combining ability and a suitable growth period.

Izhar and Chakraborty (2013) studied on Line × tester analysis involving 12 inbred lines and 5 inbred testers to evaluate the genetics of resistance against banded leaf and sheath blight in maize incited by *Rhizoctonia solani*. Out of

seventeen inbreds including five testers, three lines were resistant, twelve lines were moderately resistant and two lines were moderately susceptible. Both additive and dominance components were important in the inheritance of this disease with the predominant role of additive gene action. The inbreds, BAUIM-3, BQPM-2 and BQPM-4 were good general combiners for disease resistance as well as yield.

Madhavi *et al*. (2013) evaluated sixteen maize genotypes including five popular varieties and twelve inbred lines at Maize Research Centre, Agricultural Research Institute, Rajendra nagar during the period *rabi* 2010-11 and *kharif* 2011-12 for resistance against banded leaf and sheath blight disease under artificial inoculation conditions. None of the genotypes were found tolerant. However, of the popular varieties tested, Pinnacle was found to be moderately resistance while BPCH-44 was most susceptible. Out of 12 inbred lines, BH11 showed moderate resistance to the disease and it is suggested for breeding work for incorporating resistance into the popular high yielding varieties.

2.9 Evaluation of bioagent and fungicides against the *R. solani* under *in vitro* and *in vivo.*

Saxena (2002) tested efficacy of chemicals (*viz*, Propiconazole, 0.1%, and Carbendazim, 0.05%), by applying as foliar sprays at 30, 40 and 50th day of planting, alone or in combinations. Effectiveness of Propiconazole was markedly observed when the chemical was applied at initial stages at 30th or 40th day after planting and the second spray at 10 days after first. Foliar sprays of Carbendazim showed the ineffectiveness against BLSB.

Sharma *et al.* (2002) studied on *in vitro* evaluation, three often used fungicides, namely Bavistin, Rhizolex, and Thiophenate M, have shown absolute control of mycelial growth with 100% inhibition. It is, therefore, envisaged that under field conditions a high level of control of BLSB could be achieved using these three fungicides.

Meena *et al.* (2003) observed reduction in disease incident of BLSB when *P. fluorescens* was used in seed and soil treatment and in foliar application.

Muisaand and Quimio (2006) suppressed *Rhizoctonia solani* in microplots and increased grain yield by 27% in comparison to control, when used as seed

treatment. *B. subtilis* BR23 has a potential for commercialization as a seed treatment for the control of banded leaf and sheath blight disease (*R. solani*) in corn.

Sivakumar and Sharma (2007) studied soil application of *P. fluorescens* along with seed treatment resulted in further increase in rhizosphere population of the bacterium in glass house and field conditions. Though the seed treatment alone was quite effective in minimizing disease incidence, the application of the antagonist in combination gave a significant control of the disease in glass house and field conditions. The yield was also increased considerably due to the bacterial treatment.

Muis (2007) reported the banded leaf and sheath blight disease caused by *R. solani* in maize has become increasingly severe and economically threatened maize plants in several countries of Asia and other parts of the world and described the most of control measures like quarantine, farming practice, resistant cultivars, chemical and biological *etc*. have successfully controlled BLSB caused by *R. solani*.

Madhavi *et al.* (2011) studies on the management of banded leaf and sheath blight disease of maize (*Rhizoctonia solani*) using *fluorescent Pseudomonads.* All the *Pseudomonads* have significantly inhibited the mycelial growth and sclerotial germination of *R. solani* ranging from 48%-92% and 29%-87% respectively over controls.

Bunker *et al*. (2012) managed the disease by carbendazim, neem oil and *Trichoderma harzianum* as seed treatment and also as seed treatment plus spray in various combinations in field. Use of neem oil as seed treatment and spray could be a cost effective and eco-friendly strategy in managing the BLSB.

Divya *et al*. (2013) studied on management of maize banded leaf and sheath blight with fungicides and biocontrol agents observed in seed treatment with carbendazim and *Trichoderma viride* recorded lowest disease severity, while the lowest per cent disease incidence was observed in seed treatment with carbendazim and thiram.

Rajput (2013) studied on fungicidal evaluation at various concentration, indicated Propiconazole 25% EC and Carbendazim 50% WP were found most

effective in inhibiting the growth of the fungus. The plant extracts *viz*., nimbicidine and NSKE at 5 and 10 per cent concentrations were effective against the pathogen. Among the biocontrol agents evaluated, *Trichoderma harzianum* found effective against the pathogen. Field studies on the management of the disease revealed that, seed treatment with *Pseudomonas fluorescens*@10g kg-1 seed followed by two sprays of Propiconazole 25 EC @ 0.1% at 30 and 40 DAS found most effective treatment and resulted in lowest PDI (20.40%). This treatment increased grain yield (40.72%) and fodder yield (44.68%) over untreated check.

Rani *et al*. (2013) tested the fungicides and bio-control agents viz., benomyl, carbendazim, thiram, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* as seed and soil treatment. The lowest per cent disease incidence was observed in seed treatment with carbendazim and thiram with disease incidence 27.11% and 29.92% respectively.

CHAPTER-III
MATERIALS AND METHODS

The present study entitled **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"** was conducted during *Kharif* season of 2014-15 at Maize crop Research Area, IGKV., Raipur (C.G.). All the laboratory work was carried out in the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

In the present investigations all the material and methods followed are given below:

3.1 Source of materials

All the glassware, chemical, *viz.* streptomycin, alcohol, $HgCl_2$, different fungicides, blotting paper and other materials were obtained from Department of Plant Pathology, College of Agriculture, IGKV, Raipur.

3.2 Instrument used

The following instruments were used in the present study:

1. Autoclave for sterilization
2. BOD incubator for incubation of pathogen
3. Compound microscope for identification of pathogen
4. Hot air oven for sterilization of glassware's
5. Laminar Air Flow for isolation, purification and inoculation of pathogen
6. Anamed Electronic Digital Balance for weighing
7. Forceps, Needles, Blades, Cork borer and Inoculation needle
8. Spirit lamp for sterilization
9. Microwave oven for melting of media

3.3 Cleaning and sterilization of materials

Whenever required, the glassware's were cleaned with detergent powder and washed with tap and /or distilled water as per requirement of the experiment. The

Dried glassware's were sterilized in hot air oven at 180°C for 2 to 3 hours. The forceps, inoculation needle and other metallic instruments were sterilized by dipping in alcohol and heating over the flame before using them. Sterilization of media was done by autoclaving at 1.41 kg pressure for 20 minutes. The Plastic plates were sterilized with ethyl alcohol surface sterilization and air dried before use.

3.4 Media used

Potato Dextrose Agar (Riker and Riker, 19536) with the following composition was used.

S.No.	Ingredient		Content
1.	Distilled water	-	1000 ml
2.	Peeled potato	-	200 g
3.	Agar	-	20g
4.	Dextrose	-	20 g

3.5 Experimental site

All the *in vitro* studies on *R. solani* Kühn were conducted in laboratory of the Department of Plant Pathology, IGKV, Raipur (C.G.) and the *in vivo* studies was carried out at Maize Crop Research Area, IGKV., Raipur (C.G.)

3.6 Test varieties/entries

In general the variety NK-30 was used in the studies. The variety was grown in direct shown condition as per the requirement.

3.7 Collection and isolation of banded leaf and sheath blight pathogen

3.7.1 Collection of disease sample

Maize plants showing naturally infected symptoms of banded leaf and sheath blight, was collected from the research farm of Indira Gandhi Agricultural University and from the farmers field. The diseased specimen were kept for isolation in air tight polythene bags.

3.7.2 Isolation of the fungus

Collected samples were brought to laboratory for critical examination i.e. isolation, identification and description of the pathogen, the samples were examined under compound microscope. The diseased samples were washed thoroughly with tap water. Small pieces of infected parts containing healthy as well as diseased tissues were cut with the help of sterilized scalpel blade. These pieces were surface sterilized with 0.1% mercuric chloride solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical, dried by placing in between the two sterile blotters and finally the pieces were than transferred aseptically to petridishes containing PDA and were incubated at 27±1^{o}C. The petri-plates were examined at regular intervals for fungal growth radiating from the infected pieces. Mycelial growth of suspected *R. solani* were transferred to PDA slants and maintained the pathogen.

3.7.3 Mass inoculum of *R. solani.*

Mass inoculum of *R. solani* was prepared on Sand Maize Medium. SMM was prepared by mixing maize grain flour, sand and distilled water in 2:1:1 proportion in transparent polythene bags (5 × 9 inch) and sterilized at 15 psi for one hour followed by inoculation with 5 mm discs from the periphery of culture of the test fungus and incubated in incubator at 27± 1^{o}C temperature for 15 days.

3.8 Pathogenicity test of banded leaf and sheath blight pathogen, *Rhizoctonia solani.*

Pathogenicity of the fungus was tested in field condition and laboratory condition with the help of dessicators by following methods.

3.8.1 Laboratory condition

The healthy seedlings collected from field and selected the healthy leaves and sheaths for inoculation. Leaves and sheaths were surface sterilized with 0.1% mercuric chloride for one minute followed by three washing with sterilized water, after sterilization these healthy leaves and sheaths were kept in big size dessicators and

inoculated the mycelium bits of test fungus on leaves and sheaths. After inoculation dessicators was sealed. Symptoms were observed after two days of inoculation.

3.8.2 Field condition

The infection assays were conducted at the field on maize variety NK - 30 which were raised in field during July, 2014. Field isolate was multiplied on PDA at 27± 1^{o} C in petri dish till the formation of sclerotia. Representative *Rhizoctonia solani* isolates were tested for pathogenicity on maize. In 40 – 45day old healthy maize plant, a hyphal disk (5 mm diameter), cut from the margin of an actively growing colony of *Rhizoctonia solani* isolate, was transferred to the inner side of the upper nodal sheath of maize plants and non-inoculated plants served as control. The surface of the inoculated area was covered with a piece of water-soaked cotton to retain moisture. After 2-3 days, symptoms and lesion length on plants were recorded after full development of the lesions.

3. 9 Host range studies of the *R. solani*.

In vitro an experiment was conducted to know the host range studies of the *R. solani*. In this experiment six different crops (soyabean, rice, wheat, bean, brinjal, chilli and maize) were evaluated in glass house shade for host susceptibility under artificial inoculated condition. Seeds of all the crops were collected from IGKV, research farm, Raipur. The pots were filled with sterilized soil and mixed with the culture, The field soil was sterilized with one per cent formaldehyde solution and left for 10 days to remove chemical residue. The sterilized soil was filled in 22.5 cm diameter earthen pots. About 30 g of fungal inoculum from one bag of each isolates was added /pot separately in one set of pot filled with soil. The inoculated pots were left for one day. Different host crops seeds were treated with Bavistin (Carbendazim 50%WP). Ten seeds of the different host crops were sown/pot, watered daily and kept under observation.

In another set, plants were raised in pots filled with a sterilized soil. 15-20 days old plants were inoculated with the 3-7 days old culture of pathogen, the plugs of PDA with sclerotia were inserted to the leaf sheath near the water line. Three replications

were maintained for individual treatment and the disease symptoms was recorded from each host plant.

3. 10 Screening of hybrid maize against banded leaf and sheath blight of maize under natural field conditions

To find out the source of resistance an experiment was conducted during *kharif* season 2014 in the field of Genetics and Plant Breeding department IGKV, Raipur. The twenty one hybrid maize entries were shown in a direct sowing with a spacing of 60 cm row to row. The observations were taken up to 30 days after planting at interval for natural incidence.

Each entries, plants were observed. There were in total 21 entries assessed for their disease development, on the basis of percentage of incidence and severity of disease. Similarly, the percent disease incidence of banded leaf and sheath blight was calculated by using formula :

$$\text{Percent disease incidence} = \frac{\text{Total number of plant infected}}{\text{Total number of plant examined}} \times 100$$

The percent disease index was calculated and varietal performance rating was done as given below:

Scale

0 - No disease

1.0- Disease on the leaf sheath only; few small, non coalescent lesions are present.

1.5- Disease on two sheaths: lesions large and coalescent.

2.0- Disease up to four sheaths: lesions many and always coalescent.

2.5- As in 2.0, plus rind discolored with small lesions.

3.0- Disease on all sheaths except two internodes below the ear.

3.5- Disease upto one internode below the ear sheath; rind discoloration on many internodes with large depressed lesions.

4.0- Disease up to the internode bearing ear sheath but shank not affected.

4.5- Disease on the ear; husk leaves show bleaching, bands and caking among themselves as also of silk fibers; abundant fungal growth between and on kernel rows; kernel formation except being lusterless; ear size less than normal; some

plants prematurely dead.

5.0- In addition to 4.5, shrinkage of stalk; reduced ear dimensions; wet rot and Disorganization of ear; kernel formation absent or rudimentary; premature dead plants common; abundant sclerotial production on husk leaves, kernels, ear tips and silk.

Percent Disease intensity (PDI) was calculated by using the formula:

$$\text{Percent Disease intensity (PDI)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no.of plants asses sed}} \times \frac{100}{\text{maximum rating}}$$

Disease score given by Anshu *et al.,* (2007)

Disease Score	Reaction
0	HighlyResistant
1-30	Resistant
>30-60	Moderately resistant
>60-90	Susceptible
>90-100	Highly Susceptible

3.11 Identification of potential candidate isolate of *Pseudomonas fluorescence* ffective against *Rhizoctonia solani*.

The experimental material consisted of twenty three isolates of *Pseudomonas fluorescens* kindly provided by Dr. A. S. Kotasthane, Professor & Head, Department of Plant Pathology, College of Agriculture, IGKV, Raipur. These isolates were evaluated under *in vitro* condition against *R. solani* by implying funnel technique.

3.11.1 Procedure for funnel technique

In laboratory, funnel is commonly used to separate solids from liquids, liquids from liquids and occasionally for pouring something into a container. Our present investigation suggests a simple technique where funnel can be used to inoculate bioagent (liquid/sporulting bioagent) for confrontation assays developed by Dr. A. S.

Kotasthane personal communication (Under publication). Funnel (Borosil make, Diameter 75 mm, Plain, 60º Angle Stem) of different diameters are available and can be used as per the requirement and size of the Petridish. Edges of the glass funnel were sterilized by dipping in alcohol and flaming. Broth containing young growing cell of *Pseudomonas fluorescens* were dispensed in a sterile Petri dish (For each isolate of *Pseudomonas fluorescens* a sterilized container is required). Cool sterilized edge of the funnel was then dipped in the broth culture containing young growing cells of *Pseudomonas fluorescens*. Care was taken to remove the excess inoculums by gently shaking the dipped funnel. Plates were inoculated in the center with agar plugs (9 mm dia.) containing young growing mycelium of pathogenic fungi *R. solani* was further used for confrontation assays. Precaution was taken to keep the plugs (containing growth of pathogenic fungus) in the center. Petri-plates pre-inoculated with plant pathogenic fungi were then inoculated by the *Pseudomonas fluorescens* isolate by touching / stamping the edge of the funnel on the surface of the solid media (sterilized potato dextrose agar (PDA). Keeping the narrow stem of the funnel vertically positioned on the agar plug helped us to stamp the inoculum (present on the edge of the funnel) uniformly surrounding the plugs (containing growth of pathogenic fungus). Narrow stem of the funnel also eased the handling of funnel in all the inoculation steps. Touching/stamping with the edge of funnel (containing bacterial inoculum) uniformly transfer the bacterial inoculum surrounding the plugs (containing mycelial growth of pathogenic fungus). The plates were incubated at 27 ± 1°C. The mycelial growth of the pathogen and inhibition zone was measured after 3, 5 and 7 days of incubation. Evaluation of fungal antagonism is performed on petri dish bioassays.

Table: List of *Pseudomonas fluorescens* isolates evaluated against *Rhizoctonia solani.*

S.No.	Isolate	S.No.	Isolate	S.No.	Isolate	S.No.	Isolate
1.	P5	7.	P85	13.	P143	19.	P201
2.	P6	8.	P99	14.	P151	20.	P205
3.	P11	9.	P124	15.	P161	21.	P216
4.	P248	10.	P126	16.	P167	22.	P233
5.	P72	11.	P129	17.	P176	23.	P247
6.	P76	12.	P141	18.	P179		

3.12 Efficacy of fungicides against *Rhizoctonia solani* under *in vitro* condition.

Seven fungicide *viz*. Carbendazim 50% WP, Captan 70% + Hexaconazole 5% WP, Hexaconazole 5% SC, Thifluzamide 24% SC, Propiconazole 25% EC, Metalaxyl 72% WP, Carbendazim + Mancozeb 75%WP were used to evaluate the efficacy against *Rhizoctonia solani*. Three concentrations *i.e.,* , 10, 20 and 30 ppm for each treatment were used in poisoned food technique. The inhibitory effect of all fungicides generally increased with increase in concentration.

Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar so as to get the desired concentration of fungicide. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 90 mm size from actively growing culture of the fungus were cut by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated three. Then such plates were incubated at 27 ± 1°C in BOD incubator and observation was recorded at five, seven and nine days after inoculation for radial growth.

The efficacy of a fungicide was expressed as per inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

Table 3 : List of Fungicides evaluated against *Rhizoctonia solani.*

Sr. No.	Chemical name	Trade name	Formulation (%)	Dose (ppm)
1	Captan +Hexaconazole	Taqat	75 % WP	10,20 and 30
2	Hexaconazole	Contaf Plus	5% SC	10,20 and 30
3	Thifluzamide	Pulsor	24% SC	10,20 and 30
4	Propiconazole	Tilt	25%EC	10,20 and 30
5	Carbendazim	Bavistin	50%WP	10,20 and 30
6	Metalaxyl	Ridomil	72%WP	10,20 and 30
7	Carbendazim + Mancozeb	Saaf	75%WP	10,20 and 30

3.13 Efficacy and evaluation of fungicides and bioagent with different formulations against the *R. solani* under field conditions.

The experiment was laid in Randomized Block Design (RBD) with three replications. The test variety was "NK-30". Five fungicide *viz*. Carbendazim 50% WP, Captan 70% + Hexaconazole 5% WP, Hexaconazole 5% SC, Thifluzamide 24% SC, Propiconazole 25% EC and one bioagent *Pseudomonas fluorescens* (P167*)* were used to evaluate the efficacy against banded leaf and sheath blight disease. Field isolate was multiplied on PDA at 27 ± 1^{o} C in petri dish till the formation of sclerotia. Then each plot was artificially inoculated with *Rhizoctonia solani* by inserting sclerotia under leaf sheath of test plants and un-inoculated plants served as control. First sprays of

fungicides were done just at the appearance of the disease. Second and third sprays of fungicides were done at 10th day after the first and second spray respectively. Observations for the disease development were taken at every 10th day intervals after each spray. The different fungicide at recommended concentration in water Carbendazim 50% WP, Captan 70% + Hexaconazole 5% WP, Hexaconazole 5% SC, Thifluzamide 24% SC, Propiconazole 25% EC and *Pseudomonas fluorescens* (P167*)* was sprayed with the help of hand sprayer.

Table 3 : Composition of Fungicide and Bioagent.

Sr.No.	Treatment	Dosage/ litre of water
1	Taqat (Captan 70% +Hexaconazole 5% WP)	2.0 gm
2	Contaf Plus (Hexaconazole 5% SC)	1.0 ml
3	Pulsor (Thifluzamide 24% SC)	1.0 ml
4	Tilt (Propiconazole 25%EC)	1.0 ml
5	Bavistin (Carbendazim 50%WP)	2.0 gm
6	*Pseudomonas fluorescens* (P167*)*	1.0 ml
7	Check	Untreated

At random, plants in each plot were assessed for disease severity by measuring the total lesion length and total sheath length.

Experimental Details:

Design	-	RBD
Replication	-	3
Treatment	-	7
Variety	-	NK-30
Date of sowing	-	4 July 2014
Plot Size	-	30×10 m
Plant spacing	-	60×20 cm
No. of spray	-	Three
Interval of spray	-	10 days

Observation Details:

- Observation on disease severity of disease were recorded and calculated by using following formula :

$$\text{Disease severity}(\%) = \frac{\text{Total lesion length}}{\text{Total sheath length}} \times 100$$

Percent increase or decrease in disease severity over control was calculated as per the formula described by Vincent (1947).

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

Where,

I or D = % increase or decrease over control

C = % disease severity in control

T = % disease severity in treatment

CHAPTER -IV
RESULTS AND DISCUSSION

This chapter deals with the experimental results obtained during the course of investigation on **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"**

The results were statistically analyzed wherever required by using the analysis of variance technique and the findings are given below. The results of the experiments have been thoroughly discussed and corroborated in the light of research work done by various workers earlier.

During the present investigation a field observations were recorded to gather information on the occurrence of diseases of maize in Maize Crop Research Area, IGKV., Raipur (C.G.). Laboratory studies on isolation, pathogenicity, symptomatology and evaluation of fungicides and bioagents against the pathogen under *in-vitro* condition was carried out. Further, field experiment was layed out to study the maize banded leaf and sheath blight fungicidal management. The results thus obtained are presented in different sections under this chapter.

4.1. Symptomatology.

- The disease symptoms were observed on leaves, sheaths and the ears.
- Initial symptoms were observed on the first and second leaf sheath above the ground and eventually spreaded on the ear causing ear rot .
- Infected leaves and sheaths were brown in colour and showed water soaked concentric bands.
- Whole plant was blighted within a weeks.
- White mycelial fungal growth was seen under surface of infected leaves and sheaths and young branches.
- Sclerotia were observed on severely blighted leaves, sheaths and the ears.

Similar symptoms of banded leaf and sheath blight disease were also observed by Saxena (2002), Patra (2007) and Ahuja and Payak (1982) were also observed the ear roting on the ear. Lu *et al.*

(2012) recorded brown cottony mycelial growth and small round mustard like black sclerotia.

4. 2 Isolation and purification of pathogen.

The infected plants showed typical banded leaf and sheath blight disease symptoms were collected from the field for isolation of the pathogen in the laboratory. The pathogen was isolated from infected plant parts. The culture were purified by single hyphal tip method and were maintained on PDA at 27 ± 1^{o} C in BOD incubator. The isolated fungi were identified on the basis of following morphological characteristics.

The genus *Rhizoctonia solani* belongs to Form Class Deuteromycetes that does not make vegetative spores and present as mycelium and sclerotia. It produces shade of vegetative hypha and constriction at the point of branching and right angle branching in matured hyphae. The mature hyphae branch at right angle and sclerotia are produced abundantly in culture and on infected plant. The isolate shared typical characteristics of *R. solani* like **a)** branching near distal septum of cells in young vegetative hyphae; **b)** formation of septum in the branch near the point of origin, **c)** construction of branch; **d)** dolipore septum; **e)** no clamp connection; **f)** no conidium; **g)** sclerotium not differentiated in rind and medulla and **h)** no rhizomorph. Mostly, sclerotia are 1 to 5 mm in diameter with spherical shape, and dark brown to black colour (Singh *et al.,* 2012).

4. 3 Pathogenicity test

Pathogenicity test was performed in laboratory conditions by detached method and field conditions by attached method with the isolate derived from naturally infected plants. In laboratory condition, the healthy maize plant parts were kept in big size desiccators and inoculated the mycelium bits of test fungus on leaves and sheath and the symptoms were observed after 2 days of inoculation. Initial symptoms was started as small lesions (0.3-0.4cm), discoloured and after 5 days of inoculation symptoms were water soaked, discoloured, brown in colour. After 9 days of inoculation white mycelial fungal growth was seen under surface of leaves and sheaths. Sclerotia were observed on severely blighted leaves and sheaths at 12 days after inoculation.

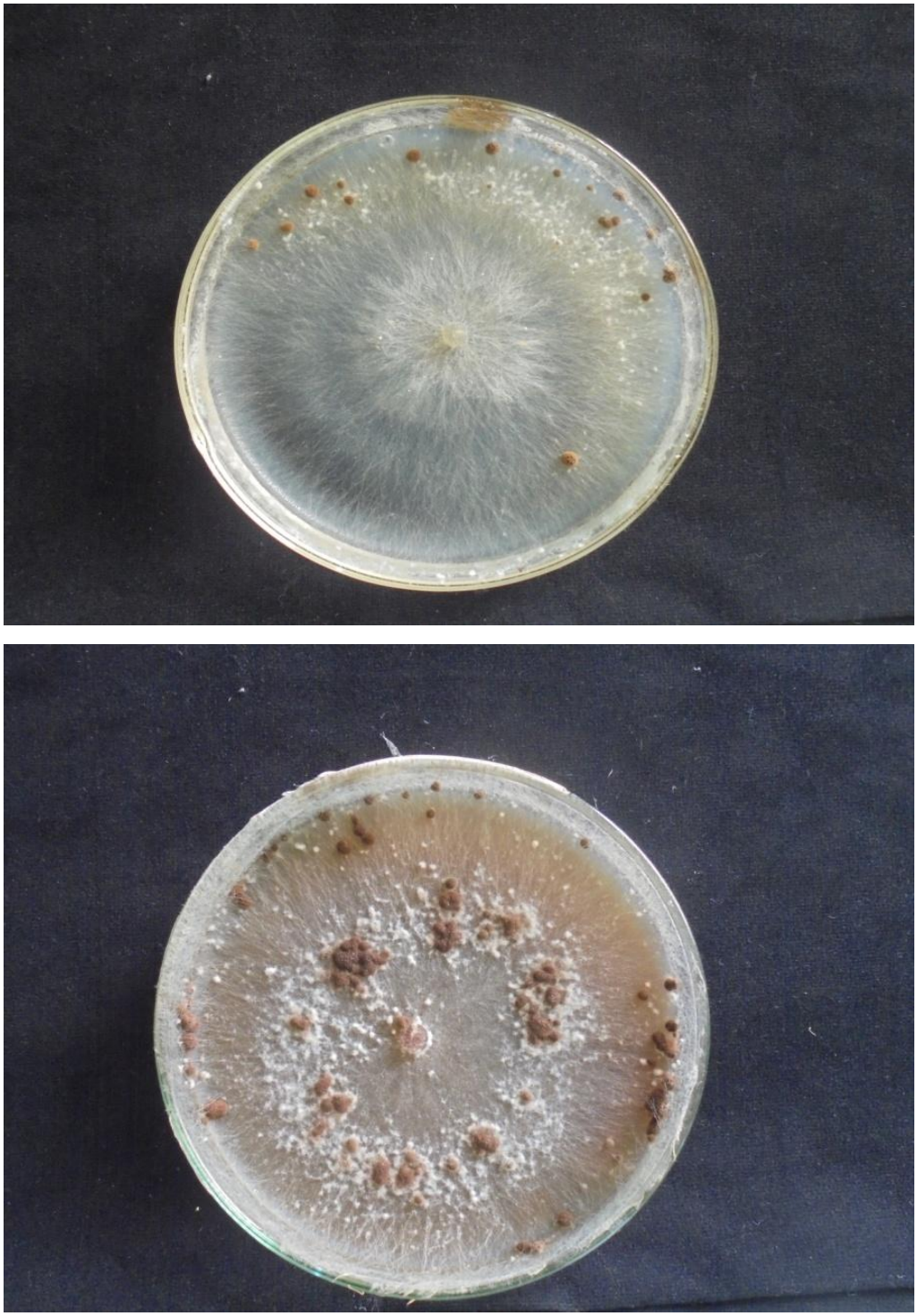
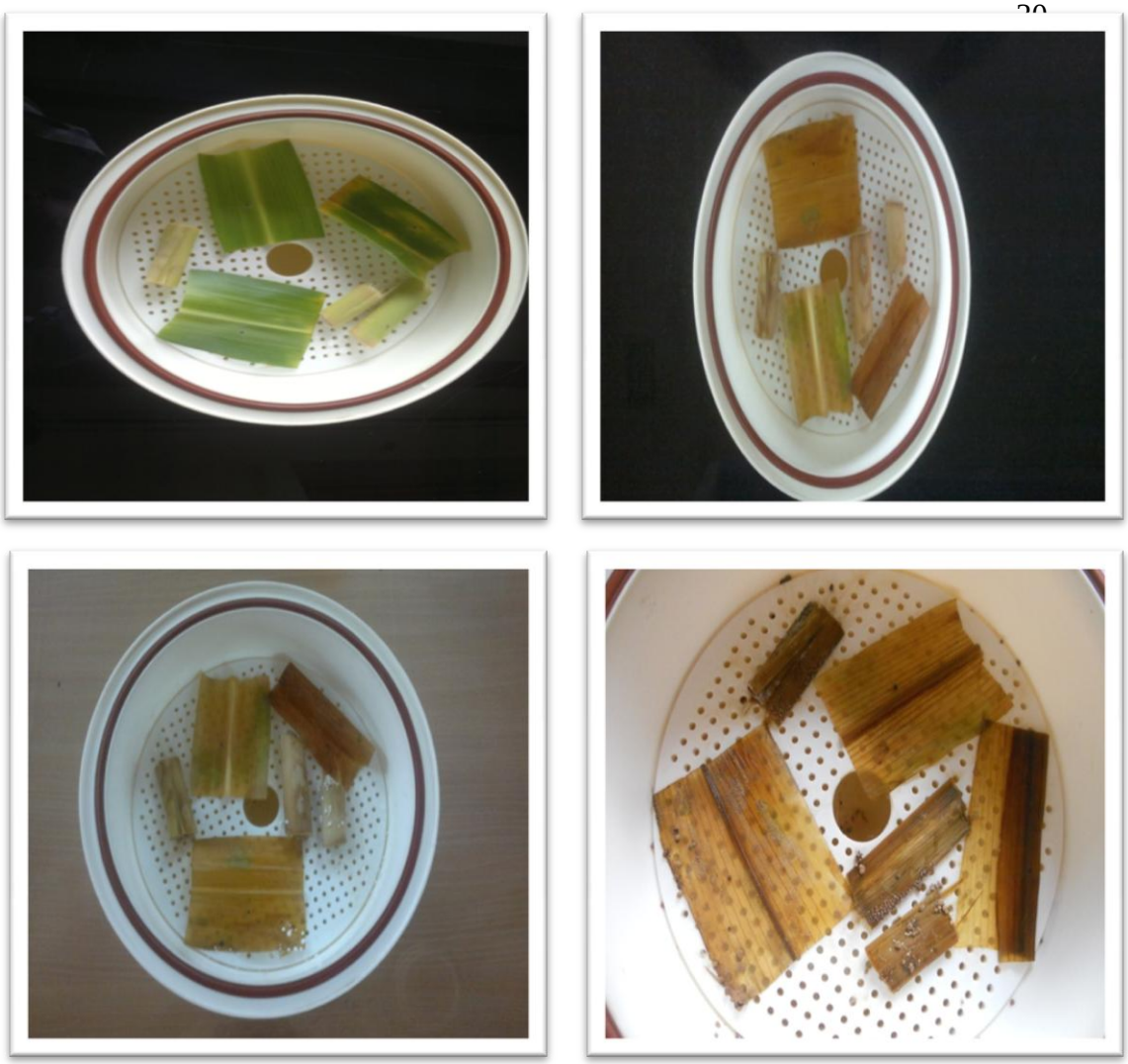


Plate 4.1 – Mycelium growth of *Rhizoctonia solani*.



(A)

(B)

Plate 4.2-(A) Pathogenicity test of *R. solani* by leaf and sheath inoculation in dessicator. **(B)** Pathogenicity test of *R. solani* by leaf and sheath inoculation under natural field condition**.**

In field condition, the healthy seedlings of variety NK-30 was selected for inoculation. The isolate was multiplied in PDA. The sclerotia produced in PDA was inoculated in healthy sheath of maize (NK-30) and the symptoms were observed after three days of inoculation. Initial symptoms was started as water-soaked, straw-coloured lesions on leaf and sheath which enlarged gradually day by day however, lesion become enlarged and discoloured areas alternating with dark bands in 7 days after inoculation. Lesion on leaf sheath was expanded from single pinpoint to entire plant parts.

Under artificial inoculation conditions the banded leaf and sheath blight pathogen showed their pathogenic ability and produced typical banded leaf and sheath blight symptoms. Fernando *et al*. (2015) confirmed the pathogenicity under field condition by inserting the sclerotia into the sheath and found well among the techniques tested. While Madhavi *et al.* (2011) found better paddy straw method in comparison to other.

4. 4 Host range study of *Rhizoctonia solani*.

All the screened host plants bean, soyabean, wheat, chilli, brinjal not showed host susceptibility within 3 days of inoculation of test fungus *R.solani* but rice crop showed susceptibility against *R.solani*. In susceptible host, small lesion appear, again small lesions appeared after 3 days then enlarged the lesion in big size after 12 days of inoculation. Some finding also confirmed by Yang *et al.* (2008), they carried out extensive study on the frequency and pathogenicity distribution of *Rhizoctonia* spp. causing sheath blight on rice and banded leaf disease on maize.

Table 4.1 Host range studies of the *R. solani*.

		Disease Symptoms		
Sr.No.	**Host Crop**	**3 DAI**	**7 DAI**	**12 DAI**
1	Bean	No Disease	No Disease	No Disease
2	Soyabean	No Disease	No Disease	No Disease
3	Wheat	No Disease	No Disease	No Disease
4	Rice	Small Lesion	Small Lesion	Large Lesion

5	Brinjal	No disease	No disease	No disease
6	Chilli	No disease	No disease	No disease
7	Maize	Small lesion	Small lesion	Large lesion

DAI- Days After Inoculation

4. 5 Screening of hybrid maize against banded leaf and sheath blight of maize under natural field conditions.

The data presented in table 4.5 showed that out of 21 hybrid maize entries, 15 entries ie. 2682, 26824, 2685, 2686, 2688, 2690, 2691, 2692, 2695, 2696, 2697, 2698, 2699, 2700 and 2701, showed highly resistant reactions against the banded leaf and sheath blight disease under natural field conditions. Remaining entries were exhibited resistant reaction against the disease. The highest disease incidence per cent was observed at all entries by 2689 (4.87%) followed by 2683 (2.63%) under natural field conditions.

Under natural field conditions 15 hybrid maize entries were found to be free from banded leaf and sheath blight disease and six entries were showed resistant reaction. Bhavana *et al*. (2011) were screened maize genotypes against banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* under artificial epiphytotic condition. Similarly Madhavi *et al*. (2013) also evaluated a sixteen maize genotypes including five popular varieties and recorded BH11 as moderate resistance to the disease.

Table 4.2 Screening of hybrid maize against banded leaf and sheath blight of maize under natural field conditions.

Sr. No.	Entries	Total Plant	Infected Plant	% Disease incidence	Percent Disease Index (%)	Reaction
1.	2681	78	1	1.28	1.15	Resistant
2.	2682	75	0	0	0	Highly Resistant
3.	2683	76	2	2.63	1.18	Resistant
4.	2684	72	0	0	0	Highly Resistant
5.	2685	77	0	0	0	Highly Resistant
6.	2686	69	0	0	0	Highly Resistant
7.	2687	81	1	1.23	1.12	Resistant

Bean

Soyabean

Chilli

Rice

Brinjal

Wheat

Maize

Plate 4.3 – Different host range studies of the *R. solani*.

Plate 4.6 - Field view of screenings of hybrid maize trial against banded leaf and sheath blight diseases

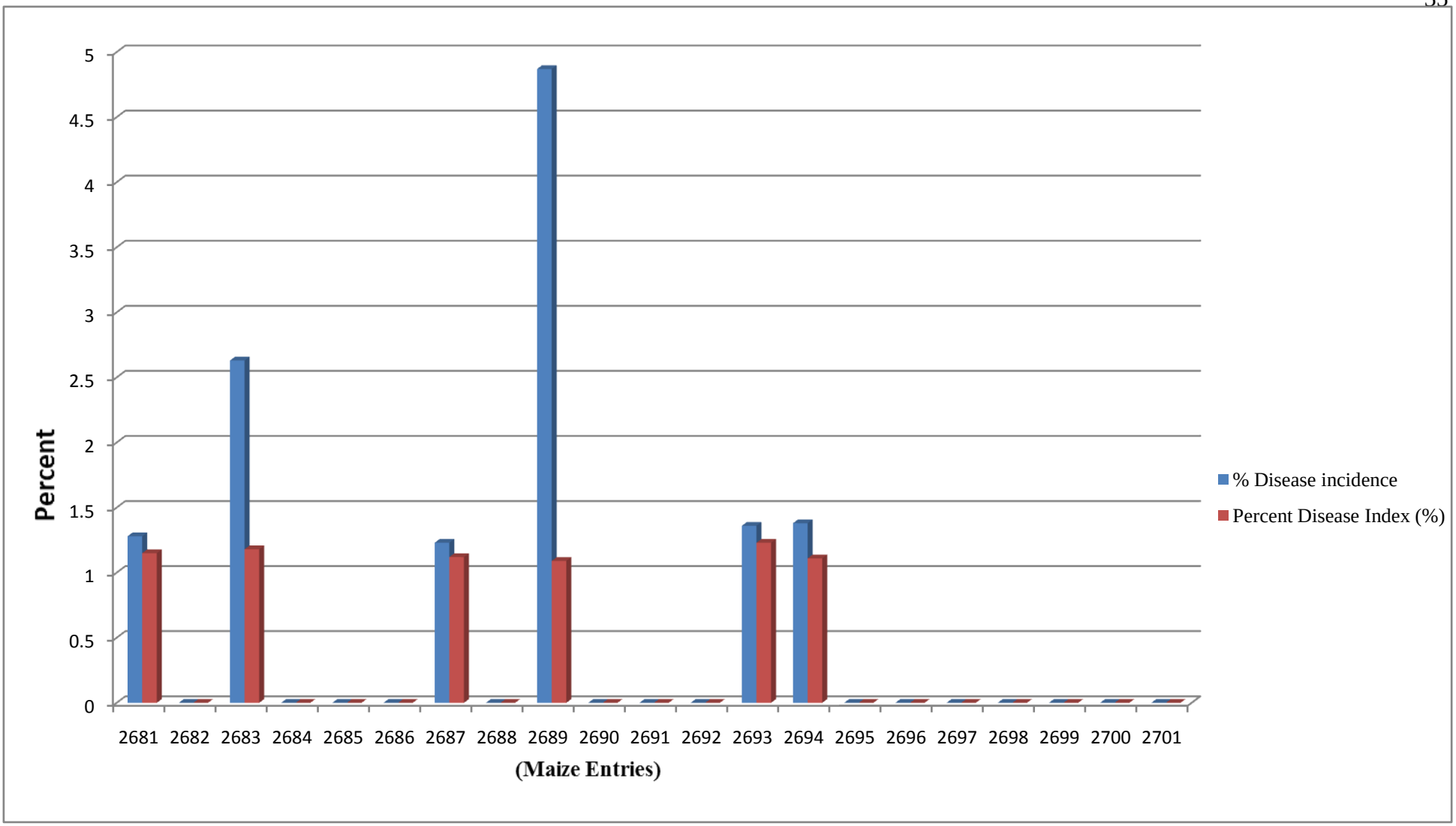


Fig.:4.1 Screening of hybrid maize against banded leaf and sheath blight of maize under natural field conditions.

8.	2688	83	0	0.00	0.00	highly resistant
9.	2689	82	4	4.87	1.09	Resistant
10.	2690	79	0	0.00	0.00	highly resistant
11.	2691	84	0	0.00	0.00	highly resistant
12.	2692	58	0	0.00	0.00	highly resistant
13.	2693	73	1	1.36	1.23	Resistant
14.	2694	72	1	1.38	1.11	Resistant
15.	2695	84	0	0.00	0.00	highly resistant
16.	2696	83	0	0.00	0.00	highly resistant
17.	2697	68	0	0.00	0.00	highly resistant
18.	2698	55	0	0.00	0.00	highly resistant
19.	2699	76	0	0.00	0.00	highly resistant
20.	2700	75	0	0.00	0.00	highly resistant
21.	2701	75	0	0.00	0.00	highly resistant

4.6 Evaluation of antagonistic *Pseudomonas fluorescens isolates* against *R. solani* under *in vitro* condition.

Pseudomonas fluorescens were evaluated against the pathogen *R. solani* (Table 4.3 and Fig. 4.2). All the twenty three isolates of *Pseudomonas fluorescens* were tested under *in vitro* conditions, maximum inhibition in radial growth of *R.solani* was observed with P72 (78.66 %) followed by P201 (72.43%), P5 (66.25%), P85 (65.02%), P141 (63.37%), P6 (62.96%), P233 (62.96%) and the least inhibition were obtained with the isolates P99, P124, P126, P143, P151, P161, P179, P205, P216, P247 and P248 (0.00 % respectvely). Similar finding were reported by Madhavi *et al.* (2011) and observed 48-98 % mycelial growth inhibition and 27-87 % sclerotial production inhibition of *R. solani* by *P. fluorescens* in dual culture.

Table 4.3 Evaluation of antagonistic *Pseudomonas fluorescens* isolates against *R. solani* under in vitro condition.

Sr. No.	Treatment	Mycelial growth of R.solani (mm)	Mycelial growth inhibition (%)
1	P5	30.37	66.25
2	P6	33.33	62.96
3	P11	60.37	32.92
4	P72	19.26	78.66

5	P76	62.59	30.45
6	P85	31.48	65.02
7	P99	90.00	0.00
8	P124	90.00	0.00
9	P126	90.00	0.00
10	P129	61.48	31.68
11	P141	32.96	63.37
12	P143	90.00	0.00
13	P151	90.00	0.00
14	P161	90.00	0.00
15	P167	81.48	9.46
16	P176	52.22	41.97
17	P179	90.00	0.00
18	P201	24.81	72.43
19	P205	90.00	0.00
20	P216	90.00	0.00
21	P233	33.33	62.96
22	P247	90.00	0.00
23	P248	90.00	0.00
24	Control	90.00	0.00
C.D.at 5%		**0.91**	
SE(m)		**0.32**	

*** Average of three replications**

4. 7 Evaluation of fungicides against *R. solani* by poison food technique.

In this study, seven fungicides were evaluated for their effect on mycelial growth of *R. solani* isolate i.e. Pulsor (Thifluzamide 24% SC), Taqat (Captan 70% +Hexaconazole 5% WP), Contaf Plus (Hexaconazole 5% SC), Tilt (Propiconazole 25% EC), Saaf (Carbendazim+ Mancozeb 75% WP), Bavistin (Carbendazim 50% WP), Ridomil (Metalaxyl 72% WP). Result presented in table - 4.4 and fig.- 4.3 indicated that all the fungicide significantly inhibited mycelial growth of *R.solani* as compared to the control.

Plate 4.3 - Antagonistic interaction between *Rhizoctonia solani* **and different isolates of *Pseudomoas fluorescens* by funnel technique**

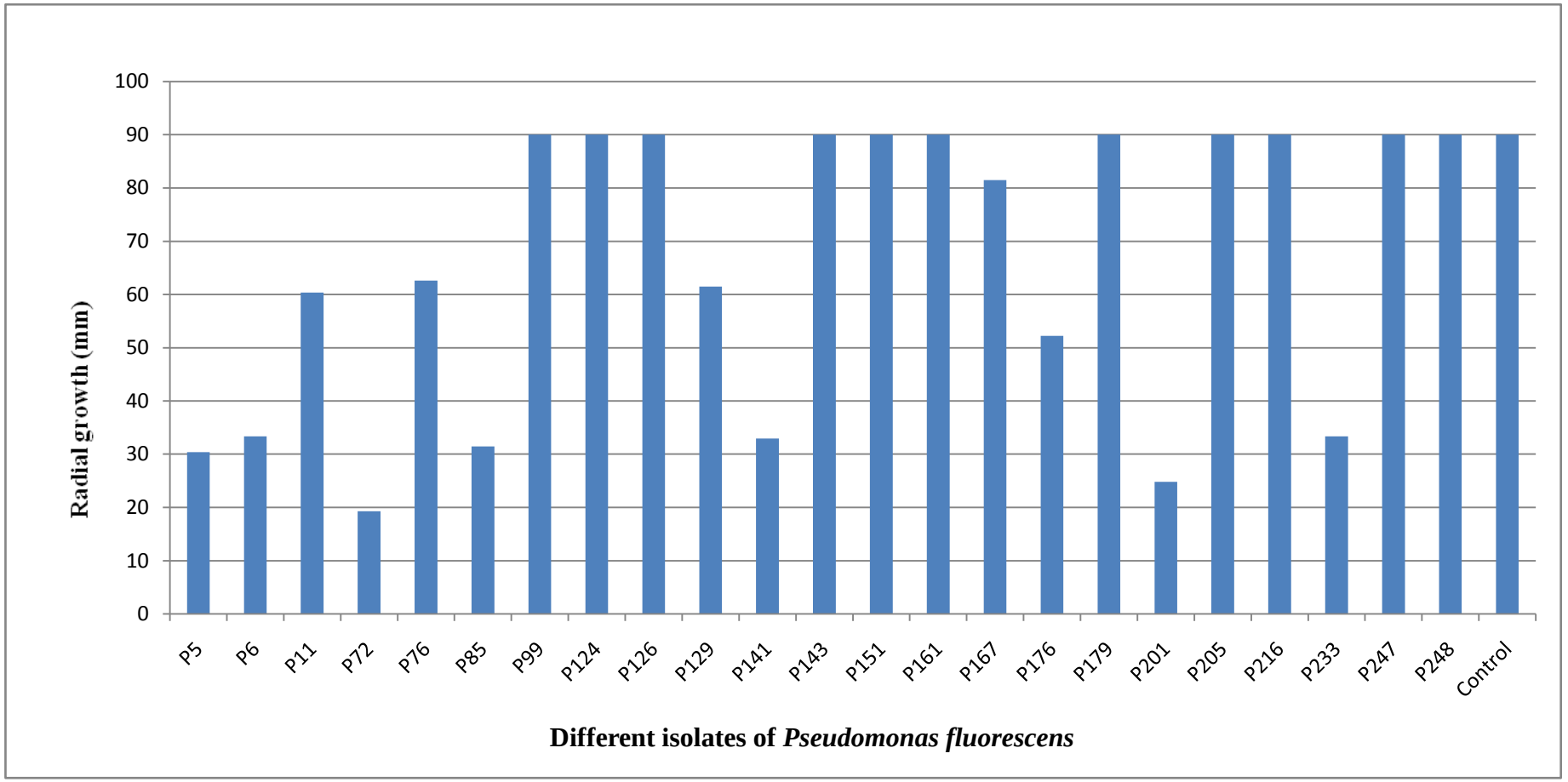


Fig : 4.2 Efficacy of Radial growth of *R. solani* against different isolates of *P. fluorescens*

Table 4.4 Evaluation of fungicides against *R.solani* by poison food Technique.

Sr. No.	Treatment	Mycelial growth (mm) *			Mycelial growth inhibition (%)		
		10 ppm	20 ppm	30 ppm	10 ppm	20 ppm	30 ppm
T_1	Pulsor(Thifluzamide 24% SC)	0.00	0.00	0.00	100	100	100
T_2	Taqat (Captan 70% +Hexaconazole 5% WP)	21.33	20.33	20.00	76.33	77.41	77.78
T_3	Contaf Plus (Hexaconazole 5% SC)	0.00	0.00	0.00	100	100	100
T_4	Propiconazole (Propiconazole 25%EC)	0.00	0.00	0.00	100	100	100
T_5	Saaf (Carbendazim+ Mancozeb 75%WP)	16.66	15.66	13.66	81.47	82.60	84.82
T_6	Bavistin (Carbendazim 50%WP)	0.00	0.00	0.00	100	100	100
T_7	Ridomil (Metalaxyl 72%WP)	22.33	21.66	10.66	75.18	75.93	88.15
T_8	Control	90.00	90.00	90.00	-	-	-
	C.D. at 5%	**0.61**	**0.87**	**0.79**	-	-	-
	SE(m)	**0.20**	**0.28**	**0.26**	-	-	-

*** Mean of three replication**

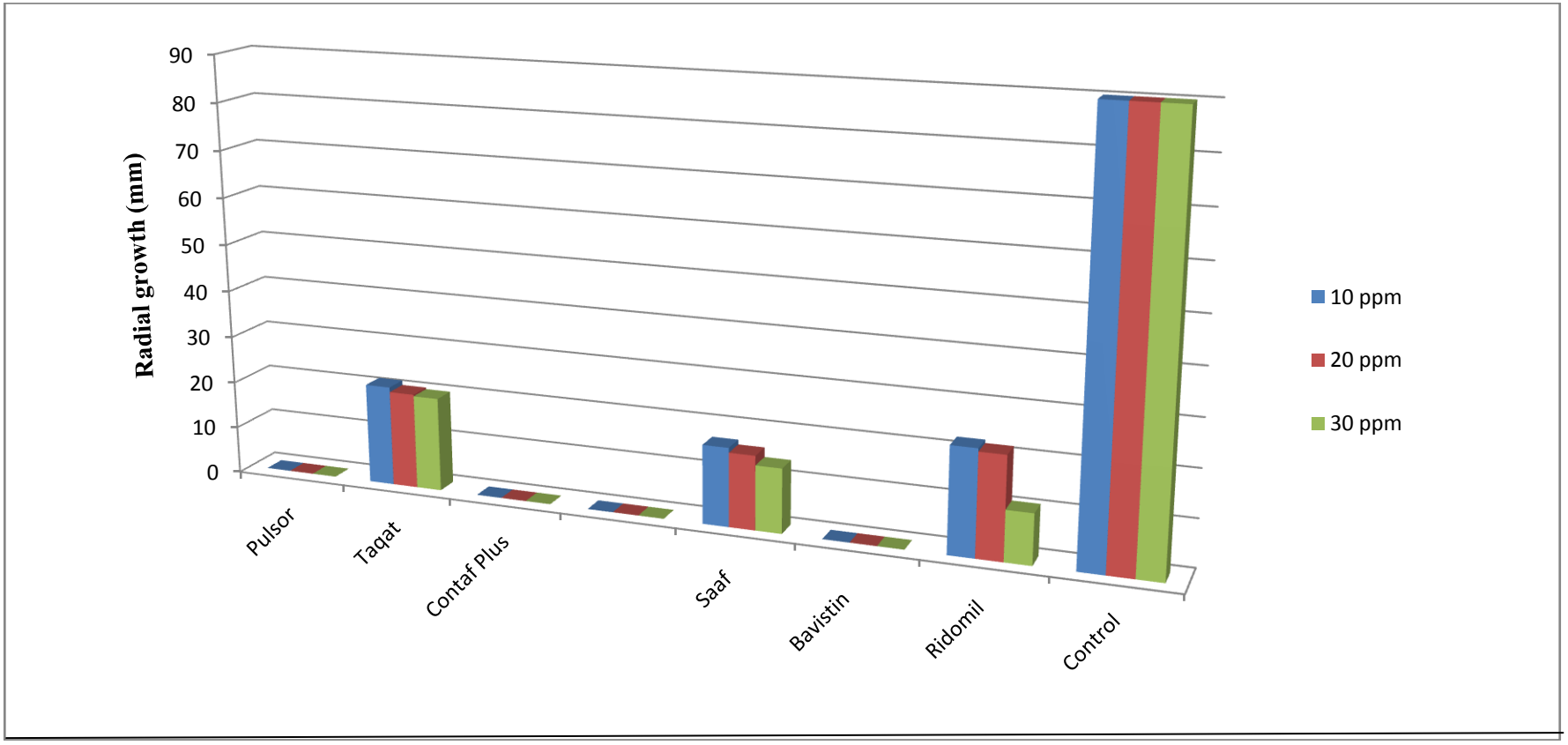


Fig: 4.3 Evaluation of fungicides against *R. solani* by poison food technique

It is clear from the table 4.4, that as the concentration of fungicides increased from 10 ppm, 20 ppm and 30 ppm there was decline in mycelial growth of *R. solani* as compared to control.

All the fungicides were significantly effective over control and among treatments at 10 ppm, 20 ppm and 30 ppm in inhibiting the radial growth of the test pathogen.

The percent inhibition of the radial growth was observed at all concentration by Pulsor (Thifluzamide 24% SC), Contaf Plus (Hexaconazole 5% SC), Tilt (Propiconazole 25%EC), Bavistin (Carbendazim 50%WP); however, Ridomil, Saaf and Taqat inhibited the mycelial growth by 88.15 %, 84.82 % and 77.78 % respectively at 30 ppm concentration of fungicides. More than 70 percent inhibition of mycelial growth were recorded at 10 ppm concentration of aforesaid fungicides.

All the three concentration of the fungicides showed inhibitory effect on mycelial growth of *R.solani.* Complete inhibition of mycelial growth were observed in case of Pulsor (Thifluzamide 24% SC), Contaf Plus (Hexaconazole 5% SC), Tilt (Propiconazole 25%EC), and Bavistin (Carbendazim 50%WP). The fungicides in the present study, viz; Ridomil, Saaf and Taqat also inhibited the mycelial growth of *R.solani* as compared to control. Similar results were reported by Sharma *et al.* (2002), Rajput (2013) and Rani *et al.* (2013) in case of Carbendazim and Propiconazole.

4.8 Efficacy and evaluation of fungicides and *Pseudomonas fluorescens* (P167*)* **with different formulations against the *R.solani* under field conditions.**

Data presented in Table 4.5, clearly indicated that after first spray of a formulation, Tilt (Propiconazole 25% EC) was found highly effective as minimum disease severity (13.02%) followed by Pulsor (Thifluzamide 24% SC) (13.83 %) and Contaf Plus (Hexaconazole 5% SC) (17.01%) as compared to the control (47.61 %).

After second spray of a formulation,, Pulsor (Thifluzamide 24% SC) and Tilt (Propiconazole 25% EC) (16.16 % and 19.44 % respectvely) were found effective and reduced the disease severity as compared to the control (91.49 %).

At final observation, after third spray of a formulation, Pulsor (Thifluzamide 24% SC) was found significantly superior and reduced the disease severity by 78.84 % followed by contaf plus, tilt, bavistin and taqat in comparison to control (95.92 %). All the fungicidal treatments were statistically as par with each other. Spray of *Pseudomonas fluorescens* (P167) isolate reduced the disease severity by 14.50% over control.

All the fungisides and *Pseudomonas fluorescens* (P167*)* tested under *in vivo* condition were more effective against *R.solani* as compared to control. Similar results were obtained by Saxena (2002) and reported that foliar application of Propiconazole was most effective fungicides.

Similarly Meena *et al.* (2003) suppressed BLSB caused by *R.solani* by foliar application of *P. fluorescens.*

Table 4.5 Efficacy and evaluation of new fungicides and *Pseudomonas fluorescens* (P167*)* with different formulations against the *R. solani* under field conditions.

Sr. No.	Treatments	Dosage/ Litre of water	Disease Severity(%)*			% Decrease over control
			10DAI	20DAI	30 DAI	
T_1	Taqat (Captan 70% +Hexaconazole 5% WP)	2.0 gm	21.89	23.20	24.60	74.35
T_2	Propiconazole (Propiconazole 25%EC)	1.0 ml	13.02	19.44	22.11	76.94
T_3	Bavistin (Carbendazim 50%WP)	2.0 gm	20.66	21.68	23.33	75.67
T_4	Pulsor (Thifluzamide 24% SC)	1.0 ml	13.83	16.16	20.29	78.84

T_5	Contaf Plus (Hexaconazole 5% SC)	2.0 ml	17.01	20.02	22.10	76.95
T_6	*Pseudomonas fluorescens* (P167)	1.0 ml	37.84	70.49	82.01	14.50
T_7	Control	Untreated	47.61	91.49	95.92	
	SE(m)				**6.52**	
	C.D.(at 5%)				**20.32**	

DAI - Days After Inoculation
*** Average of three replications**

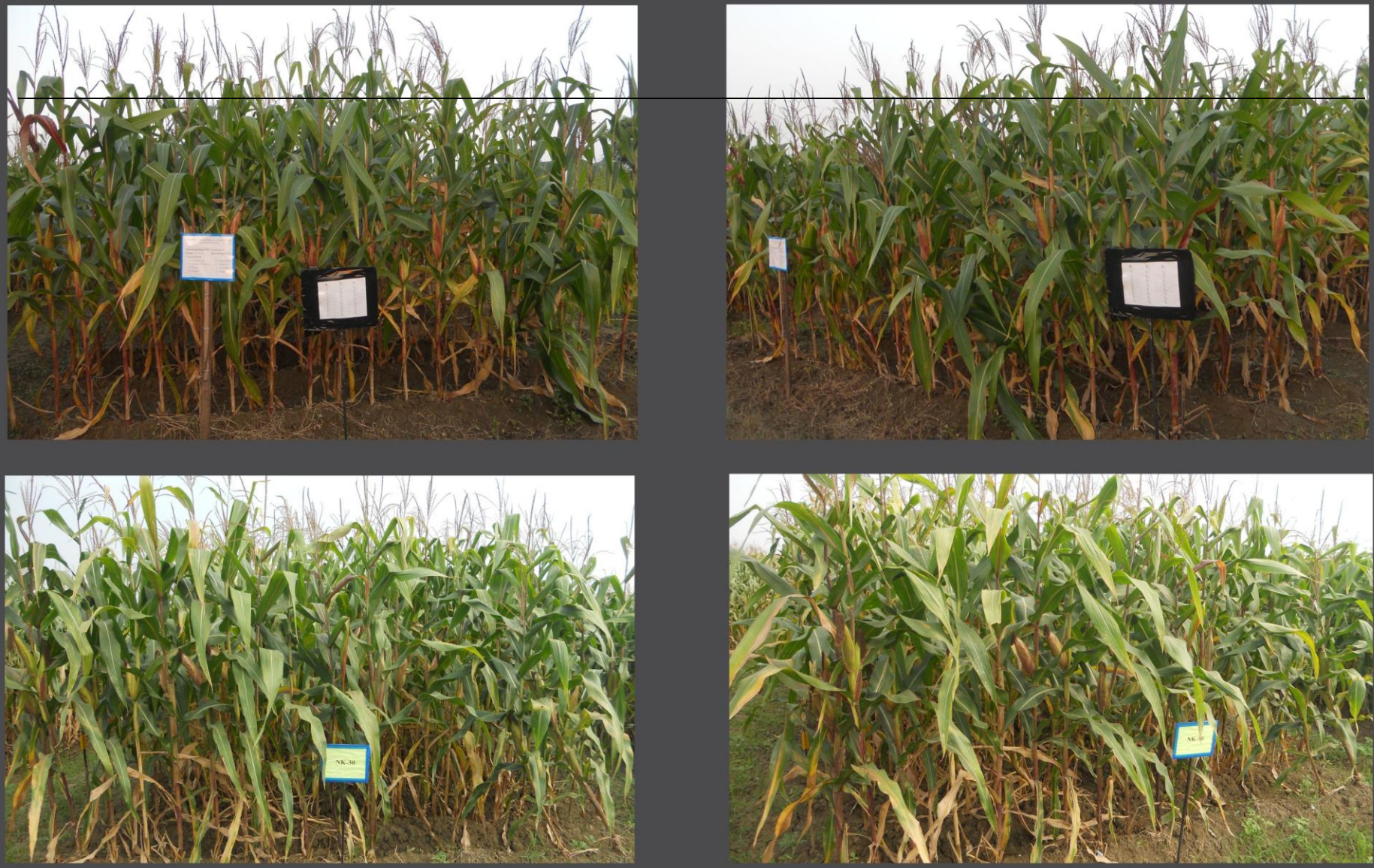


Plate 4.5- Field view of fungicides trial against bended leaf and sheath blight diseases

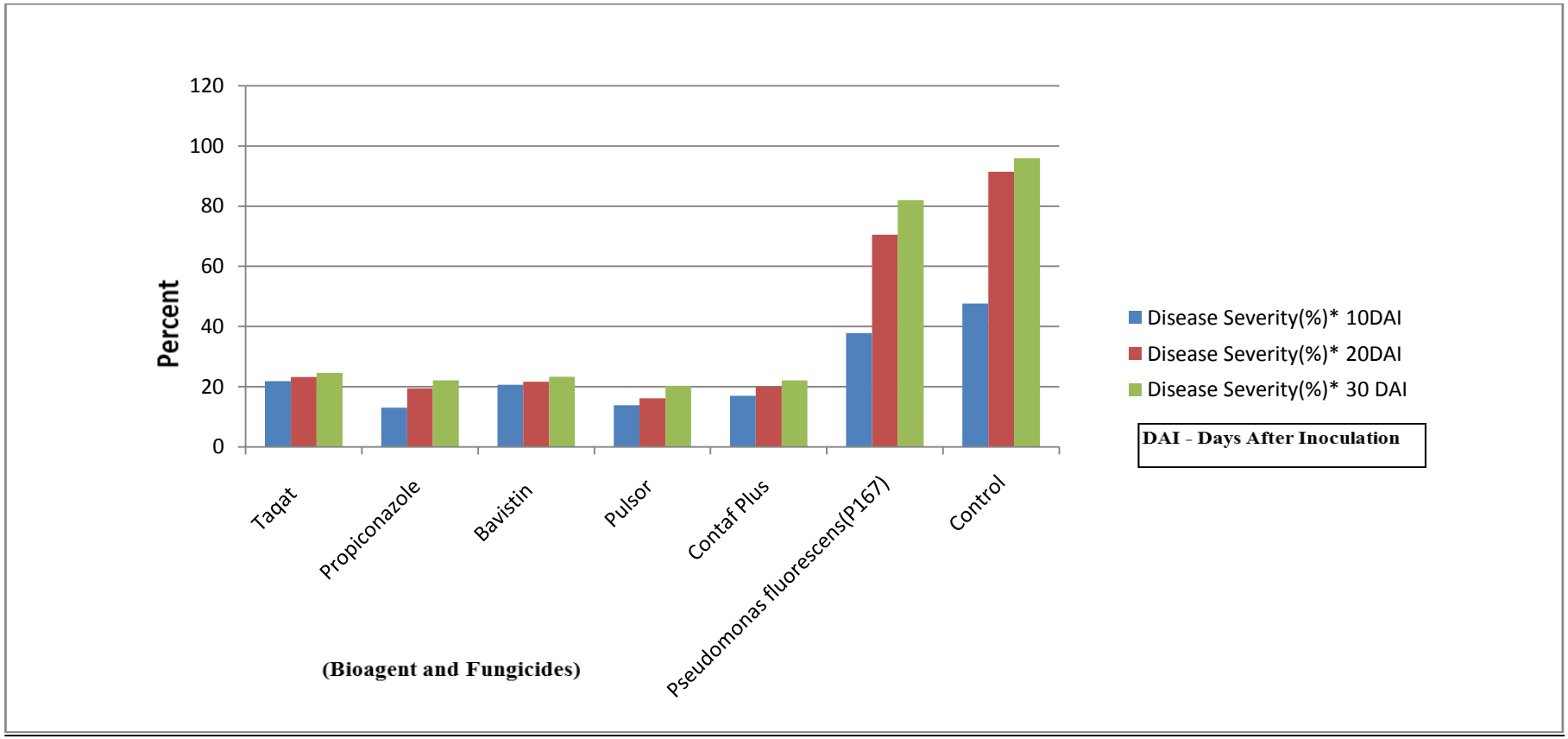


Fig.: 4.4 Efficacy and evaluation of new fungicides and *Pseudomonas fluorescens* (P167*)* with different formulations against the *R. solani* under field condition.

CHAPTER-V
SUMMARY AND CONCLUSIONS

The finding of present investigation on **"Studies on banded leaf and sheath blight (*Rhizoctonia solani* Kühn) disease of Maize"** was carried out in the Department of Plant Pathology, College of Agriculture, IGKV, Raipur (C.G.). The investigation mainly consists four objectives:

1. Collection and isolasion of *Rhizoctonia solani* from naturally infected maize plants.
2. Pathogenicity and host range studies of the *R. solani.*
3. Evaluation of maize genotypes against the *R. solani.*
4. Evaluation of bioagent and fungicides against the *R. solani* under *in vitro* and *in vivo.*

The summary and conclusion of findings of the present investigation are givenbelow:

In symptomatological studies, the initial symptoms were observed on the first and second leaf sheath above the ground and eventually spreaded on the ear causing ear rot. Infected leaves and sheaths were water soaked concentric bands and discoloured, brown in colour.Whole plant was blighted within a weeks. White mycelial fungal growth was seen under surface of infected leaves and sheaths and young branches. Sclerotia were observed on severely blighted leaves, sheaths and the ears.

The infected plants showed typical banded leaf and sheath blight disease symptoms were collected from the field for isolation of the pathogen in the laboratory. The pathogen was isolated on PDA from infected plant parts.The culture were purified by single hyphal tip method and were maintained on PDA at 27 ± 1^{o} C in BOD incubator. The isolated fungi were identified on the basis of morphological characteristics.

Pathogenicity was proved in maize under laboratory condition by deteched method and under field condition by attached method. In laboratory condition,the healthy maize plant parts were kept in big size dessicators and inoculated the mycelium bits of test fungus on leaves and sheath and the symptoms were

observed after 2 days of inoculation. Sclerotia were observed on severely blighted leaves and sheaths at 12 days after inoculation. In field condition, the healthy seedlings of variety NK-30 was selected for inoculation. The isolate was multiplied in PDA. The sclerotia produced in PDA was inoculated in healthy sheath of maize (NK-30) and the symptoms were observed after three days of inoculation. Initial symptoms was started as water-soaked, straw-coloured lesions on leaf and sheath which enlarged gradually day by day however, lesion become enlarged and discoloured areas alternating with dark bands in 7 days after inoculation.

In host range studies, 6 plant species were inoculated with pathogen. All the 6 host crop not showed host susceptibility against *Rhizoctonia solani* but rice crop showed host susceptibility against *Rhizoctonia solani.*

Twenty one entries of maize were evaluated against *Rhizoctonia solani* under natural field conditions. All the entries showed highly resistant and resistant reactions against banded leaf and sheath blight disease.

Antagonistic efficacy of *Pseudomonas fluorescens* were studied against isolates of *Rhizoctonia solani* by bacterial funnel technique, where the 78.66 % highest growth inhibition percent was found in P72 followed to 72.43% in P201 and 66.25 % in P5. The least 0.00% growth inhibition was found in P205, P126, P124, P99, P143, P151, P179, P161, P247, P248 and P216.

Antagonistic efficacy of *Pseudomonas fluorescens* were studied against isolates of *Rhizoctonia solani* by bacterial funnel technique, where the 78.66 % highest growth inhibition percent was found in P72 followed to 72.43% in P201 and 66.25 % in P5. The least 0.00% growth inhibition was found in P205, P126, P124, P99, P143, P151, P179, P161, P247, P248 and P216.

Seven fungicides (Captan 70% + Hexaconazole 5% WP, Propiconazole 25% EC, Carbendazim 50% WP, Thifluzamide 24% SC, Hexaconazole 5% SC, Metalaxyl 72% WP, Carbendazim 75% WP + Mancozeb) were evaluated *in vitro* by poisoned food techniques at three concentrations i.e. 10, 20 and 30 ppm. All the fungicides significantly effective in reducing the mycelia growth at all the three concentrations. Carbendazim 50% WP, Hexaconazole 5% SC, Propiconazole 25% EC, Thifluzamide 24% SC proved to be the best fungicides giving best mycelial growth inhibition of the test fungus (100.00%) after 9 days of inoculation followed

by Captan 70% + Hexaconazole 5% WP (77.78 %), Carbendazim 75% WP + Mancozeb (84.82%) and Metalaxyl 72% WP (88.15%).

Five fungicides were evaluated under *in vivo* conditions for banded leaf and sheath blight of maize clearly revealed that commercially available fungicides like Taqat (Captan 70% + Hexaconazole 5% WP) (24.60%), Tilt (Propiconazole 25% EC) (22.11%), Bavistin (Carbendazim 50% WP) (23.33%), Pulsor (Thifluzamide 24% SC) (20.29%), Contaf Plus (Hexaconazole 5% SC) (22.10 %) were significantly reduced the banded leaf and sheath blight disease severity over control (95.92%).

Conclusions

All the twenty one maize entries showed highly resistant or resistant reaction against the banded leaf and sheath blight disease.

Isolate P72 of *Pseudomonas fluorescens* was promising in reducing the mycelial growth of *Rhizoctonia solani.*

Fungicide Hexaconazole 5% SC, Propiconazole 25% EC, Thifluzamide 24% SC, Carbendazim 50% WP and Captan 70% + Hexaconazole 5% WP were found effective in reducing the disease.

Suggestions for future research work

- Survey for the prevalence and severity of banded leaf and sheath blight disease of maize caused by *Rhizoctonia solani* in different parts of Chhattisgarh region is necessary.
- Research should further be intensified to reduce the incidence of banded leaf and sheath blight disease of maize with parameter viz. sowing dates, sowing depth, soil characteristics, soil moisture and temperature.
- Epidemiological factors which affect the severity of banded leaf and sheath blight disease of maize under natural field condition have to be study.
- Effective plant extract should be tested in field level to manage banded leaf and sheath blight disease of maize.

- Field screening of more number of fungicides, botanicals and bio-agents and possibility of their integration need to be studied and minimize the yield loss.
- To find out the efficacy of new fungicides and its compatibility and combine efficacy with new and commercially available insecticides.

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