"STUDIES ON BACTERIAL PUSTULE (*Xanthomonas axonopodis* pv. *glycines* NAKANO) DISEASE OF SOYBEAN"

M. Sc. (Ag.) THESIS

by

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
RAIPUR (C.G.)

2015

"STUDIES ON BACTERIAL PUSTULE (*Xanthomonas axonopodis* pv. *glycines* NAKANO) DISEASE OF SOYBEAN"

Thesis

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by

Girija Shankar

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CERTIFICATE - I

This is to certify that the thesis entitled **"Effect of crop configuration and seed rate on weed dynamics and productivity of soybean [*Glycine max* (L.) Merrill] under *Vertisols* of Chhattisgarh plains"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Saurabh Kumar** under my/our 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 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.

Gupta

Chairman

Date: 21/07/2015

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTE

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Member (Dr. R. R. Saxena) — 21/7/15

CERTIFICATE - II

This is to certify that the thesis entitled **"Studies on bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean"** submitted by **Girija Shankar** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur in partial fulfilment 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.

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Approved/Not approved

Director of Instructions

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"Education plays vital role in personal and social development and teacher plays a fundamental role in imparting education. Teachers have crucial role in shaping young people not only to face the future with confidence but also to build up it with aim and responsibility. There is no substitute for teacher pupil relationship". I take this golden opportunity to express my heartful humble and deepest sense of gratitude to those who helped me to complete my research possible. These words are small acknowledgement but never fully recompensed for their great help and co-operation.

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I express my sincere thanks to my colleagues Nand Kishor Pandey, Santram Sahu, Manoj Sahu, Bhimeshwari Sahu, Krishna Marawi, Indra Kumar, Lokesh Taram, Ashok Koshariya, Miss Priyanka Prajapati, Birendra Tigga, Umakant Banjare, Harsh Mishra, Puneshwer Singh Painkra, Harish Manik, Dinesh Kumar Marapi, Om Prakash Sakewal, Prakash Raichandani, Saurabh Verma, Pankaj Bhargav and Hemkant Chandravanshi.

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Abbreviations	Description
%	Percent
CD	Critical difference
% CV	Percent Coefficient of variation
cv.	Cultivars
^{0}C	Degree Celsius
mm	Mili meter
cm	Centimetre
et al.	*et alii*
Fig.	Figure
g	Grams
ha	Hectare
i.e.	That is
Kg	Kilogram
SEm±	Standard error of means
L	Litre
BOD	Biological oxygen demand
/	Per, also means and or
ppm	Part per million
DAI	Days after inoculation
DAS	Days after sown
HAI	Hours after inoculation
OD	Optical density
V	Variety
	Micro litre

min.	Minutes
Ml	Millilitre
S.N	Serial number
NO.	Number
qt.	Quintal
e.g.	For example
@	At the rate

THESIS ABSTRACT

a)	Title of the Thesis	:	"Studies on bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean"
b)	Full Name of the Student	:	Girija Shankar
c)	Major Subject	:	Plant Pathology
d)	Name and Address of the Major Advisor	:	Dr. R.K. Dantre (Professor), Dept. of Plant Pathology College of Agril., Raipur (C.G.)
e)	Degree to be awarded	:	M.Sc. (Ag) Plant Pathology

Signature of Major Advisor
Date: 22/5/15

Signature of the Student

Signature of Head of the Department

ABSTRACT

The present investigation entitled "Studies on bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean" was conducted in the laboratory, field and polyhouse of dept. of Plant Pathology and Plant Pathology soybean research farm. IGKV, Raipur (C.G.).

The pathogen associated with bacterial pustule of soybean was identified as *Xanthomonas axonopodis* pv. *glycines* on the basis of *in vitro* studies *viz.*, isolation, morphological characterization and pathogenicity test. Pinprick method was most suitable for disease development. Screening of soybean bacterial pustule was showed that five varieties *viz.*, JS-72-44, JS-75-46, JS-71-05, JS-335 and Shivalic were free from the disease. Maximum bacterial pustule was recorded in var. Punjab-1 (64.34%) followed by NRC-7 (46.51%) and Monatta (38.73%).

In vitro evaluation of seven antibiotics were evaluated at five different level of concentrations (100, 200, 300, 500 and 1000 ppm) by "disc diffusion method". All the different level of concentrations best result found three antibiotics viz., Tetracyclin, Chloramphenicol and Streptomycin. Dual culture test with sixteen isolates indicates that Pf 18 was found to be most significantly effective to inhibite

isolates indicates that Pf 18 was found to be most significantly effective to inhibite the growth of the pathogen; this was followed by Pf 34, Pf 33 and Pf 35 after 24, 48 and 72 hrs of incubation period respectively. Bio efficacy of Fluorescent *Pseudomonas* was evaluated under field condition that designates highest disease control of pustule was recorded for variety Monatta with 29.10 per cent disease control followed by NRC-7 24.52 per cent disease control after foliar application of Fluorescent *Pseudomonas.*

In vitro evaluation of five fungicides at three different levels of concentration (0.1, 0.2 and 0.3%) by "disc diffusion method" showed that Copper sulphate, Copper oxychloride (COC) and Dithane M-45 were found significantly superior against the test pathogen after 72 hrs of incubation period.

Out of the forteen leaf extracts, the maximum zone of inhibition was recorded in leaf extract of *Adathoda vasica* (38.00 mm) followed by *Emblica officinalis* (30.33 mm).

Growth promoting activities of the soybean seed and Fluoresecent *Pseudomonas* influenced different growth parameters. The best Fluorescent *Pseudomonas* stains were Pf 39, Pf 33 and Pf 35.

सारांश

वर्तमान परीक्षण इंदिरा गांधी कृषि विश्वविद्यालय के पादप रोग विभाग के प्रयोगशाला प्रक्षेत्र और पालीघर में किया गया। जिसका शीर्षक सोयाबीन की जीवाणु जनित नामक रोग का अध्ययन था।

सोयाबीन के जीवाणु जनित फुंसी की पहचान रोगजनक जेन्थोमोनास ऑक्जेनोपोडीस पीवी. ग्लाइसीन्स के रूप में पहचान की गई। प्रयोगशाला के अध्ययन के आधार पर जेन्थोमोनास ऑक्जेनोपोडीस पीवी. ग्लाइसीन्स का पृथककरण, रूपात्माक लक्षण और रोगजनक का परीक्षण पिन चुभोवक विधि से किया गया। सोयाबीन जीवाणु जनित फुंसी का निरीक्षण यह बतलाता है कि पांच किस्में जेएस–72–44, जेएस–75–46, जेएस–71–05, जेएस–335 और शिवालिक रोगमुक्त थी। अधिकतम जीवाणुजनित फुंसी पंजाब–1 (64.34 प्रतिशत) उसके बाद एनआरसी–7 (46.51प्रतिशत) तथा मोनाटा (38.73 प्रतिशत) किस्में में दर्ज की गई।

इन विट्रों में सात जीवाणुरोधक दवाओं का मूल्यांकन डिस्क प्रसार विधि द्वारा सांद्रता (100, 200, 300, 500, एवं 1000 पी पी एम) के पांच अलग–अलग स्तर पर मुल्यांकन किया गया। तीन एंटीबायोटिक ट्रेटासाइकिन क्लोरोएमफिनीकॉल और स्ट्रेप्टोमाइसिन अलग अलग सांद्रता स्तर पर बेहतर रहीं। सोलह वियोजन का दुतियक संवर्धन प्रयोग यह बतलाता है कि पी एफ 18 सार्थक रूप से रोगाणु के वृद्धि को रोकता है। इसी क्रम में पीएफ 34 पीएफ 33 और पीएफ 39 उष्मायन अवधि क्रमशः 24, 48 और 72 घंटे के लिये सार्थक थी। फ्लोरोसेंट स्युडोमोनास पर्णी छिड़काव कर फ्लोरोसेंट स्युडोमोनास के जैव प्रभावकारिता का मूंल्याकन प्रक्षेत्र परिस्थिति पर जीवाणु जनित फुंसी की अधिकतम अवरोधकता मोनाटा किस्म में (17.80 प्रतिशत) में दर्ज की गई। इसी क्रम में एन.आरसी–7 (15.10 प्रतिशत) अवरोधकता दर्ज की गई।

डिस्क प्रसार विधि द्वारा पांच फंफूदनाशक के तीन अलग अलग सांद्रता पर इन विट्रो मूल्यांकन बताता है कि कॉपर सल्फेट, कॉपर ऑक्सीक्लोराइड और डाइथेन एफ 45 रोगाणु के 72 घंटे के उष्मान काल पर सार्थक रहीं। चौदह पौध पत्ती उत्पाद में से अधिकतम निषेध की क्षमता ऐदाथोडा वेसीका के पौध उत्पाद में दर्ज की गई इसी क्रम में इम्बीलिका ऑफिसिनेलिस रहीं। सोयाबीन बीज की वृद्धि विकास क्रियाकलाप और फ्लोरोसेन्ट स्युडोमोनास, विभिन्न पादप वृद्धि मापदंडों को प्रेरित करते है। बेहतर फ्लोरोसेंट स्यूडोमोनास रंजक पीएफ 39, पीएफ 33 और पीएफ 35 पाया गया।

CHAPTER-I

INTRODUCTION

Soybean [*Glycine max*. (L.) Merril] belonging to family Leguminaceae is designated as 'miracle bean', established its potential as an industrially vital and viable oilseed crop in many areas of India. It is the cheapest source of vegetable oil and protein. It contains about 40% protein, well balanced in essential amino acids, 20% oil rich with poly unsaturated fatty acids specially Omega 6 and Omega 3 fatty acids, 6-7% total mineral, 5-6% crude fiber and 17-19% carbohydrates (Chauhan *et al.,*1988). The protein form of soybean is equivalent to that of meat, milk products and eggs in quality. Soybean mainly on account of its dietic, industrial, agricultural and medicinal importance, its products have various uses. The Soya flour is essential in the various preparations *viz*, bread, cakes, muffins, biscuits and pastry. Due to consumer's preference, soybean demand is going to increase in coming years. India imports vegetable oil, so soybean production in the country would not only help in meeting vegetable oil requirements but also save foreign exchange (Pan, 2008). The soybean meal obtained after oil extraction and defatted soybean flour are irreplaceable sources of protein in the nutrition of livestock, poultry and fish. Soybean builds up the soil fertility by fixing atmospheric nitrogen through the root nodules, and also through leaf fall on the ground on maturity. It is able to leave residual nitrogen effect for succeeding crop equivalent to 35-40 kg N ha^{-1}.

Soybean is a world's first rank crop as a source of vegetable oil. Global area and production of soybean is 111.27 m ha and 276.4 million tonnes respectively (Anon., 2013b). In oilseed scenario of India, it occupies 1st place. And it is cultivated in area of 12.2 m ha, with production potential of 11.95 million tonnes and average productivity of 9.79 qt. ha^{-1} (Anon., 2013a). The productivity of soybean is less in India as compared to world average (24.84 qt. ha^{-1}). The major soybean producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh and Karnataka (Anonymous, 2013).

In Chhattisgarh, agriculture is mainly based on rain water; therefore most of the crops are grown as rainfed in kharif season. Soybean occupies 159.59

thousand ha area with a yield of 11.50 qt. ha^{-1} (Anon., 2015). In Chhattisgarh, major soybean growing districts are Rajnandgaon, Durg, Mungeli, Bemetara, and Kabirdham.

Soybeans are susceptible to several hundred pathogens, some of which are economically important. Fungal pathogens usually predominate, but bacterial, nematode and viral pathogens occur as well (Sinclair, 1997). The most common bacterial diseases of soybeans are bacterial blight and bacterial pustule (Sinclair, 1999).

Among the bacterial diseases bacterial pustule is the most serious problems in soybean production since they reduces the total production, and important protein. Pre-flowering appearance of disease causes economic losses in yield (Saxena, 1977). It can be estimated that soybean yield losses of 15, 21, 38 and 53% are encountered at the 10.1-25, 25.1-50, 50.1-75 and >75% infection rates, respectively (Shukla, 1994). The earliest definitive recognition of bacterial pustule came in 1992 when Hedge described the disease and isolated the organism (Hartwig and Lehman1951). The disease has been reported in most soybean-growing areas of the world where warm weather and frequent showers prevail during the growing season. It is prevalent in soybean-growing areas, especially Australia, China, India, Japan, Korea, Sudan and USA (Sinclair and Backman1989).

Small yellow-green spots with brown centers on the leaves are the first symptoms of bacterial pustule. These spots are most conspicuous on the upper surface of the leaf. A small blister-like pustule usually develops in the center of the lesions, especially on the lower leaf surface. The spots may merge, forming larger dead areas in which the tissue may fall out, giving the leaf a ragged appearance. As the pustules rupture and dry, the disease may become confused with bacterial blight.

Bacterial pustule is caused by the bacterium *Xanthomonas axonopodis* pv. *glycines* Erwin Smith who isolated a yellow bacterium from soybeans may have observed the disease as early as 1902 (Hartwig and Lehman1951). *Xanthomonas. axonopodis* pv. *glycines* (Nakano 1919) Dye is synonymously known as *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and *X. phaseoli* (smith)

Dowson var. *sojensis* (Hedge) Starr and Burkholder. The causal organism of pustule is a motile, gram-negative rod (0.5-0.9 x 1.4-2.3 μm) with a single polar flagellum. Colonies on beef infusion agar are pale yellow, become deep yellow with age, and are small, circular and smooth with an entire margin. The bacterium is slow growing in culture. The optimum temperature for growth is 30-33°C (the maximum is 38°C, the minimum is 10°C). The bacterium produces auxins, bacteriocin, and exopolysaccharides in culture. Races of the pathogen may exist (Hartman *et al.*, 1999).

The bacteria are carried over from year to year with infested residue and may be seed born. It favours warm weather and usually appearing about mid-July. The primary infection starts mainly from the seed-borne bacterium. The secondary spread of the bacteria may be through wind, wind-blown, rain splash, irrigation water, insects and other implements. The bacterium enters through natural openings and wounds caused by insect. Rain splashes play an important role in the development and spread of the disease.

The best way to manage this disease is to grow resistant varieties. Although it is an important disease which occurs severely in all soybean growing areas specially Madhya Pradesh and Chhattishgarh. But it has not been studied properly and knowledge about this disease is nill to many of us. In India itself, no proper research work has been done regarding this disease. It causes yield loss in soybean crops to a considerable amount. The disease bacterial pustule is the most serious problems in soybean production which needs to be control through various aspects. So taking into account about this and keeping in view the importance of this disease, present investigations were proposed with the following objectives:

Objectives of investigation:

1. Isolation, purification and pathogencity of *Xanthomonas axonopodis* pv. *glycines* causal agent of bacterial pustule of soybean.
2. Screening of soybean varieties against bacterial pustule disease.
3. Management of *Xanthomonas axonopodis* pv. *glycines* causal agent bacterial pustule disease of soybean.

CHAPTER – II

REVIEW OF LITERATURE

Soybean [*Glycine max* (L.) Merril] belonging to family Leguminaceae is designated as 'miracle bean', established its potential as an industrially vital and viable oilseed crop in many areas of India. It is a cheapest source of vegetable oil and protein. It contains about 40% protein, well balanced in essential amino acids, 20% oil (Chauhan *et al.,* 1988). Soybeans are susceptible to several hundred pathogens, some of which are economically important but the most common bacterial diseases of soybeans are bacterial blight and bacterial pustule (Sinclair, 1999). Among the bacterial diseases bacterial pustule is the most serious problems in soybean production since they reduces the total production of this important protein-producing legume. Keeping the wide occurrence of disease and its destructive nature of the pathogen, present investigation entitled "**Studies on Bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean"** was undertaken.

2.1 Isolation, purification and pathogenicity of *Xanthomonas axonopodis* pv. *glycines* causal agent of bacterial pustule of soybean

Montova (1992) isolated *Xanthomonas axonopodis* pv. *glycines* from diseased soybeans cultivar *Soyica* P 31 in the municipality of Villavicencio, Colombia, and confirmed pathogenicity.

Hwang *et al.* (1992) developed a rapid technique soybean cotyledon assay Soybean cotyledons detached from 10 to 14 days old seedlings grown in the greenhouse were surface sterilized with 0.5% sodium hypochloride for 5 min and washed with sterile distilled water. Pathogenic and nonpathogenic responses based on detached cotyledon assay were not distinguishable from those based on intact cotyledon assay.

Prathuangwong and Choethana (1998) studied on the pathogenic variation among 100 isolates of *Xanthomonas campestris* pv. *glycines*, the causal agent of

bacterial pustule of soybean. When bacterial suspension of each pathotype strain was sprayed onto foliage of greenhouse-grown soybean plants, variability in leaf lesion size and colony forming unit (CFU) of the pathogen per lesion were observed. The most aggressive isolate produced the largest leaf lesion size of 0.18 × 0.4 cm^2 and the highest of 8.7 × 10^9 CFU/lesion, where the least aggressive isolate produced the smallest leaf lesion size of 0.11 × 0.27 cm^2 and the lowest of 3.3 × 10^9 CFU/lesion. The result also revealed that the number of leaf lesion or infected leaf area was the most convenience to be evaluated for severity of soybean bacterial pustule regardless size and CFU of lesion.

Tung and Kuo (2000) studied that the citrus canker caused by *Xanthomonas axonopodis* pv. *citri* for disease symptom assays, 20 to 30 puncture wounds were introduced per 1cm area of citrus leaf with a standard 26-gauge needle. The bacteria had been grown overnight in TSG broth, harvested by centrifugation, and resuspended in 0.85% NaCl at a density of approximately 10^8 colony-forming units (CFU)/ml. Droplets (10 μL) were placed directly onto the puncture wound sites on the citrus leaves. A watersoaking (WS) response was induced by the CL– mutant XT37, in which an early blister-like lesion failed to form but a later watersoaked lesion, appeared surrounding the infection site. The watersoaked lesions were apparent 2 weeks after inoculation and continued to enlarge until they resembled the lesions induced by the wild-type strain except for the absence of the central residence. The reactions of plants were determined 7 days after inoculation.

Eddin *et al*. (2005) tested different methods of artificial inoculation for evaluation of cotton genotypes for resistance to bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum (*Xam*).* Among them sandpaper inoculation method recorded the maximum disease incidence of 85 per cent followed by pressurized spraying (75 per cent) and hypodermic syringe (70 per cent) methods. Symptoms were expressed 15– 21 days after inoculation in the form of typical water soaked spots and vein blight on leaves; black arm on stem and boll rot on bolls.

Ogunjobi *et al*. (2008) reviewed that the cassava bacterial blight caused by *Xanthomonas axonopodis* pv *manihotis*. A total of 75 bacteria strains were studied, made up of sixty eight (68) *Xanthomonas axonopodis* pv *manihotis* (Xam), four (4) *Xanthomonas axonopodis* pv *cassavae* (Xac) and three (3) unidentified species. The isolate were similar to one another in most of the cultural and physiological characteristics. They were all able to hydrolyze aesculin and showed a positive reaction to catalase, citrate and oxidase tests.

Dhutraj *et al.* (2008) studied on an experiment to develop a suitable isolation technique, pathogenicity test and suitable control measures using antibiotics (streptocycline and Bactosan) for *Xanthomonas axonopodis* pv. *glycines*, a seed born bacterium causing bacterial pustule of soybean. The percentage prevalence of the seed born bacterium was maximum in nutrient agar medium on sterilized or unsterilized seeds, followed by other media and found to be statistically at par. The bacterium proved pathogenic on the locally grown soybean cultivar Monetta.

Wang *et al*. (2011) also proved the pathogenicity of citrus canker caused by *Xanthomonas citri* leaves of 'Meiwa' and 'Newhall' were pinprick-inoculation either with water or the bacterial inoculum, cultured in a growth chamber and periodically observed over 7 days. As expected, inoculation with water did not cause any morphologically pathological change in the leaves

Sain and Gour (2013) isolated the bacterial pustule pathogen (*Xanthomonas axonopodis* pv. *glycines*) were isolated from naturally infected of soybean plant leaves, stems parts on nutrient agar medium after 72 hrs of inoculation. When inoculated on soybean plants, the disease symptoms on the leaves started showing typical symptoms as pale green spots in interveinal area with elevated centers which became confluent and formed large brown patches. Earliest symptom development was initiated in pin prick method (3-5 days after inoculation) as compared to carborundum method (4-6 days after inoculation)

2.2 Varietal screening of bacterial pustule of soybean

Hartwig *et al*. (1951) and Feaster (1952) reported the resistance of the US cultivar "CNS" "Clemson Non Shatter" was due to a monogenic recessive major character. The differences in degree of susceptibility among soybean strains indicated that modifying genes influenced the phenotype of the dominant allele.

Khare *et al.* (1968) found resistant cultivar of soybean cultivars *viz.*,, Bragg, Clark-83, Dare, Davis, Lee, Hampton, Hill, Dickett, Semmes and Hardee to *X. phaseoli* pv. *sojense* infection under field conditions at Jabalpur.

Verma (1970) reported that among 15 varieties of soybean screened for bacterial leaf spot disease caused by *X. p* pv. *sojense* the varieties Semmes, Hardee, Davis, Lee, Dare and Clarke-63 were highly resistant.

Khare *et al.* (1971) screened 16 soybean cultivars for bacterial pustule under natural conditions, only three cultivars *viz.*,, Bragg, Cluster and Scott were found to be highly resistant and these were suggested to be the good material for resistance breeding programme.

Patel *et al.* (1972) tested 225 soybean genotypes for resistance to *X. phaseoli.* pv. *sojense* under artificial inoculated conditions in the field. They found that 34 lines, many being US varieties, were resistant. This suggesting that there were no difference in pathogenic strains of *X. phaseoli* pv. *sojense* in India/USA and hence these US varieties could directly be adapted for cultivation in India or as resistant donars.

Sundaresh *et al.* (1981) reported DS-74-73 and DS-74-72, DS-74-1 8-2, Jupitor and PK-258 as immune, whereas, AMSS-9, UGM-30 and Improved Pelican as highly susceptible to bacterial pustule.

Carrasco *et al.* (1982) reported that the variety INIFAT 112 of soybean as tolerant to *X. p.* var. *sojens*, at AVRDC Taiwan, 41 AGS lines, 37 accessions and 26 breeding lines of soybean were evaluated following artificial inoculation for

resistance to *X. campestris* pv. *glycines,* 33 cultivars, 13 AGS lines and 20 accessions were rated as highly resistant.

Groth and Braun (1986) reported that a very high level of resistance to bacterial pustule was conferred by a recessive gene designated *"rxp"* which increases the number of bacterial cells necessary for infection rather than by restricting pathogen growth within host tissues.

Prathuangwong and Amnuaykit (1987) investigated 68 soybean cultivars/ lines of 35- 45 days old for their reactions to *X. c.* pv. *glycines* on farms in Kampangsan and Suwan and found that 20 cultivars/ lines were resistant, nine were susceptible and remaining 39 were intermediate. Yield and seed size of the susceptible cultivars/ lines differed significantly from those of the resistant ones.

Kushwaha *et al.* (1990) studied ten soybean varieties during 1983 and 1984 and concluded that Punjab-1, JS 76-205 and JS 76-188 gave the highest yields with low incidence of *X. p.* var. *sojense*.

Surin *et al*. (1993) screened twelve varieties for resistance against bacterial pustule of soybean they found that six varieties showed resistance against the test pathogen.

Sharma *et al.* (1993) screened soybean germplasm for resistance to bacterial pustule disease and recorded P4-2 and P 169-3 as highly resistant, four cultivars EC-34160, Bragg, Kalitur and PK-472 as moderately resistant and others as susceptible.

Gaikwad *et al.* (1995) evaluated 91 different varieties of soybean against *X. phaseoli* var. *sojense*, which were grown in Nagpur, Maharashtra, out of which 39 were totally free from the disease and 11 were resistant. Further 80 lines were tested under artificial inoculation and found that varieties T-NGS-57.2, T-NGS-35.1, T-NGS-38.3, T-NGS-33.0, TNGS-40.2, T-NGS-57.3, T-NGS-41.2, T-NGS-56.1, PK-472 and Glycine-16 were free from the infection by *X. p*. pv. *sojense*.

James (2000) evaluated over 200 lines with high culinary quality originating from Japan, and over 10 000 progeny, results showed that nearly all suffered from severe seed shelter at maturity and were susceptible to the disease bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*). However, some Japanese varieties had reasonable yield potential, excellent phytophthora resistance and high grain quality when grown in southern Queensland, Australia

Mali *et al.* (2004) screened 96 soybean genotypes against bacterial pustule of soybean, out of which 25 genotypes recorded as resistant, 34 as moderately resistant, 18 as susceptible and rest of them as highly susceptible.

Mahesha (2006) recorded thirteen out of 204 genotypes (Bragg, EC-245988, Hardee, Himso-1597, Lee, MACS-450, MAUS-681, NRC-2, NRC-12, PK-472, PS-1092, PS-1347 and SL-518) resistant against bacterial pustule of soybean.

Verma and Dantre (2011) conducted an experiment to record major diseases incidence on sixteen varieties of soybean at all growth stages and maximum bacterial pustule was recorded in var. PK 262 (80%) followed by Punjab 1 (60%) and JS 72-280(40%) while the nine varieties were free from the diseases.

Suryadi *et al*. (2012) evaluated resistant genotypes of soybean to three major soybean foliar diseases, *viz.,* soybean stunt virus (SSV), bacterial blight (BB) and bacterial pustule (BP) was done by screening 100 soybean accessions of ICABIOGRAD - Bogor germplasm collections. They found that 43 genotypes were resistant, 41 moderately resistant, 14 moderately susceptible and 2 were susceptible.

Nandini (2012) carried out a survey on incidence of cowpea bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* and revealed that the highest disease severity (14.32 PDI) was observed in Belgaum district followed by Gadag and Dharwad districts. Lowest disease severity (9.58 PDI) was observed in Haveri district. Jagtap et al. (2012) carried out a survey in eight districts (Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad) of Marathwada

region during June to August in Kharif, 2009 to 2010. In all, 69 soybean fields were surveyed (roving survey) for recording the severity and incidence of soybean blight. The most serious pod disease was noticed on the soybean field of Parbhani district, followed by Hingoli, Nanded, Latur and Beed. The variety JS-335 showed the maximum pod blight severity in all surveyed districts.

2.3 Effect of weather parameter on disease development of bacterial pustule

Sabet, (1967) studied on environmental factor and observed acute attack by the pathogen at an air temperature of 20-26 ^{o}C, with soil temperatures of 35^{o}C. He also observed varying soil moisture from 20 – 40 % and relative humidity between 70 – 80 % influenced the incidence of leaf spot. After the isolation of Xcs from symptomatic tissues expressing dark and light brown spots which turned to be dark brown, it was deduced that these variations in symptoms resulted from the environmental variations.

Habish and Hammad (1969) recorded initiation of disease from infected seed or from soil, the abaxial (lower) side of the cotyledons and showsed the first signs of infection, with the development of water soaked, small, dark green marginal spots. In the acute phase of attack, the cotyledons become spotted all over the upper and lower surfaces, with dark brown colored spots. Seedling death occured when the shoot apices were infected.

Malaguti (1971) stated that, bacterial blight of sesame caused by Xcs affecting plants of all ages and causing severe blight of leaves, petioles, flowers and stem, resulting in defoliation, and sterility. The problem was acute when wet weather prevailed or low levels of rain fall lead to the development of high relative humidity at night.

According to Maiti *et al*. (1985) recorded that the leaf spot of sesame (*P. syringae* pv. sesame) caused severe reductions in yield, but bacterial blight caused by the *X. campestris* pv. sesami is far more damaging during the monsoon season.

Adhikari *et al*. (1994) observed highly positive correlation between bacterial blight progressions with environmental factors such as temperature, rainfall and relative humidity.

Roberts (1997) investigated the effect of weather conditions on simultaneous local (plant to plant) spread and infection of peas (*Pisum sativum*) with bacterial blight (*Pseudomonas syringae* pv. *pisi*) by exposing susceptible bait plants for 24 h periods in infected field plots. He found that rainfall rate and wind run were the most important explanatory variables for the mean number of lesions

followed by maximum temperature, rainfall duration and rainfall in the previous week and disease incidence in the surrounding crop.

Srivastava *et al*. (1997) studied the epidemiology of bacterial blight of sesame caused by Xcs in the Kharif crops of 1993 and 1994 in Kanpur, India, recording disease intensity at weekly intervals. Highest disease intensity occurred in September when the average temperature was 29-29.4 ºC, relatively humidity was 88-90.5 percent and rainfall was 8.9 - 9.97 mm. A substantial decrease in disease intensity was recorded as humidity, rainfall and temperature declined at the end of September in both 1993 and 1994.

Khare and Khare (2003) conducted an experiment in the soybean cultivar Punjab-1 to determine the relationship between weather parameters and bacterial pustule disease (*Xanthomonas axonopodis* pv. *glycines*) development. The weather parameters comprised of temperature, vapour pressure, rain and number of rainy days, sunshine hours, and evaporation rate.

2.4 Bio efficacy of Fluorescent *Pseudomonas* on *Xanthomonas axonopodis* pv. *glycines* under *in vitro* and *in vivo*

Manmeet and Thind (2002) evaluated four antagonists, namely *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Penicillium notatum* against *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice, by dual culture method under *in vitro* and *in vivo* condition. They

found that *B. subtilis*, *P. fluorescens* and *T. harzianum* were inhibitory to the test bacterium.

Eddin *et al.* (2007) tested *in vitro* antagonistic activities of seven strains of *Pseudomonas flourescens* isolated from the rhizosphere of cotton against *Xanthomonas axonopodis* pv. *malvacearum*, the causal agent of bacterial blight of cotton. Among them *P. fluorescens* strains MMP ansd Pf 1 showed a high level of inhibition of Xam *in vitro*. Wet seed treatment with *P. fluorescens* Pf 1 significantly increased the seed germination and seedling vigour of cotton. Seed treatment followed by foliar application of Pf 1 significantly reduced the incidence of bacterial blight and recorded the percent disease index of 14.5 as against 43.8 in the control.

Manonmani *et al.* (2007) conducted an experiment to maximize the biocontrol efficacy of anatagonists by integrating diverse bioagents *viz.*,, *Pseudomonas fluorescens*, vermicompost extracts and plant extracts. Biological as well as resistance induction efficacy of *Pseudomonas fluorescens* were superior compared to other bioagents. The disease control activity of *Pseudomonas fluorescens* was enhanced by integrating ecologically and economically safer bioagents *viz.,* vermicompost extract and plant extracts by recording PROC of 63.9 and 61.3, respectively.

Giri *et al.* (2008) conducted a study during 2005-06 in Akola, Maharashtra to manage the citrus canker disease (*Xanthomonas axonopodis* pv. *citri*) with botanical and biological control agents. Among the bioagents *A. niger* recorded the maximum disease reduction followed by *P. fluorescens*.

Kumar *et al.* (2009) evaluated four bioagents against *Xanthomonas oryzae* pv. *oryzae* (BLB of rice) and results revealed that among bioagent *Fluorescens Pseudomonas* and *Trichoderma harzianum* restricted maximum growth of pathogen.

Raut *et al.* (2010) found that *Pseudomonas fluorescens* Pf-1@0.2% with 15.20 mm significantly inhibit the growth of *Xanthomonas axonopodis* pv. *malvacearum* (Bacterial blight of cotton).

Sain (2010) conducted an experiment to test the efficacy of *P. fluorescens* isolates efficacy against bacterial pustules of soybean caused by *Xanthomonas axonopodis* pv. *glycines,* both in laboratory and field conditions. They recorded that PGPR-10 (*P. fluorescens*), PGPR-3 (*B. subtilis*), PGPR-4 (*B. subtilis*) and PGPR- 12 (*P. fluorescens*) showed maximum bacterial growth inhibition (more than 30mm inhibition zone).

Gangwar and Sinha (2012) evaluated seven isolates of *Pseudomonas fluorescens* (FLP 84, FLP 88, FLP 90 and FLP 85 from rice leaves, FLP 2, FLP 3 and FLP 28 from different rice fields). All the isolates of *Pseudomonas fluorescens* were found significantly effective in reducing disease severity over the control. The maximum reduction was shown by Pf 83 and FLP 85 (62.18%), followed by FLP 90 (60.77%) and FLP 88 (58.65%). FLP 28 was the least effective, showing 49.46% reduction in disease severity. FLP 88 was the best in increasing grain yield (60.74%), followed by FLP 84 (52.35%) and Pf83 (50.67%). FLP 88 also recorded the greatest 1000-grain weight of 26.97%, followed by Pf83 (26.30%) and FLP 84 (25.30%).

Yenjerappa *et al.* (2013) conducted an experiment to evaluate the efficacy of available biological control agents (BCAs) against bacterial blight of pomegranate caused by *X. axonopodis* pv. *punicae*, both *in vitro* and *in vivo* condition. They observed that *P. fluorescens* exhibited significantly inhibition the pathogen growth.

Choorin *et al.* (2013) found that a mutated biocontrol agent, *Pseudomonas fluorescens* SP007s was affected in antimicrobial activity toward *Xanthomonas axonopodis* pv. *glycines* 12-2 (Xag12- 2) caused soybean bacterial pustule both *in vivo* and *in vitro* assays. Strain SP007s mutated in carA and carB was also reduced in their ability to form biofilm (detected by crystal violet assay) with 8% and 3% respectively. Two mutants carA and carB that lost ability to degrade

DSF(diffusible signal factor) of Xag in association with biofilm formation exhibited 10% and 29% reduction of disease severity respectively.

Tolba and Soliman (2013) screened for antagonistic activity of seventy native bacterial isolates towards plant tumorigenic *Agrobacterium tumefaciens in vitro* as well as their efficacy in reducing gall formation in in rose shoots, kalanchoe leaves and squash fruits. *Pseudomonas asplenii*, *P. viridilivd* and *P. polymyxa* reduced the incidence of crown gall up to 100% in the case of kalanchoe leaves and squash fruits, whereas, they reduced galling of rose shoots to 66.7%, 55.6% and 44.5% respectively.

Shivalingaiah and Umesha (2013) demonstrated that the *Pseudomonas fluorescens* possess antibacterial activity against the *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen of rice.

Basamma *et al.* (2014) studied bacterial blight in pomegranate (*Punica granatum* L.) caused by a dreaded pathogen *Xanthomonas axonopodis* pv. *punicae*. Among five strains of *Pseudomonas flourescens* tested, strain no. 326 (4) and 139 has recorded highest inhibition zone of 31.0 mm and 30.0 mm respectively, followed by strain pf 1 (27.75 mm), strain 134 (27.25 mm) and pf5 (26.50 mm).

Jambhulkar and Sharma (2014) evaluated antagonistic potential of *Pseudomona fluorescens s* isolate RRb-11 against bacterial leaf blight pathogen of rice *in vitro*, *in vivo*, microplot and field tests. The maximum shelf life of *P. fluorescens* was recorded in talc based bio formulation up to 150 days after storage. In rhizosphere competence study, the root rhizosphere of talc, kaolinite and barley based bio formulation treated plants showed good survivability and competence even up to 90 days after treatment. In field study, the talc based bio formulation was applied and the best results were obtained when talc based bio formulation of *P. fluorescens* RRb-11 was applied as seed treatment, seedling root dip and soil application in combination which reduced the disease by 92.3 and 88.5% over control.

2.5 Efficiency of antibiotics against disease development

Singh and Jain (1988) observed that the, seed treatment with Streptocycline at 100 ppm gave the best control of *Xanthomonas campestris* pv. *glycines.* In the field, spraying with Chloromycetin at 500 ppm or Streptocycline (200 ppm) + Copper oxychloride (2500 ppm) most effectively controlled the disease and increased grain yield.

Thrimurti *et al.* (1992) conducted a field trail for efficacy of Streptocycline in controlling bacterial pustule (*Xanthomonas campestris* pv. *sojense*) of soybean. Reported that the disease was controlled by Streptocycline in combination with any one of 3 fungicides, the best results being given by Fytolan (Copper oxychloride) at 0.3% + Streptocycline at 300 mg/litre.

Nagraj *et al.* (2001) studied *in-vitro* screening of some antibacterial chemicals (streptomycin sulfate, Streptocyclin, Paushamycin and Bacterimycin), plant products and antagonistic bacteria on the growth of bacterial blight of mulberry caused by *X. campestris pv. moricola* through inhibition zone assay technique and reported that, Paushamycin was the most effective (23.13 mm) followed by Bacterimycin (19.5 mm) both at 500 ppm, and Streptomycin sulfate was the least effective. Among the plant products tested, *Ocimum* oil was the most effective (28.67 mm) followed by Lemon oil and Garlic extract of the three antagonistic bacteria tested (*Pseudomonas fluorescens*, *P. aeruginosa* and *Bacillus subtilis*), *P. fluorescences* was found to be superior (25.33 mm) followed by P. aeruginosa (21.67 mm).

Ravikumar and Khan (2000) found that, Streptomycin was moderately effective against *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato under *in vivo* condition.

Ingole *et al.* (2001) observed *in vitro* evaluation of antibiotics and fungicide at different concentrations (each at 100, 200 and 300%), against *Xathomonas axonopodis* pv. *glycines* was conducted three antibiotics Paushamycin,

Streptomycin sulfate and Streptocycline. Streptomycin sulfate and Streptocycline were the most effective at 24, 48 and 72 hrs.

Ingole *et al.* (2004) studied *in vitro* to evaluate the effect of antibiotics Streptocyclin, Streptomycin sulphate and Paushamycin (each at 0.2, 0.25 and 0.3%) against *Xathomonas axonopodis* pv. *glycines*, causing leaf pustules in soybean. The antibiotics were tested in combination with Copper oxychloride at different concentrations (25, 50 and 100 ppm) for Streptocyclin and Streptomycin sulphate, and 50, 100 and 150 ppm for Paushamycin. Among the concentrations, streptomycin sulfate and copper oxychloride at 0.25%+100 ppm, streptocycline and copper oxychloride at 0.3%+50 ppm and 0.25%+100 ppm, and paushamycin combined with copper oxychloride at 0.3%+50 ppm and 0.25%+150 ppm were found the most effective.

Khan *et al.* (2005) found that, Copper oxychloride performed best followed by Vitigran blue with 43.25 and 48.19% disease incidence compared with 71.08% in the control, causing 39.15 and 32.20% disease reduction, respectively. Streptomycin exhibited moderate efficacy against the bacterial blight of rice disease whereas all other test treatments, alone or in combination, were least effective against the disease.

Kumar *et al.* (2011) studied bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh). The study revealed that sprays with Streptocycline (500 ppm)+Copper oxychloride (2000 ppm) were found very effective in reducing the mean disease incidence (25.5%) followed by Bromopal (500 ppm)+Copper oxychloride (2000 ppm) (33.3%), when compared with control (78.5%) after the 8^{th} spray.

Pawar and Papdiwal (2011) studied mango bacterial canker disease caused by *Xanthomonas campestris* pv. *mangiferaeindicae*. The *in vitro* studied have been performed by using octodiscs to examine the activity of antibiotics on 11 strains of Xcmi. Amongst the 26 antibiotics tested, 17 antibiotics have shown inhibitory effect while 9 antibiotics could not exhibit any inhibitory activity against the Xcmi strains under investigation.

Jagtap *et al.* (2012) revealed that all the 5 antibiotics tested *in vitro* applying poisoned food technique against *Pseudomonas syringae* significantly inhibited the growth of the test pathogen over untreated control. However, antibiotic, Streptocycline + Copper oxychloride recorded minimum mean colony diameter (10.47mm) and maximum mean inhibition (83.65 mm).

Raju *et al.* (2012) conducted an experiment on bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* to screen the different bactericides, bioagents and botanicals to inhibit the pathogen. Among the different chemicals, Streptocycline + COC with an inhibition zone of 3.3 cm exhibited superior efficacy followed by Streptocycline (2.80 cm) and COC (2.65 cm). From the botanicals tulsi leaves followed by neem seed oil, garlic bulb extract and Patchouli leaves were found effective.

Khan *et al.* (2012) found in best antibiotics Benzylpenicillin for isolate no. 21.3 and 17.1, Amplicillin for isolate no. 16.5 and Kanamycin for isolate no. 77.2 and 2.6 of Xoo. The present study shows that antibiotics can effectively control of BLB of rice caused by *Xanthomonas oryzae* pv. *oryzae* disease.

Xue Feng *et al.* (2012) recorded that among the five chemicals *viz.,* Streptomycin, Zhongshengmycin, Peracetic acid, Copper hy-droxide ziram and Bouillie bordelaise used Streptomycin showed better control effects on citrus canker disease in the field.

Khatua *et al.* (2013) conducted an experiment on bacterial pathogen of betelvine. *Xanthomonas axonopodis* pv. *betlicola* to observed 31 antibacterial antibiotics and 6 antibacterial compounds to inhibit the pathogen. Among the different antibiotics Penicillin G, Pipercellin and Cephalexin did not inhibited growth of the bacterium. Amphicillin, Cefotaxime and Cetriaxone were least effective of the bacterium.

Islam *et al.* (2014*)* observed that the *X. axonopodis* was found 100% resistant to cefotaxime and 77.77% to bacitracin. Chloramphenicol was found most effective as all the isolates were sensitive to it.

Naqvi *et al.* (2014) reported that the chloramphenicol proved to be the most effective antibiotic against bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*) as it suppressed the bacterial growth to greater extent and only the 6.25 mean bacterial colonies were appeared in the petri plates, followed by the ampicillin trihydrate which showed to be the second most effective antibiotic against the pathogen growth and retarded to 12.00 mean bacterial colonies. The maximum diameter of inhibition zone (28.31 mm) was showed by the Chloramphenicol at 100 ppm followed by ampicillin trihydrate which gave proved to be second most effective antibiotic to control the pathogen and gave maximum inhibition zone (25.02 mm) at 100 ppm concentration.

Pawar *et al*. (2015) noticed that out of various antibiotics tested, 4 antibiotics *viz.,*, Chloramphenicol, Tetracycline, Gentamicin and Streptomycin were selected for *in-vivo* experiment. Tetracycline showed maximum activity against Mango bacterial canker disease (MBCD) caused by *Xanthomonas campestris* pv. *mangiferaeindicae*.

2.6 *In vitro* evalution of various fungicides against *Xanthomonas axonopodis* pv. *glycines*

Kawale *et al.* (1989) used 6 chemicals at an early stage of disease soybean crop. Copper oxychloride (Blitox-50) and Streptocycline+Copper sulphate sprays reduced infection by *Xanthomonas campestris* pv. *phaseoli*. However, they had no effect on crop yield.

Ravikumar and Khan (2000) reported that Copper oxychloride and Copper sulphate significantly control the bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*.

Ingole *et al.* (2001) evaluated fungicides and antibiotics at different concentrations against *Xanthomonas axonopodis* pv. *glycines*. Treatments compried 3 fungicides Copper oxychloride (0.20, 0.25 and 0.30%), Dithane M-45 (0.20, 0.25 and 0.30%), Carbendazim (0.05, 0.10 and 0.15%) Among the

fungicides, Dithane M-45 (0.20%) was the most effective at 24 and 48 h followed by other concentrations.

Khan *et al.* (2005) observed that among the test fungicide Copper oxychloride performed the best followed for controlling bacterial leaf blight of rice.

Gholve *et al.* (2007) tested the efficacy of fungicides/antibiotics in the laboratory by poisoned food technique against *X. axonopodis* pv. *malvacearum*. The fungicides/ antibiotics significantly decreased the number of colonies of the bacteria at 10 and 100 ppm compared to the control. Streptocycline and Captan were the most promising.

Sahi *et al.* (2007) tested various toxicants *viz.,,* Agrimycin–100, Cupravit, Bavistin, Dithane M-45, Vitavax, Daconil, Antracol, Benlate and Nimrod at 1% concentration against multiplication of *Xanthomonas campestris* pv. *citri.* Agrimycin –100, Cupravit, Bavistin, Dithane M-45 and Vitavax proved more effective as compared to other toxicants *in vitro*. All the toxicants @ 1, 0.1 and 0.01% concentrations inhibited the multiplication of the bacterium however, Agrimycin-100 was found to be most effective while Cupravit, Bavistin, Dithane M–45 and Vitavax in that order, were effective against the multiplication of bacterium at 0.01, 0.1 and 1% concentrations. Agrimycin–100, Cupravit, Bavistin, Dithane M-45 and Vitavax at 0.2% concentration were sprayed on the field grown citrus plants and then inoculated with *Xanthomonas campestris* pv. *citri* for the control of citrus canker disease. Agrimycin–100, Cupravit, Bavistin, Dithane M-45 and Vitavax in the order proved effective also in reducing the disease intensity as compared to inoculated control.

Khalid and Sinha (2008) studied the sensitivity of 5 isolates of *X. oryzae* pv. *oryzae* (Xoo 5, Xoo 9, Xoo 10, Xoo 11 and Xoo 14) to various antibiotics (Streptocycline, Kasu B and Bacterinashak), fungicides (Copper oxychloride and Benomyl) and botanical pesticides (Neem Gold, Neemazol and Tricure). Xoo 9 was the most sensitive to 2000 ppm Streptocycline (inhibition zone of 25.49 mm) and Kasu B (inhibition zone of 23.48 mm). Copper oxychloride

exhibited superior inhibitory effects. Xoo 9 and Xoo 11 were highly sensitive to copper oxychloride. The isolates were less sensitive to Benomyl than to copper oxychloride. Xoo 9 and Xoo 10 were the most and least sensitive to the botanical pesticides, respectively. Neem Gold resulted in the greatest inhibition zone for all isolates, followed by Neemazol and Tricure. The variation in inhibition zones between Xoo 5 and Xoo 11 was not significant.

Raut *et al*. (2010) reported that Copper hydroxide 46.1% @ 0.25% was found effective in inhibiting the *Xanthomonas axonopodis* pv. *malvacerum* with 19.67 mm zone of inhibition followed by Copper oxychloride 0.3%+Streptomycin sulphate 100 ppm (16.62mm), Streptomycin sulphate alone (15.83mm). Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacerum* (Smith). Among bioagents, botanicals and chemicas,

Sajid *et al.* (2013) observed that the bacterial blight of Cotton after 48 hours of treatment, ascending trend was seen in Agrimycine, zone area was increased with respect to 100ppm, 300ppm, and 600ppm doses. In rest of treatments (Plant Protector and Copper oxy Chloride) no change was noticed at 100ppm and 300ppm concentrations. Plant protector at 600ppm was more effective. Agrimycine was more effective than Copper oxy chloride but do not differ statistically.

Khatua *et al.* (2013) *in vitro* screened the fungicides against *Xanthomonas axonopodis* pv. *betlicola*, bacterial pathogen of betelvine and recorded that there was no growth inhibition was achieved by Bavistin but Copper oxychloride found to be moderately effective

Basamma *et al.* (2014) evaluated the nine micronutrients against bacterial blight in pomegranate (*Xanthomonas axonopodis* pv. *punicae*). Among them $CuSO_4$ was recorded maximum inhibition zone of 0.60 mm at 0.05%, 0.75 mm at 0.1% and 0.95 mm at 0.2% 12.5 mm at 0.5% concentrations respectively.

Pawar *et al.* (2015) recorded that spraying of Copper-oxychloride completely inhibited the mango bacterial canker disease caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Xcmi).

2.7 Botanical extracts

Opara *et al.* (2008) observed 12 aqueous plant extracts to inhibit the growth of bacterial spot pathogen (*Xanthomonas compestris* pv. *vesicatoria*). Data obtained showed that in the *in vitro* experiment, all the plant extracts inhibited the growth of the bacterium varying degrees when compared with the untreated control. *Azadirachta indica* seed aqueous extract was the most effective (40.33%) while the least effective was *Chromolaena odorata* extract (17.33%).

Govindappa *et al.* (2011) found that the leaf extract of *Acasia vasica* was more effective in the suppression of Xoo growth in comparison to the control. When compared to bactericide, chloramphenicol the *A. vasica* showed more antibacterial activity against Xoo. However, the leaf extract of L. camera showed least antibacterial activity among the three plant species tested.

Reddy *et al*. (2012) studied on the *in vitro* efficacy of different aqueous extracts of vermicompost prepared from leaves of *Azardirachta indica, Lantana camera, Parthenium hysterophorous* for the management of the pathogen. Since the aqueous extract of vermicomposted neem showed better suppression of the pathogen.

Sajid *et al.* (2013) evaluated 3chemicals (Plant Protector, Agrimycine and Copper oxychloride) and plant extracts (*Citrullus colocynthis*, *Nicotiana tobaccum* and *Curcuma Lunga*) against colony growth of *Xanthomonas axonopodis* pv. *malvacearum*. Plant Protector was found most effective at 600 ppm after 72 hrs of treatment.

Islam *et al.* (2013) evaluated the herbal sensitivity of *X. axonopodis* with the plant extract of *Allium cepa*, *Allium sativum*, *Litchi chinensis*, *Vitis amurensis* and *Syzygium cumini*. Reportd that the pathogens were found most sensitive to *Allium sativum* and *Syzygium cumini* and resistant to *V. amurensis*.

Pawar and Pandit (2014) screened fresh leaf extracts of *A. indica* plants against 25 strains of Xcmi. The maximum activity was recorded against Xcmi.09 (Mean activity zone 21.86 mm) followed by Xcmi.07 (Mean activity zone 21.55 mm) and minimum against Xcmi.23 (Mean activity zone 18.30 mm) strain.

Pawar *et al.* (2014) investigated 42 different plants in *in vitro* condition against *Xanthomonas axonopodis* pv. *punicae*. Water extracts of fruits of five plants, *Mesua ferrea, Terminalia beleria, Piper nigrum, Emblica officinalis* and *Terminalia arjun*, inhibited all four isolates. MIC of these water extracts ranged between 2.25 to 0.15 mg/ml.

Gargade *et al.* (2014) noticed that the herbal extracts, fresh cow urine and fresh butter milk and various combinations of these materials showed antibacterial activity against *Xanthomonas axonopodis* pv. *punicae*. The highest antibacterial activity was observed for admixture of aqueous extract of *Datura metel* with aqueous extracts of *Acacia nilotica* and least activity was observed for ethanol extracts of *Acacia nilotica*.

Naqvi *et al.* (2014) showed the results of the poison food technique, *Mentha Piperita* to control the pathogen by inhibiting the mean bacterial colonies to 6.33 at (P=0.05) on 75ppm concentration followed by the *Aloe vera* with 12.66 at (P=0.05), *Azadirachta indica* and *Syzygium cumini* with 19.33 and 20.33 at (P=0.05) respectively while the disk diffusion technique showed also *Mentha Piperita* to produce best results against the pathogen by showing (7.91mm) inhibition zone followed by *Aloe vera* (7.21mm), *Azadirachta indica* (7.16mm), *Moringa oleifera* (6.43mm) and *Syzygium cumini* (6.13mm) at (P=0.05) on the same concentration.

Pawar *et al.* (2014) tested leaf extract of 37 plants against Xcmi. Out of them, leaf extract of *Ocimum sanctum* L. gave promising results. The maximum activity was recorded against Xcmi.21 (Mean activity zone of 20.36 mm) followed by Xcmi.07 (Mean activity zone of 20.11 mm) and minimum against Xcmi.14 (mean activity zone of 16.27 mm) strain under investigation.

Bhagwat and Datar (2014) evaluated *in vitro* antibacterial activity of herbal extracts against five plant pathogenic bacteria (*viz., Xanthomonas campestris*, *Xanthomonas axonopodis* pv. *punicae*, *Erwinia spp*., *Pseudomonas syringae* and *Xanthomonas citri*). Herbal extracts of leaves and rinds of *Garcinia indica*, rhizomes of *Curcuma aromatica*, roots of *Glyccyrrhiza glabra*, leaves of *Nyctanthes arbor-tristis* and seeds of *Vernonia anthelmintica* were used for screening. It was observed that extracts of *C. aromatica*, *G. indica* and *G. glabra* have shown lowest MBC values among other tested plant extracts.

2.8 PGPRs activities of Fluorescent *Pseudomonas* on soybean

Girish and Umesha (2005) studied that use of plant growth promoting rhizobacteria in managing bacterial canker disease of tomato. Tomato seeds were treated with PGPR strains *viz.,*, *Bacillus pumilus* INR7, *Bacillus pumilus* SE34, *Bacillus pumilus* T4, *Bacillus subtilis* GBO3, *Bacillus amyloliquefaciens* IN937a and *Brevibacillus brevis* IPC11 were subjected for seed germination and seedling vigor. Among the PGPR strains tested, only three strains (IN937a, GBO3 and IPC11) which showed enhancement in the seed quality parameters includes seed germination and seedling vigor.

Fallahzadeh and Ahmadzadeh (2010) studied on the ability of *Pseudomonads fluorescens* of cotton rhizosphere in induction of systemic resistance against disease bacterial blight of cotton and reported that all the isolates significantly suppressed the disease on plant. Suppression of disease in 5 of them was more than the reference strain *Pseudomonas aeruginosa* 7NSK2. 35Q and 7NSK2 had highest effect on growth of cotton plants as growth of plants treated with these isolates, were more than or equaled to healthy control plants. 10AQ drastically suppressed the disease but it had negative effect on plant and significantly decreased the growth factors of the plants.

Wahyudi *et al.* (2011) evaluated that *Pseudomonas spesies* are one of the rihizobacterial group that have an important role in plant growth promoter and plant health. Fourteen isolates identified as a non-pathogenic *Pseudomonas sp* that

produced IAA and Promoted enhancement of root length, shoot length, or number of lateral root.

Meera *et al.* (2012) isolated thirty five (Pf1 to Pf35) isolates of *Pseudomonas fluorescens* from the rizosphere of rice fields. Among these isolates Pf 13 and Pf 04 showing on par root length and shoot length. Both the Pf13 and Pf04 isolates were found significantly superior than other isolates in increasing the shoot length and root length over control. The isolates Pf13 was recorded high vigor index (3830) followed by Pf04 (3648). The least vigor index was recorded by Pf08 (2631).

Khendkar and Deshpande (2014) isolated 48 actinobacterial PGPRs obtained by spread plate technique. Actinobacterial isolates were tested for antagonistic activity against Rhizoctonia bataticola by dual culture method. Total 11 out of 48 actinobacterial isolates exhibited antagonistic activity against Rhizoctonia bataticola. Plant growth promoting activity of these 11 isolates was tested on soybean seeds by evaluating the seedling vigor index (SVI) by paper towel method. Total 8 isolates demonstrated significant antagonistic activity and plant growth promoting potential out of which ASG35 and ASG27 of Streptomyces were more effective.

CHAPTER-III

MATERIALS AND METHODS

The present study entitled **"Studies on Bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean"** was carried out at the Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, (C.G.).

The materials used and methods implied during the work of this studies are following as -

Experimental site

All the field experiments were conducted during *Kharif* 2014 at the experimental field of Department of Plant Pathology situated in the Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). Besides these field experiments, all the laboratory and polyhouse experiments were carried out at the laboratory and polyhouse (mist chamber) of Department of Plant Pathology College of agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

Experimental designs

The designs used all the field experiments and laboratory experiments were done by Randomized Block Design (RBD) and Complete Randomized Block Design (CRD) respectively.

3.1 Materials

3.1.1 Glass wares and plastic wares

Whenever required, the Borosil glassware, Tarson plastic plates, blotter paper of standard grade and chemicals of standard grade (Merck, Qualigens, S.D. fine ***etc.)*** were used during the course of investigation. All the glass wares, polythene bags, ethyl alcohol, formalin, sterile disc, Whatman filter paper, chemicals and other materials were procured from the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G.).

3.1.2 Equipments used

The following equipments or materials used in present investigation were-

1. Autoclave for media sterilization
2. BOD incubator for incubation

3. Binocular microscope
4. Compound microscope
5. Hot air oven for glasswares sterilization
6. Forceps, needles, blades, inoculation needle
7. Growth chamber
8. Laminar air flow for isolation and purification
9. Spirit lamp
10. Electronic weighing balance
11. Refrigerator
12. Microwave oven for melting the media
13. Thermo-hygrometer
14. Mortar and pestle
15. Vortex shaker for shaking of sample

3.1.3 Chemicals used

Analytical grade chemicals supplied by different manufacturers and some of the chemicals were procured from Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G.).

3.1.4 Medium used for bacterial culture

During the entire studies modified Wakimoto (1954) potato sucrose agar medium (PSA) was used having the following composition.

Peeled potato	250 g
Sucrose	20 g
Peptone	5 g
Sodium di-hydrogen orthophosphate	2 g
Calcium nitrate	0.5 g
Agar- agar	15 g
Distilled Water	1000 ml

Nutrient agar with the following composition was used for detection of seed-borne bacteria.

Beef extract	3 g
Bacto peptone	5 g
Glucose	5 g
Sodium chloride	5 g
Agar- Agar	15 g
Distilled water	1000 ml

For multiplication of *Pseudomonas fluorescens,* King's B (KMB) with following composition was used.

Protease peptone	20 g
Disodium hydrogen phosphate	1.5 g
Magnesium sulphate	1.5 g
Glycerol	10 ml
Agar	15 g
Distilled water	1000 ml
pH	7.2

Maintenance of cultures

The bacterial culture selected for the study was maintained on potato sucrose agar and bio control agent was maintained on King's B (KMB) agar slants.

3.1.5 Antibiotics and fungicides

Efficacy of seven antibiotcs and five fungicides presented in the (Table3.1 and 3.3) respectively were tested against *Xanthomonas axonopodis* pv. *glycines*.

3.1.6 Biocontrol agents used

Sixteen isolates of Fluorescent *Pseudomonas* presented in the (Table 3.2) were evaluated to test their efficacy against *Xanthomonas axonopodis* pv. *glycines*.

3.1.7 Botanical plant extracts used

Botanical extracts of different plant species presented in the (Table 3.4) were evaluated to test their efficacy against *Xanthomonas axonopodis* pv. *glycines*.

such as Neem, Adathoda, Ginger, Aonla, Onion, Jarayan, Gulmohar, Tulsi, Garlic, Kala jamun, Bhringraj, Karanz, Lemon grass and Mint.

3.2 Methods

3.2.1 Cleaning and sterilization of materials

Before to use, glasswares were kept for 24 hrs in cleaning solution containing 60.0 g of potassium dichromate ($K_2Cr_2O_7$), 60.0 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water and were washed with soap powder followed by washing in running tap water and then finally rinsed with distilled water. The dried glasswares were sterilized in hot air oven at 160ºC for 2 to 3 hrs. 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 1.1 Kg cm-² pressure for 20 minutes. The plastic plates were sterilized with ethyl alcohol surface sterilization and air dried before use.

3.2.2 Isolation and purification of the pathogen (*Xanthomonas axonopodis* pv. *glycines*)

During the *Kharif* season of 2014 bacterial pustule diseased samples were collected from the experimental field of the Department of Plant Pathology and carried to the laboratory for isolation of the pathogen.

The entire work of isolation was done in isolation chamber and laminar flow, which were sterilized by ethyl alcohol and UV radiation, prior to use. Fresh infected leaves of soybean plants showing bacterial pustule symptoms were surface sterilized with 95% ethyl alcohol for 30 seconds and washed properly with sterilized distilled water. Small pieces of infected portion of leaf was cut with the help of sterilized blade in such a manner that half of each piece consisted of healthy and the other half diseased portion. These pieces were surface sterilized with 1:1000 mercuric chlorides ($HgC1_2$) solution for 1 minute followed by 3 changes in sterilized water to remove traces of $HgCI_2$. Three to four pieces were taken on the sterilized slide with a drop of sterilized distilled water and kept inside the moist chamber for 48 hrs at room temperature (25 ± 2ºC). After 48 hrs “ooze test” was done then these pieces were chopped with the help of sterilized blade and loop of extract was inoculated on nutrient agar (NA) by the streak plate method

(Bray and Thorpe, 1954). Bacterial growth of *Xanthornonas axonopodis* pv. *glycines* was identified with the help of morphological characters and on the basis of descriptions given in the monograph of.*Xanthornonas axonopodis* pv. *glycines*. As soon as the characteristics colonies of the bacterium were observed in the plates, pure cultures were established by single colony isolation. They were maintained at 5ºC in refrigerator by sealing of 48 hrs old cultures on potato sucrose agar (PSA) medium with sterile paraffin oil for further work.

3.2.2.1 Identification of the pathogen (*Xanthomonas axonopodis* pv. *glycines*)

The bacterium was identified as *Xanthornonas axonopodis* pv. *glycines* on the basis of the colony colour and colony characters of the bacterium by confirming with standard reports Ishiyama (1922), Wakimoto (1955, 1967), Isaka (1970) and Lelliot and Stead (1987).

3.2.2.2 Pathogenicity Test

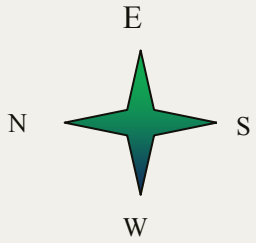
Pathogenicity test of the isolated pathogen was tested *in vivo* by the standard procedure as follows. 20 days old soybean plants, which were grown in hard plastic pots, were selected for pathogenicity test. The leaves of these plants were first surface sterilized with 95% ethyl alcohol for 30 seconds and washed 4 times with sterilized distilled water to remove residuals. Now sterilized leaves were inoculated with bacterial suspension of 0.3 OD by “pinprick method” during evening hrs. Later seedlings transferred to growth chamber that was preset to 25±2°C temperature and 95-96% relative humidity, for a period of 5 days. The seedlings were observed for the infection and symptoms production. Controls were maintained by spraying the plants only with distilled water. The pathogen was reisolated from the infected leaves and compared with the original culture to confirm its identity.

3.2.3 Screening of soybean varieties against bacterial pustule

A field experiment was conducted to evaluate the 16 varieties for its resistance or response against bacterial pustule disease of soybean during *kharif* 2014 under natural field condition at AICRP Soybean, College of Agriculture, Raipur.

Layout of experimental details are given below

Replication- 1	Replication- 2	Replication- 3
T1	T16	T10
T2	T15	T8
T3	T14	T6
T4	T13	T2
T5	T12	T4
T6	T11	T14
T7	T10	T16
T8	T9	T12
T9	T8	T1
T10	T7	T3
T11	T6	T9
T12	T5	T7
T13	T4	T11
T14	T3	T13
T15	T2	T5
T16	T1	T15



No. of genotype – 16
Design -- RCBD
Replication -- Three
Spacing:-
Rep. to Rep. – 1 m
Plot to Plot -- 60 cm
Row to Row - 30 cm
Plant to Plant – 10 cm
Date of sowing - 24/06/14
Observations: --

1. Disease incidence
2. Disease severity

1 m

3 m

The crop was raised as per recommended package of practices and protective irrigation was given as and when required. Per cent disease was calculated as per the standard area diagram developed by Mayee and Datar (1986).

For recording the disease intensity at field condition, 1 to 9 disease rating scale developed by Mayee and Datar (1986) was used. For this purpose five leaves located at the bottom, five middle and five top of the plant were chosen and scored.

Measurement of disease severity was carried out on five randomly selected plants in each plot after initiation of the disease. Observations on disease incidence and disease severity were recorded and calculated by using following formula. Effect of weather parameters on development of pustules in infected genotypes was also recorded.

$$\textbf{Per cent incidence (PI)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

$$\textbf{Per cent disease index (PDI)} = \frac{\text{Sum of observed numerical ratings}}{\text{No. of leaves observed x maximum grade}} \times 100$$

The data were statistically analyzed and interpretation of data was carried out in accordance with Walter (1997). The level of significance used in ‘F’ and ‘T’ test was P=0.05 and P=0.01. Critical differences were calculated wherever ‘F’ test was significant. The values percent disease index was subjected to angular transformation according to the table given by Sundarraj *et al*. (1974).

Observations of selected genotypes for their level of resistance were also measured by following the scale given by (Mayee and Datar 1986) which is as follows.

Rating	Description	Category
1	Normal, no spots, no lesions	Resistant
3	Spots on–few plants, up to 6% leaf area covered	Moderately resistant
5	Spots on many plants, 6-15% leaf area covered, no defoliation	Tolerant
7	Spots on all plants, 16-50% leaf area covered, dropping of few leaves	Moderately susceptible
9	Full size lesions / spots on all plants, more than 50% leaf area covered, defoliation and death of plant is common	Susceptible

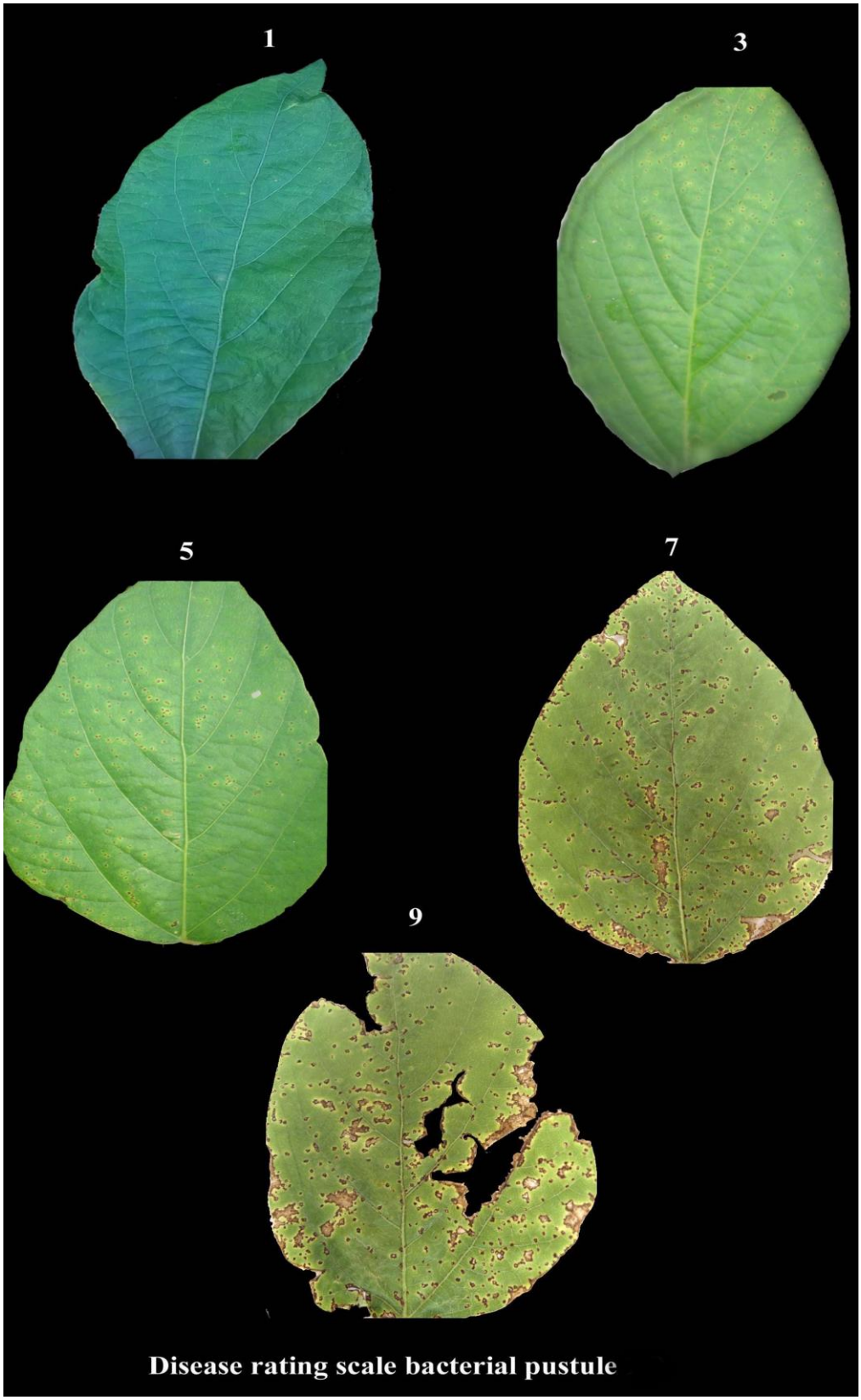


Plate 3.1 Disease rating scale of soybean pustule disease for screening of varieties

3.2.3.1 Effect of weather parameter on disease development of bacterial pustule

Three varieties *viz.*, Panjab-1, Monatta and NRC-7 were selected for studies on effect of weather parameters on disease development as well as spread of the disease within the plant and between the plants. The observations on among the plant and within the plant disease spread were recorded on ten days interval. First observation (D_1), second observation (D_2), third observation (D_3) and forth observation (D_4) were recorded on 1st August, 11st August, 21st August and 31st August, respectively.

Experimental details:-

1. **Varities** – a) Panjab-1
   b) Monatta
   c) NRC-7
2. **Design** - RCBD
3. **Repliction** – Threee
4. **Plot size** - 3x1 m
5. **Distane** -
   Plant to plant -- 10 cm
   Row to Row -- 30 cm

6. **Observation** – Observations were started just after initiation of disease and tagged the plants. We count the infected leaves within the plant for vertical disease spread and infected plants within 60 cm^2 area around the tagged plant for horizontal disease spread, than these counts were converted into disease incidence within the plants and among the plants with the help of following formula:-

$$\textbf{Per cent incidence (PI)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

Analysis of variance (ANOVA) was used to test the difference between the means. Means of the treatments were distinguished using the least significance difference (LSD) test.

MANAGEMENT

3.2.4 *In vitro* evaluation of various antibiotics against *Xanthomonas axonopodis* pv. *glycines*

Efficacy of seven antibiotics *viz.,* Streptomycin, Tetracyclin, Chloramphenicol, Cefotaxime, Ceftriaxone, Ampicillin and Penicillin were evaluated at different level of concentrations (100, 200, 300, 500 and 1000 ppm) against *Xanthomonas axonopodis* pv. *glycines* by using filter paper disc technique described by Cruickshank *et al*. (1975). First of all Bacterial suspension of *Xanthomonas axonopodis* pv. *glycines* was prepared. One milliliter of this suspension was poured in sterilized Petri dishes on to which about 20 ml of sterilized potato sucrose agar (PSA) medium was poured then allowed to solidify for one day. Bacterial suspension of 0.3 OD was uniformly spread with the help of L-spreader over the medium. Filter paper discs of 6 mm diameter were prepared by cutting Whatman filter paper with the help of punch device and sterilized in an autoclave at 1.1 Kg/cm^2 for 15 minutes. These discs were then dipped with the solution of antibiotics then kept at the middle of plates. Four replications were maintained for each treatment. Control was similarly included with discs dipped in sterilized water. These Petri dishes were then incubated at 27±1ºC. The diameter of zone of inhibition was measured at different intervals *viz.,* 24, 48 and 72 hrs. Finally data were statistically analyzed by using completely randomized design (CRD).

Table 3.1: List of antibiotics evaluated against *Xanthomonas axonopodis* pv. *glycines*

S. N.	Antibiotics	Chemical formula
1	Streptomycin	$C_{21}H_{39}N_7O_{12}$
2	Tetracyclin	$C_{22}H_{24}N_2O_8 \bullet HCl$
3	Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$
4	Cefotaxime	$C_{16}H_{17}N_5O_7S_2$
5	Ceftriaxone	$C_{18}H_{18}N_8O_7S_3$
6	Ampicillin	$C_{16}H_{19}N_3O_4S$
7	Penicillin	$C_9H_{11}N_2O_4S$

3.2.5 Evaluation of Fluorescent *Pseudomonas* against *Xanthomonas axonopodis* pv. *glycines* under *in vitro* condition

3.2.5.1 Well diffusion method

The antagonistic activity of sixteen isolates of Fluorescent *Pseudomonas* towards *Xanthomonas axonopodis* pv. *glycines* was tested by the well diffusion technique suggested by Zeller and Brulez (1987). A suspension of *Xanthomonas axonopodis* pv. *glycines* prepared from an overnight shaken culture, was spread over the surface of plates containing King's B agar medium. After drying, 0.05 ml of an overnight culture of Fluorescent *Pseudomonas* was pipetted into 8 mm well. Plates were then incubated at 25±2^{0}C for 1day and examined for inhibition zones. There were four replicates for each treatment. The diameter of zone of inhibition was measured at different intervals *viz.*, 24, 48 and 72 hrs. Finally data were statistically analyzed by using completely randomized design (CRD).

Table 3.2: Fluorescent *Pseudomonas* obtained from soil sample collected from different geographical locations of Chhattisgarh.

S.No.	Isolate No.	Geographical Location
1.	Pf 3	Rajnandgoan
2.	Pf 4	Abhanpur
3.	Pf 8	Raigarh
4.	Pf 14	Khadsiya
5.	Pf 18	Bhattapara
6.	Pf 20	Abmikapur
7.	Pf 22	Udyapur
8.	Pf 25	Karawdha
9.	Pf 27	Dhamtari
10.	Pf 29	Raipur
11.	Pf 33	Lohara
12.	Pf 34	Saragoan
13.	Pf 35	Thankhamariya
14.	Pf 36	Dhamdha
15.	Pf 38	Balod
16.	Pf 39	Patan

3.2.6 Bio efficacy of Fluorescent *Pseudomonas* against bacterial pustule under natural field condition

Three genotypes *viz.,* Panjab-1, Monatta and NRC-7 were selected for studies on bio efficacy of Fluorescent *Pseudomonas* against bacterial pustule under natural field condition. Single spray of isolates Pf 18 (Fluorescent *Pseudomonas*) was given at the time of first appearance of the symptoms, Selected on the basis of *in vitro* performance. Fluorescent *Pseudomonas* suspension (@ 10^8cfu/ml) was thoroughly sprayed on the foliage with the help of automizer. The treatment was continued until the fine droplets of isolates Pf 18 appeared on the foliage (Mew and Rosales, 1986). The disease intensity/index was recorded after 10 days of spray. Per cent disease control (PDC) was calculated by the following formula:

$$PDC = \frac{\text{Infection index in control plants} - \text{infection index in treatment plants}}{\text{Infection index in control plants}} \times 100$$

Data of five randomely infected plants were recorded in each plot after initiation of the disease, than plant is tagged. Per plant total number of leaves and infected number of leaves counted before and after foliar spray.

3.2.7 *In vitro* evaluation of various fungicides against *Xanthomonas axonopodis* pv. *glycines*

Efficacy of five fungicides *viz.,* Copper oxychloride, Dithane-M-45, Copper sulphate, Bavistin and Shri-saaf were evaluated at different level of concentrations (0.1, 0.2 and 0.3 per cent) against *Xanthomonas axonopodis* pv. *glycines* by using filter paper disc technique described by Cruickshank *et al.* (1975). First of all Bacterial suspension of *Xanthomonas axonopodis* pv. *glycines* was prepared. One milliliter of this suspension was poured in sterilized Petri dishes on to which about 20 ml of sterilized potato sucrose agar (PSA) medium was poured then allowed to solidify for one day. Bacterial suspension of 0.3 OD was uniformly spread with the help of L-spreader over the medium. Sterile filter paper discs of 10 mm diameter were then dipped with the solution of fungicides then kept at the middle of plates. Four replications were maintained for each treatment.

Control was similarly included with discs dipped in sterilized water. These Petri dishes were then incubated at 25±2ºC for 72 hrs. The diameter of zone of inhibition was measured in 3-4 angles and mean was considered for accuracy. Finally data were statistically analyzed by using completely randomized design (CRD).

Table 3.3: List of fungicides evaluated against *Xanthomonas axonopodis* pv. *glycines*

S. N.	Fungicides	Chemical formula	Trade name
1	Corbox 50% WP	Cu2(OH)3Cl	Copper oxychloride
2	Mencozeb 75 WP	(C4H6MnN2S4)x(Zn)y	Dithane-M-45
3	Bluestone	$CuSO_4 \cdot 5H_2O$	Copper sulphate
4	Carbendazim 50%WP	$C_{15}H_{21}N_5O_2S_4$	Bavistin
5	Carbendazim 12 WP + Mencozeb 63 WP		Shri-saaf

3.2.8 Botanical extracts

Extracts prepared from fourteen different plants were evaluated to find out their antimicrobial activity against *Xanthomonas axonopodis* pv. *glycines*.

3.2.8.1 Preparation of aqueous extract

Fresh plant leaves, bulb and rhizomes were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized mortar and pestle by adding 100 ml sterile water (1:1 w/v) to get hundred per cent concentration. The extract was filtered through double layer of muslin cloth. Finally filtrate thus obtained was used as stock solution.

3.2.8.2 Evaluation of botanical extracts by Cup Plate Method

It is a method of testing antibacterial activity. For this, the bacterial suspension was prepared by adding 10 ml sterile distilled water to 2 days old potato sucrose agar. Five drops of bacterial cell suspension were poured in sterilized petridishes (90 mm diameter) onto which 20 ml of potato sucrose agar was poured and slowly mixed and then allowed to solidify (Pawar & Papdiwal, 2012). In the centre of the medium, a cup cavity (well) of 8 mm diameter was made with sterilized No. 4 cork borer. This cup was filled with 0.05 ml of the leaf

extract. The petridishes were incubated for 24 hrs at 25±2°C. Cup cavity filed with sterile distilled water was also used as control in all the experiments. All the treatments were replicated for four times. The data was recorded by measuring the inhibition zones after 72 hrs. Diameter of the activity zone was measured in 3-4 angles and mean was considered for accuracy. Finally data were statistically analyzed by using completely randomized design (CRD).

Table 3.4 List of plant species evaluated against *Xanthomonas axonopodis* pv. *glycines*

S.N.	Common Name	Botanical Name	Plant parts used
1	Garlic	*Allium sativum*	Bulb
2	Onion	*Allium cepa*	Bulb
3	Kala jamun	*Syzygium cumini*	Leaf
4	*Adathoda*	*Adathoda vasica*	Leaf
5	Gulmohar	*Caesalpinia pulcherrima*	Leaf
6	*Lantana camara*	*Lantana camara*	Leaf
7	Tulsi	*Ocimum sanctum*	Leaf
8	Bhringraj	*Eclipta alba*	Leaf
9	Neem	*Azadirachta indica*	Leaf
10	Karanz	*Pongamia pinnata*	Leaf
11	Lemon grass	*Cymbopogon citratus*	Leaf
12	Aonla	*Emblica officinalis*	Leaf
13	Mint	*Mentha Piperita*	Leaf
14	Ginger	*Zingiber officinale*	Rhizome

3.2.9 PGPRs activities of Fluorescent *Pseudomonas* isolates on soybean

The isolates of Fluorescent *Pseudomonas* was grown in conical flasks (250 ml) containing 100 ml of King's B broth for 48 h on a rotary shaker (150 rev /min) at 25±2ºC. Cells were removed by centrifugation at 8000 rpm for 10 min at 4ºC and washed in sterile distilled water which was repeated for three times. The optical density of the suspension was adjusted to 0.45 at A660 nm using UV-visible spectrophotometer and this suspension was used for further studies. Seeds of soybean (cv. Punjab-1) were surface sterilized with 2% sodium hypochlorite for 30 sec, rinsed in sterile distilled water and dried overnight. 20 numbers of seeds was soaked for 4 hrs in 20 ml of antagonist inoculum taken in Petridish. These seeds were subjected for determing seed quality parameters like seed germination per cent and seedling vigour according to the standard procedure of ISTA (2005). Three replications for each treatment were also maintained. The seed germination, root length and shoot lengths of individual seedlings were measured. The Germination percentage was calculated by using following formula.

$$G = \frac{NGS}{TNS} \times 100$$

Where,

G = Germination %

NGS = Number of germinated seeds

TNS = Total number of seeds

Vigour index was calculated by using the formula as described by Abdulbaki and Anderson (1973); Vigour index (VI) = (Mean root length + Mean shoot length) × Germination (%).

CHAPTER-IV

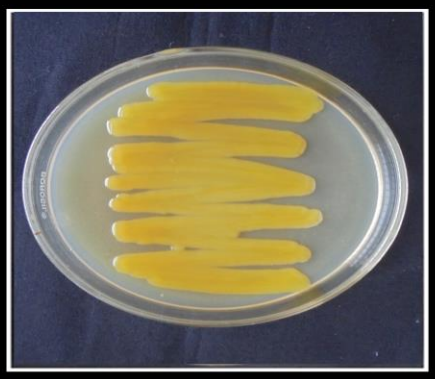
RESULTS AND DISCUSSION

Among the different seed and soil born diseases of soybean, bacterial pustule caused by *Xanthomonas axonopodis* pv. *glycines* is one of the important major disease. Hence, the present investigation entitled "Studies on bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean" was carried out in the laboratory as well as in the field during 2014 at Department of Plant Pathology, College of Agriculture, Raipur.

During the present investigation field observations were recorded to gather information on the occurrence of diseases of soybean in Instructional cum Research Farm, IGKV, Raipur (C.G.). Laboratory studies on isolation, pathogenicity and evaluation of antibiotics, botanicals, fungicides and bioagents against the pathogen under *in-vitro* condition was carried out. The results thus obtained are presented in different sections under this chapter.

4.1 Isolation, Pathogenicity and Purification

The bacterium was isolated from naturally infected soybean plant leaves parts on nutrient agar media (NA) after 72 hrs of inoculation. Single yellow, circular and semi-transparent colonies purified bacterium, when inoculated on soybean plants, the disease symptoms on the leaves started showing typical symptoms as pale green spots in interveinal area with elevated centers which became confluent and formed large brown patches. Symptom developed after 5 days of inoculation on the basis of pin prick method (Fig. 4.2). Nishiyama *et al.* (1986) collected an isolate from diseased soybean and identified *X. campestris* pv. *glycines*, as causal agent of bacterial pustule. Montova (1992) isolated *X. axonopodis* pv. *glycines* from diseased soybean cultivar Soyica P 31 and its pathogenicity was confirmed. Dhutraj *et al.* (2008) proved pathogenicity *X. axonopodis* pv. *glycines* on the locally grown soybean cultivar *Monetta*. Sain and Gour (2013) revealed that the *X. axonopodis* pv. *glycines* was isolated from naturally infected soybean plant leaves, stem parts on nutrient agar medium after 72 hrs of inoculation. Earliest symptom development was initiated in pin prick method (3-5 days after inoculation) as compared to carborundum method (4-6 days after inoculation).



1. *Xanthomonas axonopodis* pv. *glycines*

2. Field view

3 *Pseudomonas fluorescens broth culture*

Plate 4.1 1.*Xanthomonas axonopodis* pv. *glycines* culture, 2.Field view, 3. Fluorescent *Pseudomonas* broth culture

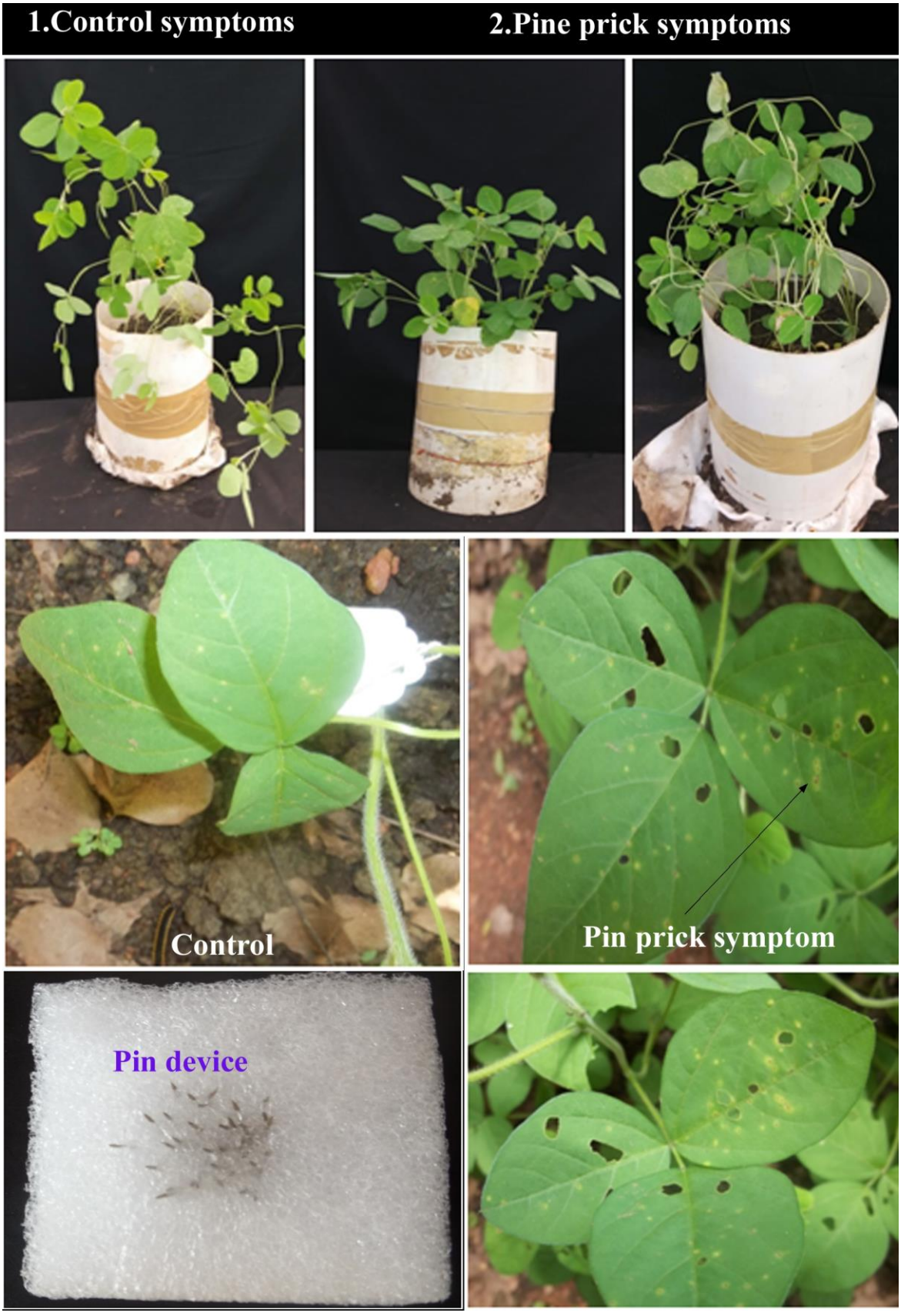


Plate 4.2 Testing of the pathogenicity by pin prick method

4.2 Screening of soybean varieties against bacterial pustule

Sixteen varieties were sown to record the per cent incidence of bacterial pustule of soybean at last growth stages. Result presented in Table 4.1 indicated that five varieties *viz.,* JS-72-44, JS-75-46, JS-71-05, JS-335 and Shivalic were free from the disease. Maximum disease incidence was recorded in var. Punjab-1 (64.34**%**) followed by NRC-7 (46.51**%**) and Monatta (38.73**%**).Varieties such as PK-262 (5.70%), JS-72-280 (4.66%), MACS (4.15%), PK-472 (3.57%), KHSB-2 (3.55%), VLS-58 (3.10%) and JS-93-05 (1.95%) showed analogous results. The results are in agreement with the findings of Verma and Dantre (2011) they recorded major disease incidence of sixteen varieties of soybean and found that nine varieties out of total entries showed nil disease incidence in case of bacterial pustule. Nandini (2012) carried out a survey on incidence of cowpea bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* and revealed that the highest disease severity (14.32 PDI) was observed in Belgaum district followed by Gadag and Dharwad districts. Lowest disease severity (9.58 PDI) was observed in Haveri district. Jagtap *et al*. (2012) carried out a survey in 8 districts (Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad) of Marathwada region during June to August in Kharif, 2009 to 2010. In all, 69 soybean fields were surveyed (roving survey) for recording the severity and incidence of soybean blight. The most serious pod disease was noticed on the soybean field of Parbhani district, followed by Hingoli, Nanded, Latur and Beed. The variety JS-335 showed the maximum pod blight severity in all surveyed districts.

Table 4.1 Disease incidence of different varieties of soybean

S. No.	Genotype	Bacterial pustule (Per cent incidence)
1.	JS-72-44	0.00
2.	JS-75-46	0.00
3.	JS-71-05	0.00
4.	JS-72-280	4.66
5.	PK-262	5.70
6.	PK-472	3.57
7.	MACS-58	4.15
8.	JS-93-05	1.95
9.	Punjab-1	64.38
10.	Bragg	3.12
11.	Monatta	38.73
12.	KHBS-2	3.55
13.	NRC-7	46.51
14.	VLS-58	3.10
15.	JS-335	0.00
16.	Shivalic	0.00

Screening of sixteen varieties of soybean was sown to record the per cent of severity. Among sixteen varieties, eight varieties exhibited resistant reaction, five varieties were moderately resistant, no any variety showed moderately susceptible reaction and three varieties showed susceptible reaction, whereas none varieties had tolerant reaction. The results are coincided with the findings of Mahesha (2006) who recorded that thirteen out of 204 genotypes (Bragg, EC-245988, Hardee, Himso-1597, Lee, MACS-450, MAUS-681, NRC-2, NRC-12, PK-472, PS-1092, PS-1347 and SL-518) were resistant against bacterial pustule of soybean. Surin *et al*. (1993) screened twelve varieties for resistance against bacterial pustule of soybean they found that six varieties showed resistance against the test pathogen. Suryadi *et al*. (2012) evaluated resistant genotypes of soybean to three major soybean foliar diseases, viz., Soybean Stunt Virus (SSV), Bacterial

Blight (BB), and Bacterial Pustule (BP) was done by screening 100 soybean accessions of ICABIOGRAD - Bogor germplasm collections. They found that 43 varieties were resistant, 41 moderately resistant, 14 moderately susceptible and 2 were susceptible. Gaikwad (1995) screened 91 varieties of soybean against bacterial pustules of these, 39 were totally free of the disease and 11 were resistant.

Table.4.2: Per cent d**isease severity of different varieties of soybean**

S. No.	Rating	Category	Varieties
1.	1	Resistant	JS-72-44, JS-75-46, JS-71-05, PK-472, JS-93-05,VLS-58, Bragg JS-335,
2.	3	Moderately resistant	JS-72-280, PK-262, MACS-58, KHBS-2, Shivalic
3.	5	Tolerant	Nil
4.	7	Moderately susceptible	Nil
5.	9	Susceptible	Punjab-1, NRC-7 ,Monatta
Total Entries			16

4.2.1 Spread of bacterial pustules disease

The observations on disease spread among the plants and within the plants were recorded on ten days interval. First observation (D_1), second observation (D_2), third observation (D_3) and forth observation (D_4) were recorded on 1st August, 11th August, 21th August and 31th August, respectively.

In case of disease spread among the plants, during initial days of disease appearance, first observation (D_1) was recorded with 6.0, 3.9 and 4.1 per cent disease spread in varieties Punjab-1, Monatta and NRC-7, respectively. At second observation (D_2) disease incidence 29.5, 21 and 24 per cent were observed in varieties Punjab-1, Monatta and NRC-7, respectively. At third observation (D_3) 40.5, 33.5 and 37.0 per cent progress in disease among the plants were observed in varieties Punjab-1, Monatta and NRC-7, respectively. At last observation (D_4) it was 56.0, 39.5 and 41.5 per cent progress in disease spread among the plants were

observed varieties Punjab-1, Monatta and NRC-7, respectively. In overall view maximum disease spread among the plant was found in genotypes between 1st August to 10th August, while within the plants maximum progess of disease was observed in genotype Monatta betwwen 21th to 30th August. Based on above observations maximum disease spread was recorded in varieties Punjab-1 followed by NRC-7 and Monatta.

Disease spread within the plants during initial days of disease appearance was recorded with 7.3, 3.6 and 4.2 per cent in varieties Punjab-1, Monatta and NRC-7, respectively at first observation (D_1). At the time of second observation (D_2) 24, 21.7 and 22.7 per cent disease incidence was observed in genotypes Punjab-1, Monatta and NRC-7, respectively. At third observation (D_3) spread in disease within the plants was observed 34.4, 33.5 and 37.1 per cent in varieties Punjab-1, Monatta and NRC-7, respectively. At last observation (D_4) disease incidence 60.0, 64.8 and 65.6 per cent were recorded in genotypes Punjab-1, Monatta and NRC-7, respectively. Based on above observations maximum disease spread was recorded in varieties Punjab-1 followed by NRC-7 and monatta. It can be noticed that disease spread of within the plant was higher than disease spread of among the plant.

Table 4.3 Disease spread among the plants in terms of percentage incidence in three varieties *viz.*, Punjab-1, Monatta and NRC-7

S. No.	Date of observation	% disease incidence		
		Punjab-1	**Monatta**	**NRC-7**
1.	1 st Aug.(D_1)	6	3.9	4.1
2.	11th Aug.(D_2)	29.5	21	24
3.	21th Aug.(D_3)	40.5	33.5	37
4.	31th Aug.(D_4)	56	39.5	41

*Average of three replication

Table 4.4 Disease spread within the plants in terms of percentage incidence in three varieties *viz.*, Punjab-1, Monatta and NRC-7

S. No.	Date of observation	% disease incidence		
		Punjab-1	**Monatta**	**NRC-7**
1.	1 st Aug.(D_1)	7.3	3.6	4.2
2.	11th Aug.(D_2)	24.0	21.7	22.7
3.	21 th Aug.(D_3)	34.4	33.5	37.1
4.	31 th Aug.(D_4)	60.0	64.8	65.6

*Average of three replication

From Table 4.5 disease indicated significant positive correlation between maximum temperature & disease spread for all the varieties Monatta (0.98*), NRC-7 (0.97*) and Punjab-1 (0.96*) while there was highly significant negative correlation between morning relative humidity (%) and disease spread, Monatta (-0.99**), NRC-7 (-0.99**) and Punjab-1 (-0.99**). There was significant positive correlation between minimum temperature and disease spread in case of two varieties, NRC-7 (0.98*) and Monatta (0.96*) and morning vapour pressure and horizontal spread for same varieties (0.96* & 0.96*) for NRC-7 and Monnatta repectivily. There was significant negative correlation between rainfall, evening relative humidity (%) with disease spread among the plant for two varieties NRC-7 (-0.97* & -0.97*) and Monatta (-0.96* and -0.97*).

The experimental data presented in Table 4.6 revealed that there was no significant correlation between spread of disease within the plant and all weather parameter only were wind velocity parameter showed negatively significant correlation with disease spread of bacterial pustule within the plant for all the varieties, Punjab-1(-0.97*), NRC-7 (-0.97*) and Monatta (-0.96*). So we may conclude that by all the weather parameters except wind velocity did not significantly increase or decrease the disease spread of bacterial pustule within the plant during the observation period.

These results were contradicted with the findings of Naqvi (2012) he suggested that there was significant correlation among incidence of bacterial blight

of sesamum caused by *Xanthomonas campestris* pv. *sesami* with environmental parameters such as maximum and minimum temperature, relative humidity and rainfall at 15 variety/line. Roberts (1997) investigated the effect of weather conditions on simultaneous local (plant to plant) spread and infection of peas (*Pisum sativum*) with bacterial blight (*Pseudomonas syringae* pv. *pisi*) by exposing susceptible bait plants for 24 hrs periods in infected field plots. He found that rainfall rate and wind run were the most important explanatory variables for the mean number of lesions followed by maximum temperature, rainfall duration and rainfall in the previous week and disease incidence in the surrounding crop. Shrivastava and Singh (1997) conducted an experiment to find out correlation between disease incidence of bacterial blight of sesamum (*Xanthomonas campestris* pv. *sesami*) and observed that temperature (29 -29.4 ºC), rainfall (8.90-9.97 mm), relative humidity (88–90%) and 3 to 4 cloudy days enhances the disease severity. Adhikari *et al*. (1994) suggested that there was highly positive correlation between bacterial blight progressions with environmental factors such as temperature, rainfall and relative humidity.

Table: 4.5 Correlation coefficient of disease spread among the plants and weather parameters in varieties Punjab-1, Monatta and NRC-7

Weather parameter	Panjab- 1	Monatta	NRC-7
Maximum Temperature (^{0}C)	0.96*	0.98*	0.97*
Minimum Temperature (^{0}C)	0.93	0.96*	0.98*
Rainfall (mm)	-0.95*	-0.96*	-0.97*
Morning Relative humidity (%)	-0.99**	-0.99**	-0.99**
Evening Relative humidity (%)	-0.94	-0.97*	-0.97*
Morning Vapor pressure (mm)	0.91	0.96*	0.96*
Evening Vapor pressure (mm)	0.49	0.48	0.43
Wind velocity (WS km/h)	-0.93	-0.92	-0.89
Wind velocity (Ep km/h)	0.69	0.79	0.80
Sunshine hours (hours)	0.76	0.85	0.87

Degree of freedom = 2
* r at 0.05% = 0.95
** r at 0.01% = 0.99

Table: 4.6 Correlation coefficient of disease spread within the plants and weather parameters in varieties Punjab-1, Monatta and NRC-7

Weather parameter	Punjab-1	Monatta	NRC-7
Maximum Temperature (^{0}C)	0.92	0.90	0.91
Minimum Temperature (^{0}C)	0.75	0.72	0.74
Rainfall (mm)	-0.78	-0.76	-0.77
Morning Relative humidity (%)	-0.89	-0.87	-0.86
Evening Relative humidity (%)	-0.86	-0.84	-0.86
Morning Vapor pressure (mm)	0.79	0.76	0.78
Evening Vapor pressure (mm)	0.70	0.71	0.71
Wind velocity (WS km/h)	-0.97*	-0.96*	-0.97*
Wind velocity (Ep km/h)	0.53	0.49	0.52
Sunshine hours (hours)	0.55	0.50	0.53

Degree of freedom = 2
* r at 0.05% = 0.95
** r at 0.01% = 0.99

4.3 *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines*

Efficacy of seven antibiotics were evaluated against the pathogen *Xanthomonas axonopodis* pv. *glycines* at five different level of concentrations (100, 200, 300, 500 and 1000 ppm) by "disc diffusion method". The data presented in Table 4.7 and fig. 4.2, revealed that most effective zone of inhibition was achieved by Streptomycin with 15.75 mm zone of inhibition followed by Tetracyclin (14.25 mm) and Chloramphenicol (12.25) at 1000 ppm level of concentrations after 24 hrs of incubation. Streptomycin, Tetracyclin and Chloramphenicol showed almost similar type of results at 300 ppm and same result at 500 ppm level of concentration 11.75 mm zone of inhibition. It was also revealed from the data, that Chloramphenicol showed almost similar type of results at 500 ppm and 1000 ppm level of concentrations. It means we can say that using 500 ppm of Chloramphenicol will be better rather than the 1000 ppm concentration. Cefotaxime did not inhibit the growth of the pathogen at 100, 200 and 300 ppm level of concentrations and also was not found so much effective

against the pathogen at 500 (4.50, 8.50 and 15 mm) and 1000 ppm (5.50, 9.50 and 19 mm) after 24, 48 and 72 hrs of incubation respectively. Streptomycin, Tetracyclin and Chloramphenicol was not found so much effective against the pathogen at concentrations of 100, 200 and 300 ppm, whereas Ceftriaxone, Amplicillin and Penicillin did not inhibit the growth of the pathogen for all the concentrations at 24, 48 and 72 hrs of incubation. It can be concluded that they are not effective against the test pathogen. Xue Feng *et al*. (2012) recorded that among the 5 chemicals *viz.,* Streptomycin, Zhongshengmycin, Peracetic acid, Copper hydroxide, Ziram and Bouillie bordelaise used, Streptomycin showed better results over control on citrus canker disease in the field. The results are corroborated with the findings of Khatua *et al*. (2013). They found that Penicillin G did not inhibit the growth of the pathogen. Amplicillin, Cefotaxime and Cetriaxone were least effective against *Xanthomonas axonopodis* pv. *betlicola* bacterial pathogen of betelvine. Islam *et al*. (2014) reported that Cefotaxime did not exhibit any inhibition on growth of *Xathomonas axonopodis*. The results are in agreement with the findings of (Khan *et al*., 2012). They found that, Streptomycin and Chloramphenicol significantly inhibited the growth of *Xanthomonas oryzae* pv. *oryzae* (Xoo). Workers like Ingole *et al*. (2001) and (2004), Khan *et al*. (2005), and Khatua *et al*. (2013) observed almost similar type of findings.

Among the antibiotics, Tetracyclin exhibited highest inhibition zone (26 mm) followed by Chloramphenicol (25.50 mm) and Streptomycin (21.25 mm) at concentration of 1000 ppm after 48 hrs of incubation (Data presented inTable 4.8 and fig 4.2). Tetracyclin and Chloramphenicol showed analogous results at 500 ppm and 1000 ppm level of concentrations. Chloramphenicol showed highest zone of inhibition 17.25 mm at 300 ppm followed by Tetracyclin 16.50 mm and Streptomycin 14.50 mm. However, Tetracyclin was showed as highest inhibition zone 11.50 mm and 5.50 mm at 200 and 100 ppm level of concentrations. Ravikumar and Khan (2000) found that streptomycin was moderately effective against *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato under *in vivo* condition. Naqvi *et al*. (2014) achieved maximum inhibition zone (28.31 mm) by Chloramphenicol at 100 ppm followed by Amplicillin trihydrate

proof to be second most effective antibiotic to control *Xanthomonas oryzae* pv. *oryzae*.

Table 4.7 *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* after 24 hrs of incubation

Treatment	Antibiotic	Zone of Inhibition (mm), at different level of concentration %				
		100	200	300	500	1000
T1	Streptomycin	3.07	5.50	9.75	11.75	15.75
T2	Tetracyclin	3.75	6.25	9.50	11.75	14.25
T3	Chloroamphenicol	0.50	1.50	9.00	11.75	12.25
T4	Cefotaxime	0.00	0.00	0.00	4.45	5.50
T5	Ceftriaxone	0.00	0.00	0.00	0.00	0.00
T6	Amplicillin	0.00	0.00	0.00	0.00	0.00
T7	Penicillin	0.00	0.00	0.00	0.00	0.00
T8	Control	0.00	0.00	0.00	0.00	0.00
	Mean	0.91	1.66	3.53	4.40	5.28
	Sem±	0.46	0.33	0.53	0.42	0.42
	CD (5%)	1.361	0.98	1.57	1.24	1.24

It was revealed from the experimental data presented in the Table 4.9 and fig 4.2 that Tetracyclin found to be most effective for inhibiting the growth of the pathogen (40 mm and 34.25 mm) followed by Chloramphenicol (37.25 mm and 31.75 mm) and Streptomycin (27.75 mm and 21.50 mm) after 72 hrs of incubation at 1000 and 500 ppm concentrations respectively. Chloramphenicol and Tetracyclin inhibited the growth of the pathogen in similar manner with 28.75 mm and 27.50 mm zone of inhibition respectively at 300 ppm of concentration.

Table 4.8 : ***In vitro*** **evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* after 48 hrs of incubation**

Treatment	Antibiotic	Zone of Inhibition (mm), at different level of concentration %				
		100	**200**	**300**	**500**	**1000**
T1	**Streptomycin**	5.50	10.00	14.50	17.25	21.25
T2	**Tetracyclin**	7.50	11.50	16.50	21.75	26.00
T3	**Chloroamphenicol**	1.00	2.50	17.25	21.00	25.50
T4	**Cefotaxime**	0.00	0.00	0.00	8.50	9.50
T5	**Ceftriaxone**	0.00	0.00	0.00	0.00	0.00
T6	**Amplicillin**	0.00	0.00	0.00	0.00	0.00
T7	**Penicillin**	0.00	0.00	0.00	0.00	0.00
T8	**Control**	0.00	0.00	0.00	0.00	0.00
	Mean	1.75	3.00	6.03	7.50	10.28
	Sem±	0.72	0.50	0.98	0.63	0.63
	C.D. (5%)	2.11	1.46	2.88	1.89	1.86

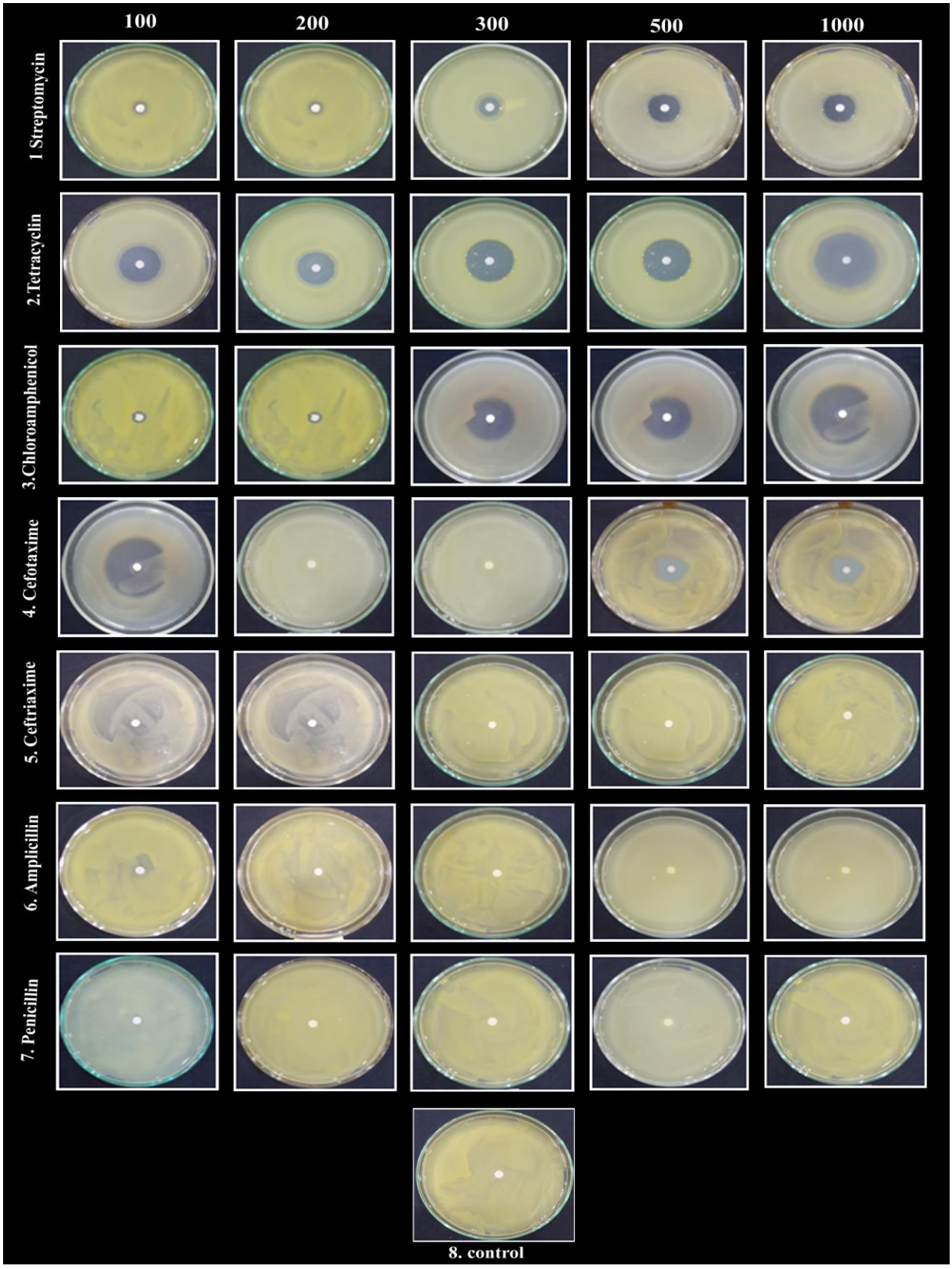


Plate 4.3 *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* after 72 hrs of incubation

Tetracyclin found most effective at all the concentrations and all the incubation period (24, 48 and 72 hrs). This was followed by Chloramphenicol and Streptomycin. The results are lined with the findings of Khatua *et al*. (2013), they evaluated 31 antibacterial antibiotics and 6 antibacterial among them Tetracyclin and Chloromphenicol were found to be most effective against *Xanthomonas axonopodis* pv. *betlicola*, bacterial pathogen of betelvine. Pawar (2015) observed that Tetracyclin was comparatively more effective to control mango bacterial canker disease. However, Chloramphenicol and Streptomycin were effective against the development of mango bacterial canker disease on fruits. Islam *et al*. (2014) reported that Chloramphenicol was most effective for all the isolates of *Xathomonas axonopodis* pv. *citri* causal agent of citrus canker. Khan *et al*. (2005) recorded that Streptomycin exhibited moderate efficacy against the bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) of rice. It means we may conclude that best three Tetracyclin, Chloramphenicol and Streptomycin can be applied for the control of the disease after the field evaluation of these antibiotics.

Table 4.9 *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* after 72 hrs of incubation

Treatment	Antibiotic	Zone of Inhibition (mm), a t different level of concentration %				
		100	200	300	500	1000
T1	Streptomycin	7.50	13.75	18.25	21.50	27.75
T2	Tetracyclin	12.75	20.00	27.50	34.25	40.00
T3	Chloroamphenicol	3.50	5.75	28.75	31.75	37.25
T4	Cefotaxime	0.00	0.00	0.00	15.00	19.00
T5	Ceftriaxone	0.00	0.00	0.00	0.00	0.00
T6	Amplicillin	0.00	0.00	0.00	0.00	0.00
T7	Penicillin	0.00	0.00	0.00	0.00	0.00
T8	Control	0.00	0.00	0.00	0.00	0.00
	Mean	2.96	4.93	9.31	12.81	15.50
	Sem±	1.10	0.97	1.31	1.34	1.54
	C.D. (5%)	3.23	2.86	3.84	3.93	4.54

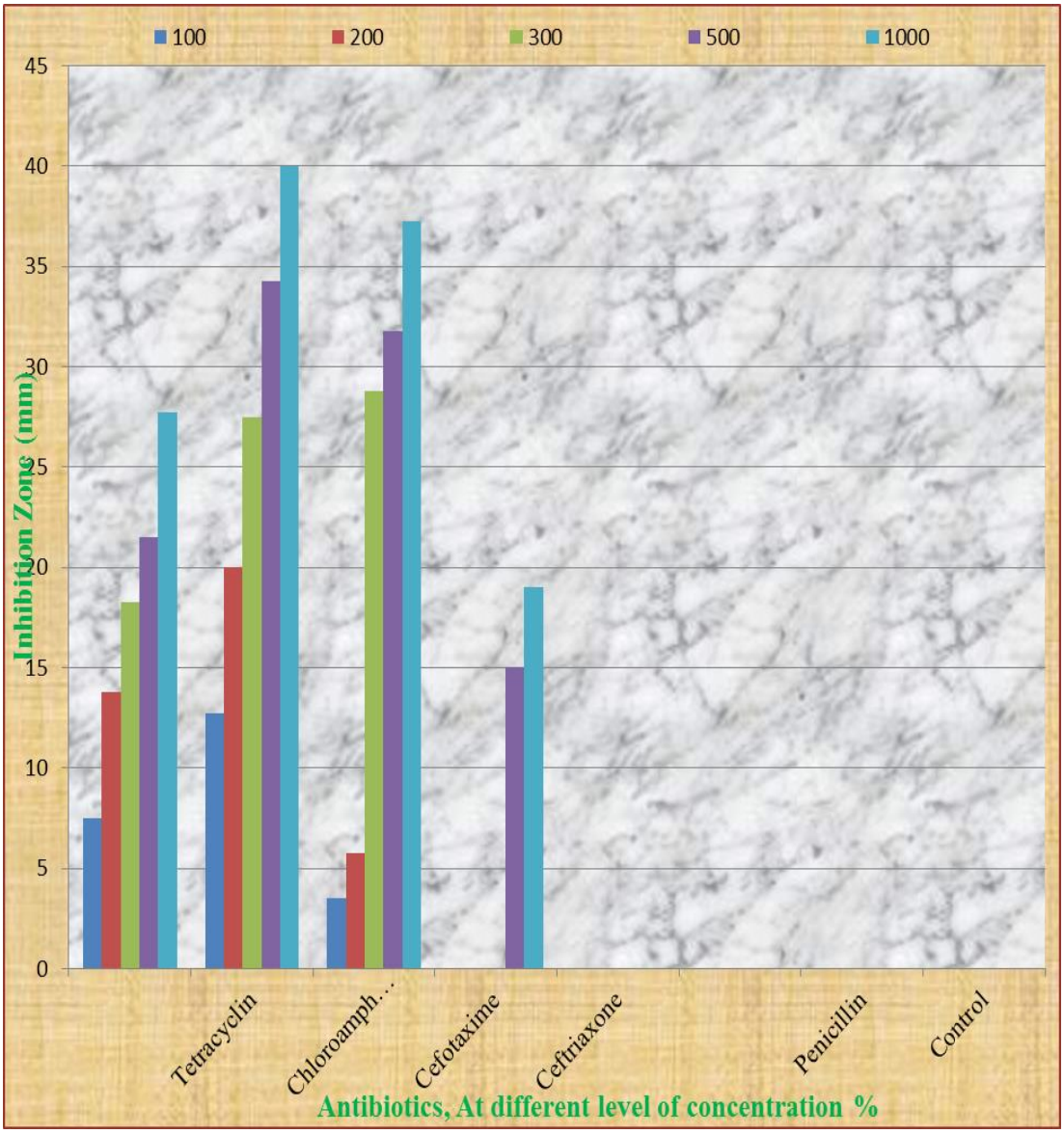


Fig. 4.1 *In vitro* evaluation of antibiotics against *Xanthomonas axonopodis* pv. *glycines* different hrs

4.4 *In vitro* evaluation of Fluorescent *Pseudomonas* against *Xanthomonas axonopodis* pv. *glycines*

Antagonistic activity of sixteen isolates of Fluorescent *Pseudomonas* were evaluated against *Xathomonas axonopodis* pv. *glycines* under *in vitro* condition. All the bacterial isolates significantly inhibited the growth of the test pathogen. Among the sixteen isolates, Pf 18 was found to be most significantly effective in inhibiting the growth of the pathogen and with inhibition zone of 37.33 mm, 41.00 mm and 46.00 mm after 24, 48 and 72 hrs of incubation period respectively. This was followed by Pf 34 (34.33 mm) Pf 33 (37.00 mm) and Pf 35 (43.00 mm) after 24, 48 and 72 hrs of incubation period respectively. Isolates such as Pf 25 (30.00 and 35.33 mm), Pf 33 (31.00 and 37.00 mm), Pf 34 (34.33 and 36.67 mm) and Pf 35 (30.00 and 36.33 mm) were at par with Pf 18 (37.33 and 41.00 mm) after 24 and 48 hrs of incubation respectively. Similarly isolated such as Pf 22 (36.33 mm), Pf 25 (39.33 mm), Pf 27 (39.00 mm) and Pf 33 (41.33 mm), Pf 34 (41.00 mm) and Pf 35 (43.00 mm) were at par to Pf 18 (46.00 mm) after 72 hrs of incubation. Least inhibition zone was achieved by Pf 4 (8.00, 9.33 and 12.33 mm) followed by Pf 36 (17.33, 20.33 and 23.67 mm) after 24 ,48 and 72 hrs of incubation period respectively. The results are coincided with the findings of Gangwar and Sinha (2012). They evaluated seven isolates of *Pseudomonas fluorescens* and found that all the isolates significantly reducing disease severity over the control. Manmeet and Thind (2002) reported that *Pseudomonas fluorescens* was inhibitory to the test *Xanthomonas oryzae* pv. *oryzae*. Kumar *et al*. (2009) evaluated four bioagents against *Xanthomonas oryzae* pv. *oryzae* (BLB of rice) and results revealed that among bioagent *Pseudomonas fluorescens* and *Trichoderma harzianum* restricted maximum growth of pathogen. Raut *et al*. (2010) observed that *Pseudomonas fluorescens* (Pf-1) significantly inhibited the growth of *Xanthomonas axonopodis* pv. *malvacearum* (Bacterial blight of cotton). Workers like Giri *et al*. (2008) and Yenjerappa *et al*. (2013) observed almost similar type of findings.

Table 4.10 ***In vitro*** **evaluation of Fluorescent** ***Pseudomonas*** **against** ***Xanthomonas axonopodis*** **pv.** ***glycines***

Treatment	Fluorescent *Pseudomonas* isolates no.	Zone of inhibition (mm), at different incubation period		
		24 hrs	48 hrs	72 hrs
T1	**Pf 3**	22.67	27.00	28.67
T2	**Pf 4**	8.00	9.33	12.33
T3	**Pf 8**	22.00	26.00	33.00
T4	**Pf 18**	37.33	41.00	46.00
T5	**Pf 22**	24.33	30.67	36.33
T6	**Pf 24**	20.33	22.67	24.67
T7	**Pf 25**	30.00	35.33	39.33
T8	**Pf 27**	26.00	30.33	39.00
T9	**Pf 29**	22.67	27.00	31.67
T10	**Pf 30**	24.67	30.00	35.33
T11	**Pf 33**	31.00	37.00	41.33
T12	**Pf 34**	34.33	36.67	41.00
T13	**Pf 35**	30.00	36.33	43.00
T14	**Pf 36**	17.33	20.33	23.67
T15	**Pf 38**	20.33	23.33	27.33
T16	**Pf 39**	28.00	31.67	34.67
T17	**Control**	0.00	0.00	0.00
	Mean	23.470	27.33	31.61
	Sem (+/-)	2.543	3.07	3.566
	CD (5%)	7.346	8.86	10.299

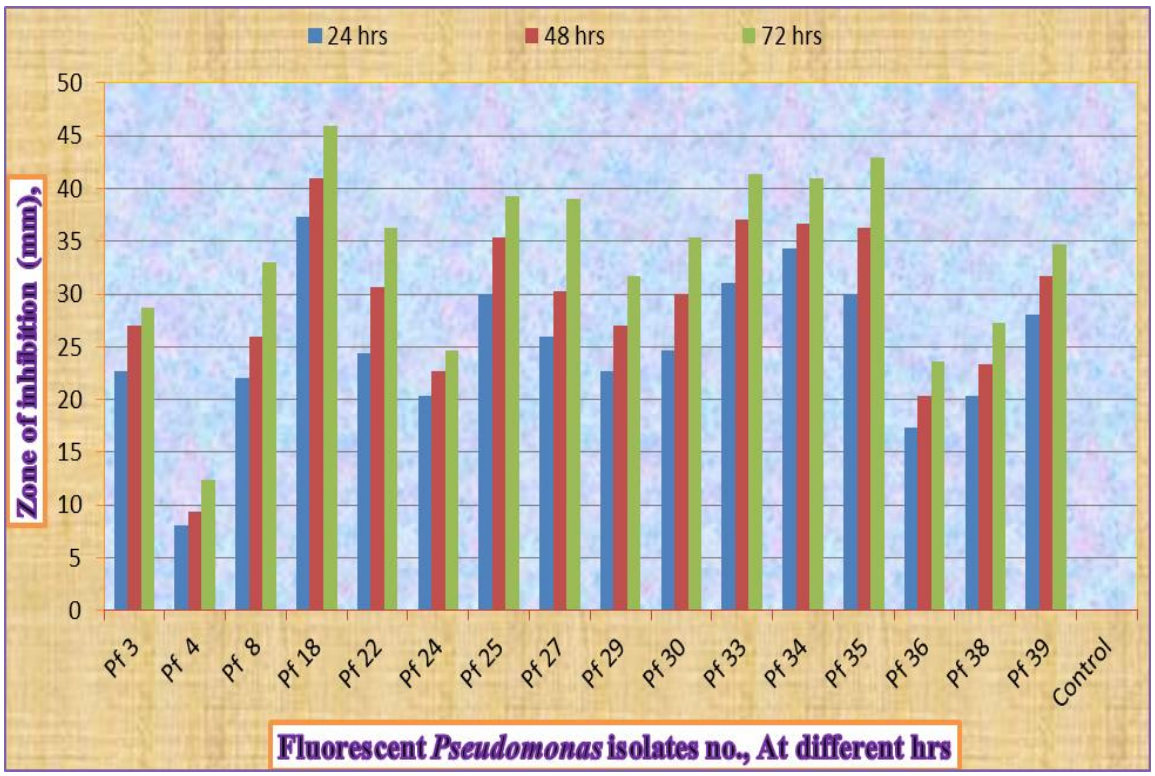


Fig. 4.2 *In vitro* evaluation of Fluorescent *Pseudomonas* against *Xanthomonas axonopodis* pv. *glycines*

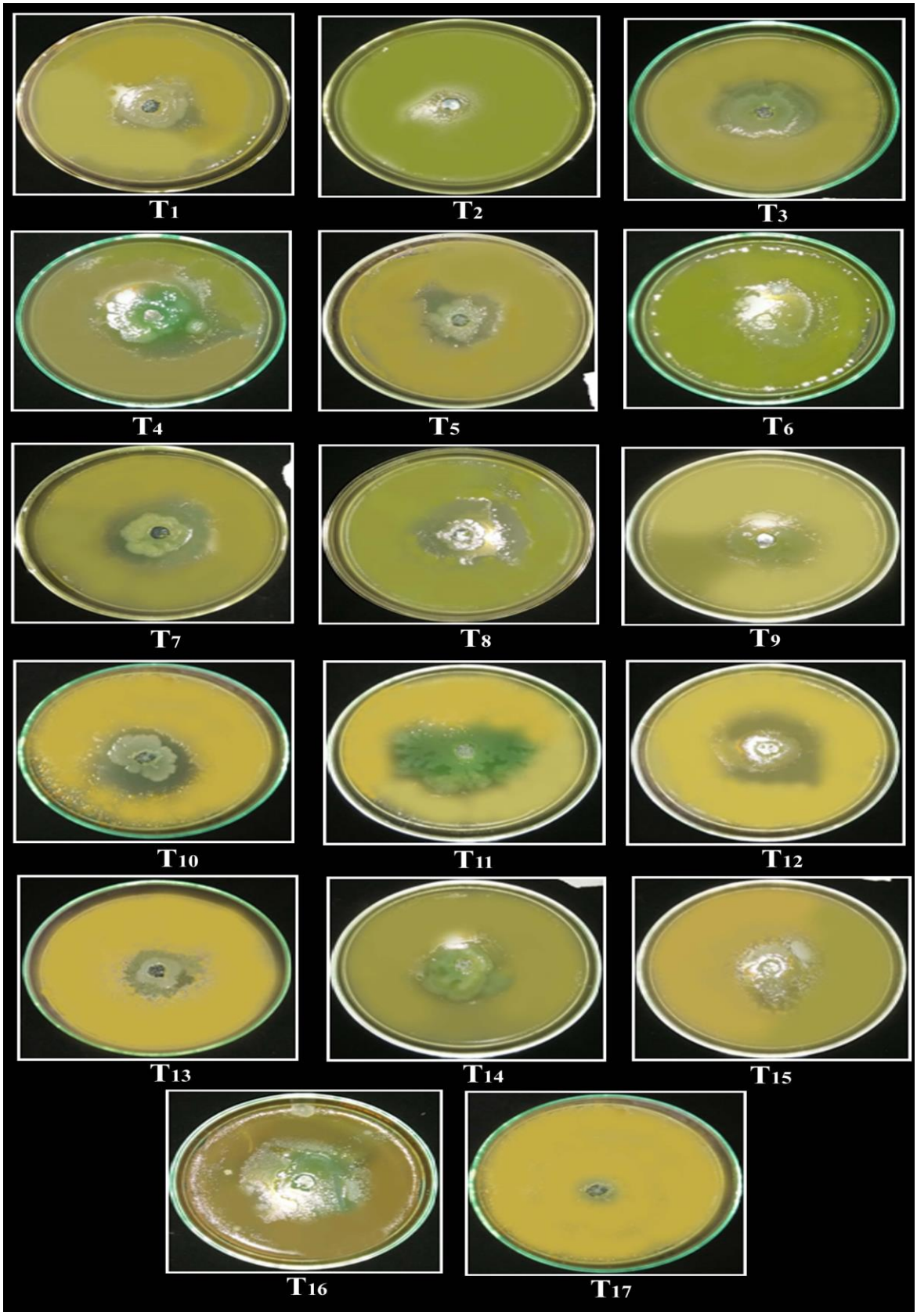


Plate 4.4 *In vitro* evaluation of Fluorescent *Pseudomonas* against *Xanthomonas axonopodis* pv. *glycines*

4.5 Bio efficacy of Fluorescent *Pseudomonas* against bacterial pustule under field condition

Bio efficacy of Fluorescent *Pseudomonas* was evaluated against bacterial pustule of soybean under field condition. It was revealed from the experimental data presented in the Table 4.11 and fig 4.3 that highest inhibition of pustule was recorded for variety Monatta with (17.80%) inhibition followed by NRC-7, (15.10%) inhibition after foliar application of Fluorescent *Pseudomonas*. Least inhibition was found in case of Punjab-1 (12.07%). It was also revealed from the data that only a single variety, Monatta was least infected with the disease. Hence we may say that variety, Monatta is the best over two other varieties. The results are coincided with the findings of Sain (2010) who reported that *Pseudomonas fluorescens* significantly inhibited the pustules in soybean plant under field condition. Lodha (2001) recorded inhibition of bacterial blight intensity and increment of seed yield in cluster bean after foliar application of bacterial antagonists. Ganeshan and Kumar (2005) suggested that application of *Pseudomonas fluorescens* reduces severity of various diseases. The results are in agreement with the findings of workers like Manonmani *et al*. (2007), Singh and Singh (2007), Raut *et al*. (2010) and Yenjerappa *et al*. (2013).

Table 4.11 Bio efficacy of Fluorescent *Pseudomonas* against bacterial pustule under field condition

S. No.	Varieties	PDI		% disease control
		Before application	After application	
1.	Punjab-1	11.60	32.81	12.07
2.	Monatta	10.19	30.86	17.80
3.	NRC-7	10.93	31.66	15.10
	Control	12.03	37.30	0
	Mean	11.18	33.15	14.99

4.6 *In vitro* evaluation of fungicides against *Xanthomonas axonopodis* pv. *glycines*

Potency of five fungicides were evaluated against the pathogen *Xanthomonas axonopodis* pv. *glycines* at three different level of concentrations (0.1, 0.2 and 0.3%) by "disc diffusion method". It was revealed from the Table 4.12 and fig. 4.4 that Bavistin and Shri-suff did not inhibit the growth of the pathogen at all the concentrations (0.1, 0.2 and 0.3%). Copper oxychloride (COC) found to be significantly superior against the test pathogen at 0.1 per cent concentration (23.25 mm) followed by Dithane M-45 (21.75 mm) and Copper sulphate (15.25 mm). Dithane M-45 was at par with the Copper oxychloride, but it was slightly inferior to the Copper oxychloride. Khatua *et al*. (2013) *in vitro* screened the fungicides against *Xanthomonas axonopodis* pv. *betlicola*, bacterial pathogen of betelvine and recorded that there was no growth inhibition achieved by Bavistin but Copper oxychloride found to be moderately effective. Pawar (2015) recorded that spraying of Copper-oxychloride completely inhibited the mango bacterial canker disease (MBCD) caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Xcmi). Findings of Ravikumar and Khan (2000) also supported the above results. They significantly controlled the bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by Copper oxychloride and Copper sulphate. Dithane M-45 (27.50 mm) and Copper oxychloride (27.25 mm) showed analogous results at 0.2 per cent concentration. Least inhibition zone was achieved by Copper sulphate at 0.1 and 0.2 per cent concentrations (15.25 and 21.25 mm). However, at 0.3 per cent concentration Copper sulphate found to be most effective against the test pathogen with mean inhibition zone of 41.00 mm followed by Copper oxychloride (30.25 mm). Copper oxychloride and Dithane M-45 inhibited the growth of the pathogen in similar manner at 0.3 per cent concentration. Over all results showed that best three fungicides significantly inhibited the pathogen almost in similar pattern. Sajid *et al*. (2013) found that Copper-oxychloride significantly inhibited the growth of *Xanthomonas axonopodis* pv. *malvacearum* causing bacterial blight of cotton . Ingole *et al*. (2001) conducted an *in vitro* study to evaluate three fungicides Copper oxychloride (0.20, 0.25 and 0.30%), Dithane M-45 (0.20, 0.25 and 0.30%) and Carbendazim (0.05, 0.10 and

0.15%) against *Xanthomonas campestris* pv. *glycines*. Among the fungicides, Dithane M-45 (0.20%) was the most effective at 24 and 48 hrs followed by other concentrations. At 72 hrs, Dithane M-45 (0.30%) was the most effective. Khan *et al*. (2005) observed that among the test fungicide Copper oxychloride performed best for controlling bacterial leaf blight of rice. Similarly, Khalid and Sinha (2008) recorded that Copper oxychloride was more effective against the isolates of *Xanthomonas oryzae* pv. *oryzae* than Benomyl.

Khan *et al*. (2005) tested four fungicides [Kasuran, Kasumin (Kasugamycin), Copper oxychloride and Vitigran blue (Copper oxychloride)] and three antibiotics (Oxytetracycline, Streptomycin and Chloramphenicol) as foliar spray for their effect on bacterial leaf blight under artificial inoculated conditions for three years (2001-03). Among the test chemicals (fungicides and antibiotics), Copper oxychloride performed the best followed by Vitigran blue with 43.25 and 48.19% disease incidence compared with 71.08% in the control, causing 39.15 and 32.20% disease reduction, respectively. The highest paddy yield among the test treatments was recorded in Copper oxychloride (3.63 t ha^{-1}), followed by Vitigran blue, Kasumin, Streptomycin, Oxytetracycline, Chloramphenicol, Copper oxychloride +Streptomycin, yielding 3.58, 3.58, 3.57, 3.57, 3.55, 3.55 t ha^{-1}, respectively, compared with 3.32 t ha^{-1} paddy yield in the control.

Table 4.12 *In vitro* evaluation of fungicide against *Xanthomonas axonopodis* pv. *glycines* after 72 hrs of incubation

Treatment	Fungicide	Zone of inhibition (mm), at different level of concentration %,		
		0.1	**0.2**	**0.3**
T1	**Copper oxychloride**	23.25	27.25	30.25
T2	**Dithane M-45**	21.75	27.50	29.25
T3	**Copper sulphate**	15.25	21.25	41.00
T4	**Bavistin**	0.00	0.00	0.00
T5	**Shri-suff**	0.00	0.00	0.00
T6	**control**	0.00	0.00	0.00
	Mean	10.04	12.83	16.70
	Sem±	1. 34	1.54	2.65
	C.D (5%)	4.03	4.63	7.93

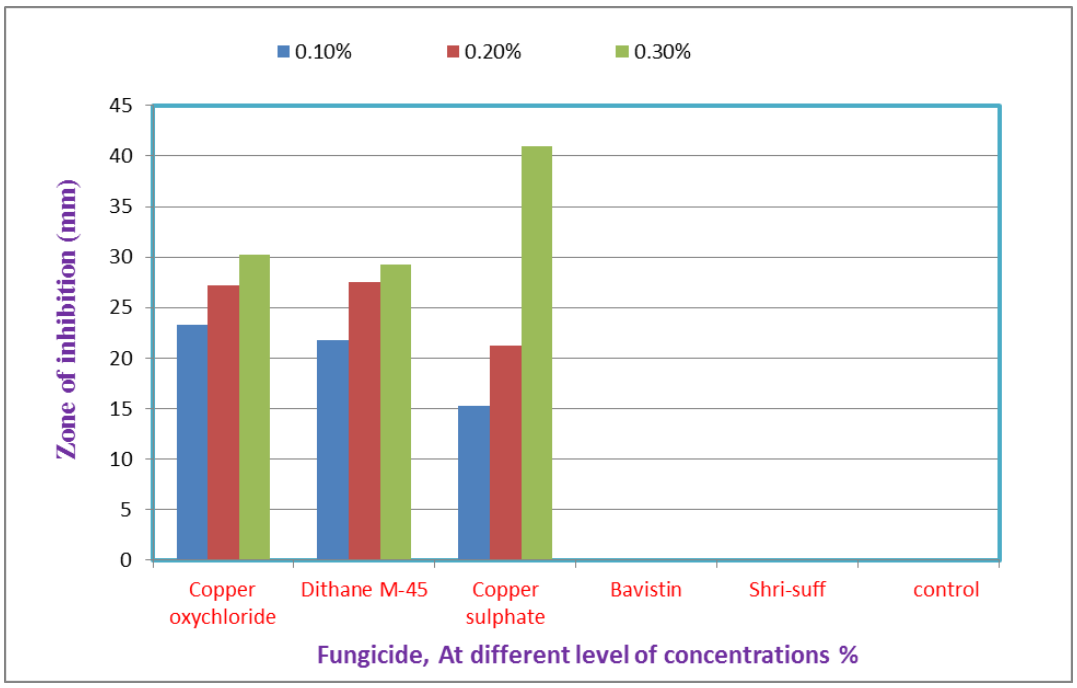


Fig. 4.3 *In vitro* evaluation of fungicide against *Xanthomonas axonopodis* pv. *glycines*

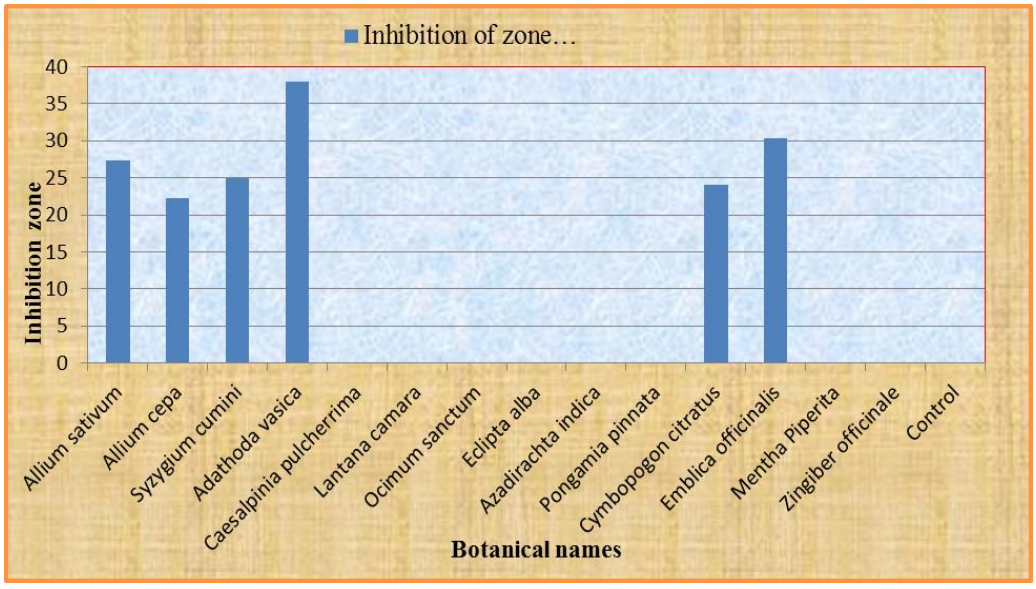


Fig. 4.4 Effective botanical extracts against *Xanthomonas axonopodis* pv. *glycines*

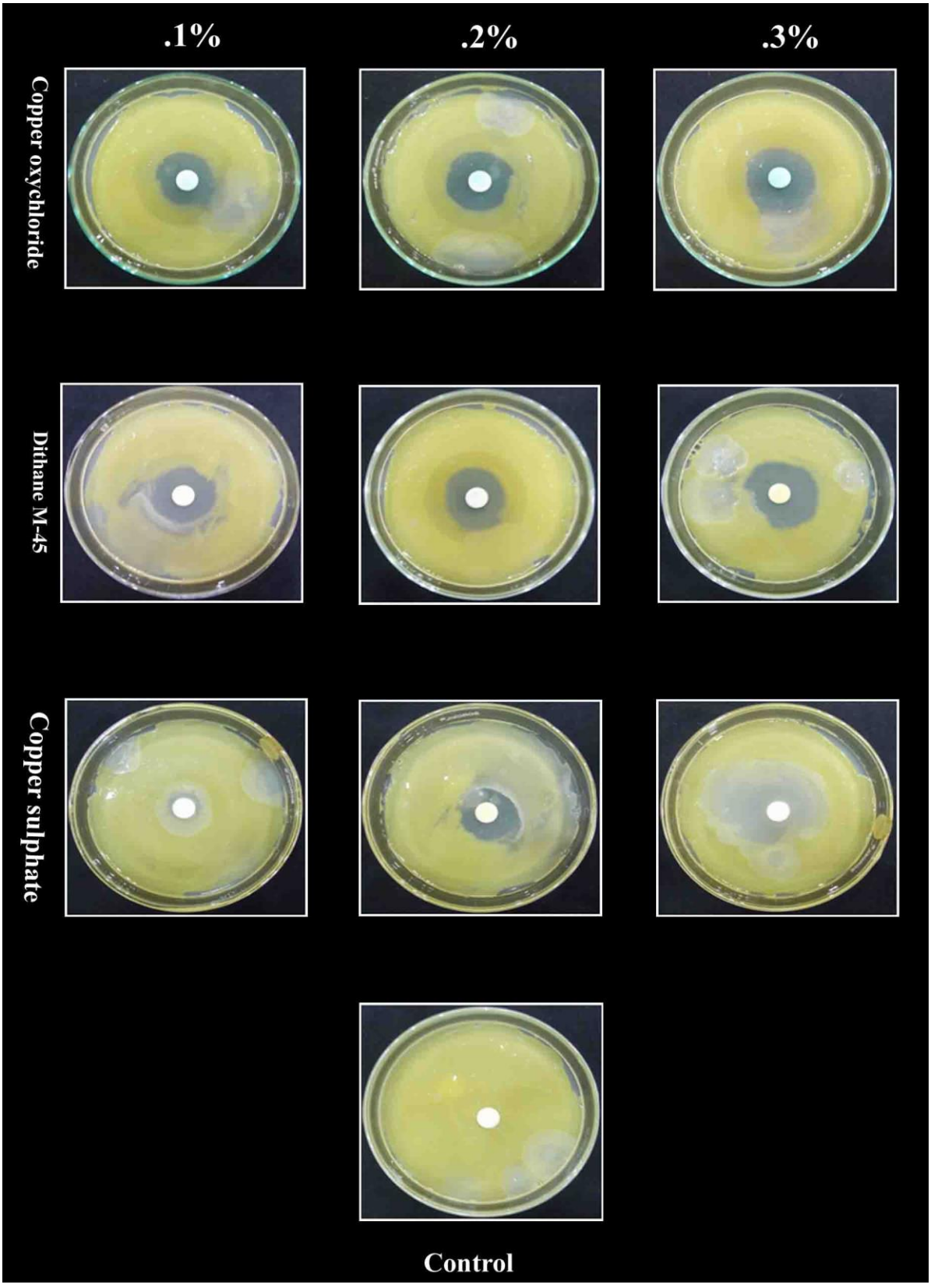


Plate 4.5 *In vitro* evaluation of fungicide against *Xanthomonas axonopodis* pv. *glycines* after 72 hrs of incubation

4.7 *In vitro* evaluation of botanical extracts against *Xanthomonas axonopodis* pv. *glycines*

Extracts prepared from forteen plants of different families were evaluated to find out their antibacterial activity against *Xanthomonas axonopodis* pv. *glycines* and mean zone of inhibition was recorded. Extract from eight plants *viz., Cesalpinia pulcherrima*, *Lantana camara*, *Ocimum sanctum, Eclipta alba*, *Azadirachta indica*, *Pongamia pinnata*, *Mentha Piperita*, and *Zingiber officinale* did not inhibit the growth of the pathogen and rest of botanical extracts showed significant results (Table 4.13 and fig. 4.5). Significantly superior mean zone of inhibition (38 mm) was recorded in leaf extract of *Adathoda vasica* followed by *Emblica officinalis* (30.34 mm). The results are in agreement with the findings of Govindappan *et al*. (2011) who had obtained highest growth inhibition of *Xanthomonas oryzae* pv. *oryzae* by aqueous leaf extract of *Adathoda vasica*. Pawar *et al*. (2014) reported the significant growth inhibition of *Xanthomonas axonopodis* pv. *punicae* (bacterial blight of pomegranate) by *Emblica officinalis*. Extract from bulb of *Allium sativum* and leaf extract of *Syzygium cumini* showed similar result of 27.34 mm and 25 mm zone of inhibition, the result was coincided with the results of Islam *et al*. (2014) and Naqvi *et al*. (2014). While leaf extract of *Cymbopogon citratus* and bulb of *Allium cepa* also showed similar type of result with 24 mm and 22.34 mm zone of inhibition respectively (Opara and Wokocha, 2008). The well diffusion test merely indicated that if the organisms are susceptible, then control may be successful if extract concentration similar to those achieved in laboratory could be applied in the field (Cheesbrough, 1991 and Barley *et al*., 1998).

Table 4.13 *In vitro* evaluation of botanical extracts against *Xanthomonas axonopodis* pv. *glycines* after 72 hrs of incubation

Treatment	Botanical Name	Zone of inhibition (mm)
T1	***Allium sativum***	27.33
T2	***Allium cepa***	22.33
T3	***Syzygium cumini***	25.00
T4	***Adathoda vasica***	38.00
T5	***Caesalpinia pulcherrima***	0.00
T6	***Lantana camara***	0.00
T7	***Ocimum sanctum***	0.00
T8	***Eclipta alba***	0.00
T9	***Azadirachta indica***	0.00
T10	***Pongamia pinnata***	0.00
T11	***Cymbopogon citratus***	24.00
T12	***Emblica officinalis***	30.33
T13	***Mentha Piperita***	0.00
T14	***Zingiber officinale***	0.00
T15	**Control**	0.00
	Mean	**11.13**
	Sem (+/-)	**0.803**
	CD (5%)	**2.318**

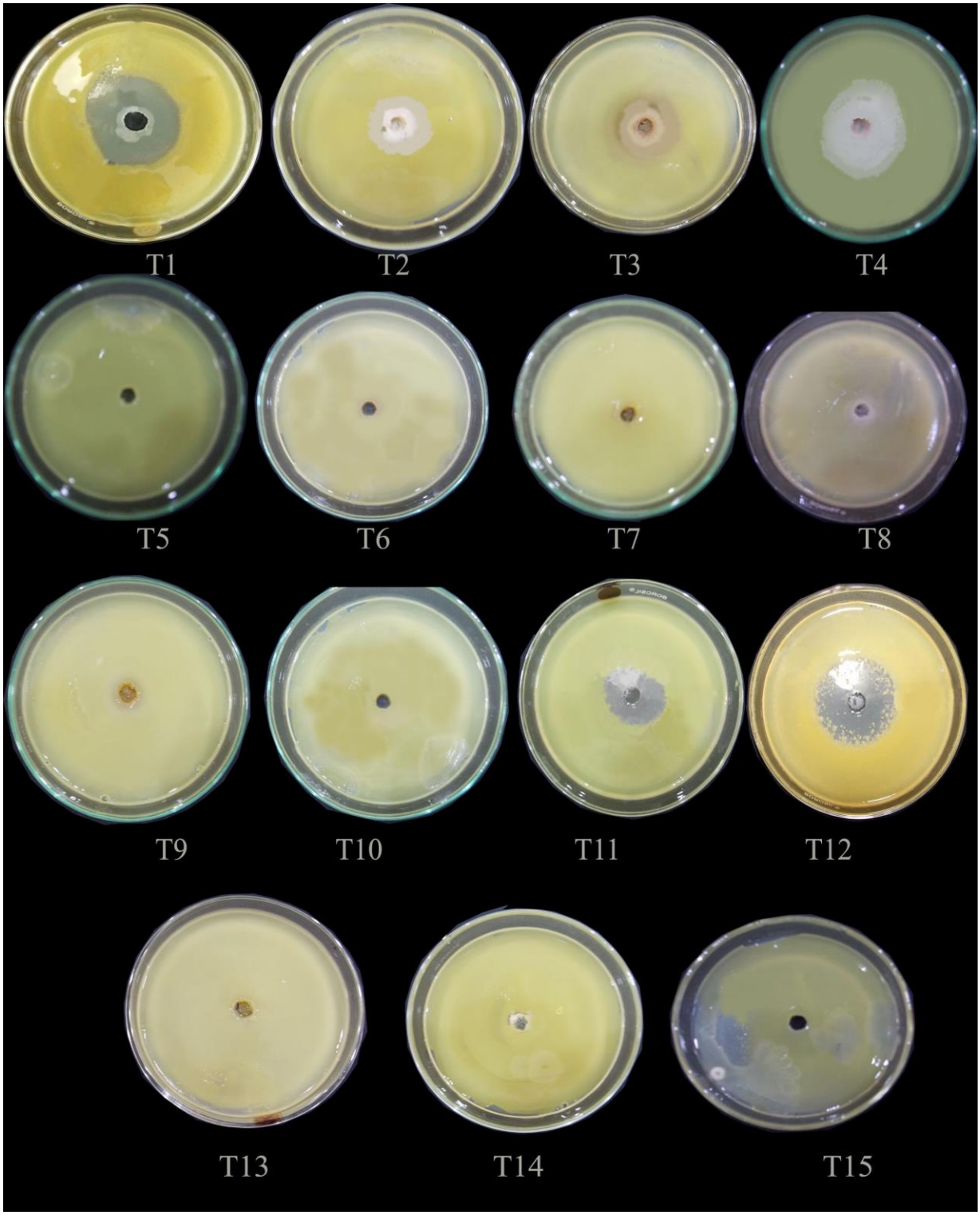


Plate 4.6 ***In vitro*** **evaluation of botanical extracts against** ***Xanthomonas axonopodis*** **pv.** ***glycines*** **after 72 hrs of incubation**

4.8 PGPRs activities of Fluorescent *Pseudomonas* isolates on soybean

Plant growth promoting activities of sixteen isolates of Fluorescent *Pseudomonas* were evaluated in germination seedling growth of soybean in *in vitro* condition. The data presented in the Table 4.14 showed significant improvement in seed germination and seedling vigour upon Fluorescent *Pseudomonas* seed treatment. This improvement was strain specific and not uniform with all the Fluorescent *Pseudomonas* strains.

Among the sixteen isolates of Fluorescent *Pseudomonas* evaluated eleven isolates significantly enhanced the seed germination of soybean seeds compared with the untreated control. Pf 39 recorded to be highest germination percentage with 99.10 per cent seed germination. This was followed by Pf 35 (97.43%) and Pf 33 (97.10%). Least germination was recorded for Pf 24 with 90.33 per cent seed germination.

There was significant increase in mean root length (Pf 33) when compared with control treated with Fluorescent *Pseudomonas* strains, followed by Pf 35 and least root length was found in Pf 4. Mean shoot length of seedlings increased significantly when the seeds were treated with Fluorescent *Pseudomonas* strains Pf 39 (12.50 cm average shoot length). This was followed by Pf 33 and Pf 35. Overall increases in mean root length, mean shoot length and germination (%) resulted in the increased vigour. Pf 4 recorded to be highest average fresh weight (5.07 g) followed by Pf 33 (5.43 g) and Pf 3 (5.07 g). Highest dry weight accumulation was recorded when seeds were treated with Pf 4 (731 mg) which was followed by Pf 33 (686 mg) and Pf 29 (679 mg).

Among the sixteen Fluorescent *Pseudomonas* strains treated, three of them significantly enhanced the vigour index when compared to control as tabulated in the (Table 4.7). The maximum vigour index was recorded in the Pf 33 with 2197.6 followed by Pf 39 (2176.9) and Pf 35 (1955.1) in comparison with untreated control (1632.8).

The growth promoting substance produced by *Pseudomonas fluorescens* might have exerted a synergistic action and enhanced the growth promotion. *Pseudomonas fluorescens* was reported to produce amino acids, salicylic acid and IAA (Sivamani and Gnanamanickam, 1988; O'Sullivan and O'Gara, 1992) which

might have improved the plant growth and seedling vigour. Production of indole acetic acid (IAA) by the strains of *Pseudomonas fluorescens* responsible for increasing root elongation was also reported (O' Dowling and O' Gara, 1994).

The results are in agreement with the findings of Sain (2010) who evaluated the plant growth promoting activity of twelve different plant growth promoting rhizobacteria (PGPRs) belonging to *Pseudomonas fluorescens* and *Bacillus subtilis* under *in vitro* condition. He observed highest seed germination (above 95%) occurred with the treatment of PGPR-4 (*B. subtilis*), PGPR- 10 (*P. fluorescens*), PGPR -1 (*B. subtilis*), PGPR-7 (*P. fluorescens*), PGPR-11 (*P. fluorescens*) and PGPR-5 (*B. subtilis*), while plant vigour increased with PGPR-12 (*P. fluorescens*), PGPR-5 (*B. subtilis*), PGPR-11 (*P. fluorescens*) and PGPR-3 (*B. subtilis*). Assis *et el*. (1995) and Mondal *et al*. (1999) indicated that PGPRs possess the plant growth promoting activity, which not only acts as antibacterial, but also enhance PVI and seed germination. *B. subtilis*, *Pseudomonas spp* and *Bacillus sp*. have been reported to be highly efficient against *X. campestris* pv. *malvacearum*. The results are also coincided with the findings of Wahyudi *et al*. (2011) they recommended 5 isolates of *Pseudomonas sp* which were Crb-3, Crb-16, Crb-17, Crb-44 and Crb-94 as potential isolates of *Pseudomonas sp* that could be applied as inoculants of soybean plant. Meera *et al.* (2012) observed that thirty five (Pf 1 to Pf 35) isolates of *Pseudomonas Fluorescens* were isolated from the rizosphere of rice fields. Among these isolates Pf 13 and Pf 04 showing on par root length and shoot length. Both the Pf 13 and Pf 04 isolates were found significantly superior than other isolates in increasing the shoot length and root length over control. The isolates Pf 13 was recorded high vigor index (3830) followed by Pf 04 (3648). The least vigour index was recorded by Pf 08 (2631). Zhang *et al*. (1997) observed that application of plant growth promoting rhizobacteria (PGPR) increased legume growth and development under optimal temperature conditions, and specifically to increase nodulation and nitrogen fixation of soybean. Workers like Maurhofer *et al*. (1998), Lodha (2001) and Athinuwat *et al*. (2014) also worked on PGPR.

Table 4.14 Effect of different Fluorescent *Pseudomonas* on germination of seeds and growth parameters of seedling

S. No.	Treatment	Seed Germination (%)	Average shoot length(cm)	Average root length(cm)	Average fresh weight (g)	Average dry weight (mg)	Vigor index
1	**Pf 3**	91.70	6.11	5.70	5.07	496	1082.4
2	**Pf 4**	94.08	9.33	5.47	5.80	731	1391.7
3	**Pf 8**	94.38	8.30	6.83	3.63	470	1428.2
4	**Pf 18**	95.50	7.93	9.07	4.47	644	1623.6
5	**Pf 22**	91.67	7.10	5.80	3.27	359	1182.5
6	**Pf 24**	90.33	6.10	6.77	3.60	477	1162.3
7	**Pf 25**	92.17	6.57	6.73	3.13	389	1225.9
8	**Pf 27**	93.90	8.23	8.90	3.59	617	1608.8
9	**Pf 29**	95.98	9.16	5.49	4.93	679	1406.4
10	**Pf 30**	94.78	8.17	8.50	4.49	498	1579.6
11	**Pf 33**	97.10	12.40	10.23	5.43	686	2197.6
12	**Pf 34**	91.26	6.73	7.60	3.28	334	1308.1
13	**Pf 35**	97.43	10.37	9.70	4.74	432	1955.1
14	**Pf 36**	95.61	8.97	7.93	3.42	342	1615.8
15	**Pf 38**	93.26	8.70	6.60	3.69	350	1426.9
16	**Pf 39**	99.10	12.50	9.47	4.87	474	2176.9
17	**Control**	90.04	9.87	8.27	4.41	527	1632.8
	Sem (+/-)	0.83	0.410	0.548	0.348	36.42	
	CD (5%)	2.39	1.182	1.582	1.006	105.14	

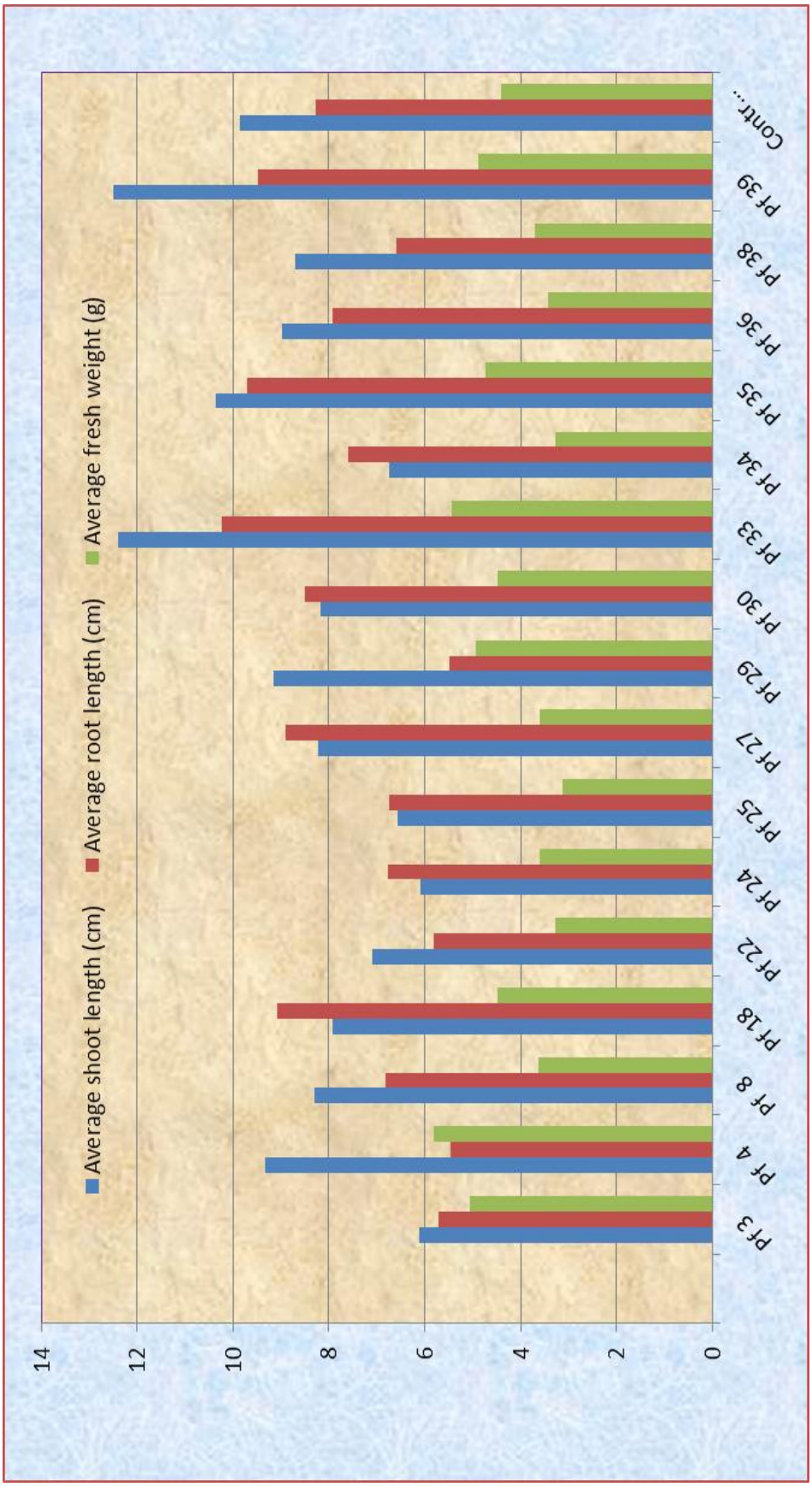


Fig. 4.5 Effect of different PGPRs on shoot length, root length and fresh weight of growth parameters of seedling

CHAPTER - V

SUMMARY AND CONCLUSIONS

The findings of the present investigation entitled **"Studies on bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* Nakano) disease of soybean"** was conducted during *Kharif* 2014 at the experimental field of Department of Plant Pathology, College of Agriculture situated in the Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The designs used for all the field and laboratory experiments were done by Randomized Block Design (RBD) and Complete Randomized Block Design (CRD), respectively.

Isolation, pathogenicity and purification of the bacterium were done from naturally infected soybean leaves on nutrient agar media (NA) after 72 hrs of inoculation. The developed colonies of purified bacterium were observed as single yellow, circular and semi-transparent. Symptom developed after 5 days of inoculation on the basis of pin prick method.

Screening of sixteen varieties of soybean was sown to record the per cent of incidence and severity. Results indicated that five varieties *viz.,* JS-72-44, JS-75-46, JS-71-05, JS-335 and Shivalic were free from the disease. Maximum bacterial pustule was recorded in varieties Punjab-1 (64.34%) followed by NRC-7 (46.51%) and Monatta (38.73%). Among sixteen varieties, eight varieties exhibited resistant reaction, five varieties were moderately resistant, reaction and three varieties showed susceptible reaction, whereas none varietie have moderately susceptible and tolerant reaction.

Correlation studies were carried out to determine the inter relation between various weather parameters and disease spread under the present investigation. Disease spread within the plants was found to be higher as compared to disease spread among the plants. Among three varieties studied Punjab-1 was more susceptible for among the plant as well as within the plant disease spread. There was significant positive and negative association of disease spread among the plants with maximum temperature and relative humidity, respectively for all

varieties. Morning vapor pressure was significant and positively correlated with disease spread among the plant for two varieties *viz.,* NRC-7 and Monatta whereas, rainfall and evening relative humidity was negatively associated with disease spread among the plant for same varieties. Minimum temperature was significant negatively correlated with disease spread among the plant for all three varieties. There was non significant correlation between diseases spread on wthin the plant and weather parameters except wind velocity which was negatively associated for all three varieties.

In vitro evaluation of seven antibiotics were evaluated at five different levels of concentration *viz.*, 100, 200, 300, 500 and 1000 ppm by "disc diffusion method". Streptomycin, Tetracyclin and Chloramphenicol showed almost similar type of results at 300 ppm and 500 ppm concentrations with 11.75 mm zone of inhibition. Whereas Streptomycin achieved 15.75 mm zone of inhibition followed by Tetracyclin (14.25 mm) and Chloramphenicol (12.25 mm) at 1000 ppm level of concentrations after 24 hrs of incubation. After 48 hrs of incubation, Chloramphenicol showed highest zone of inhibition (17.25 mm) at 300 ppm followed by Tetracyclin (16.50 mm) and Streptomycin (14.50 mm). However, Tetracyclin exhibited highest inhibition zone (26.00 mm) followed by Chloramphenicol (25.50 mm) and Streptomycin (21.25 mm) 1000 ppm concentration, whereas Tetracyclin and Chloramphenicol showed analogous results at 500 ppm and 1000 ppm level of concentrations, respectively. Although, Tetracyclin found to be most effective for inhibiting the growth of the pathogen (40 mm and 34.25 mm) followed by Chloramphenicol (37.25 mm and 31.75 mm) and Streptomycin (27.75 mm and 21.50 mm) after 72 hrs of incubation at 1000 and 500 ppm concentrations, respectively.

In dual culture test, among the sixteen isolates, Pf 18 was found to be most significantly effective in inhibiting the growth of the pathogen and with inhibition zone of 37.33 mm, 41.00 mm and 46.00 mm after 24, 48 and 72 hrs of incubation period, respectively. This was followed by Pf 34 (34.33 mm), Pf 33 (37.00 mm) and Pf 35 (43.00 mm) after 24, 48 and 72 hrs of incubation period, respectively.

Bio efficacy of Fluorescent *Pseudomonas* was evaluated under field condition. Highest per cent disease control of pustule was recorded for variety Monatta (17.80%) inhibition followed by NRC-7 (15.10%) inhibition after foliar application of Fluorescent *Pseudomonas*.

In vitro evaluation of five fungicides at three different levels of concentration (0.1, 0.2 and 0.3%) was done by "disc diffusion method". Copper oxychloride (COC) was found to be significantly superior against the test pathogen at 0.1% concentration (23.25 mm) followed by Dithane M-45 (21.75 mm) and Copper sulphate (15.25 mm). Dithane M-45 (27.50 mm) and Copper oxychloride (27.25 mm) showed analogous results at 0.2%. Copper sulphate (0.3%) found to be most effective against the test pathogen with mean inhibition zone of (41.00 mm) followed by Copper oxychloride (30.25 mm).

In evaluation of plant leaf extracts by poisoned food technique, forteen plants of different families were evaluated to find out their antibacterial activity. Significantly superior mean zone of inhibition (38 mm) was recorded in leaf extract of *Adathoda vasica* followed by *Emblica officinalis* (30.34 mm).

Growth promoting activities of local isolate of Fluorescent *Pseudomonas* on different plant parameters in soybean crop were evaluated. Among the sixteen isolates of Fluorescent *Pseudomonas* evaluated elevan isolates significantly enhanced the seed germination of soybean seeds compared with the untreated control. Pf 39 (99.10%) recorded to be highest seed germination percentage followed by Pf 35 (97.43%) and Pf 33 (97.10%). Least germination was recorded for Pf 24 (90.33%) seed germination. Considerably increased average shoot length (12.50 cm); mean root length (10.23 cm) and vigour index (2197.6) and average fresh and dry weight (5.80 g, 731 mg) and was noticed, when the seeds were treated with PGPR strains Pf 39, Pf 33 and Pf 4, respectively.

CONCLUSIONS:-

From the results of present investigation the following conclusion could be drawn;

1. Varieties *viz.,* JS-72-44, JS-75-46, JS-71-05, JS-335 and Shivalic were free from the disease. Maximum disease was recorded in genotype Punjab-1.
2. There was significant positive and negative association of disease spread among the plant with maximum temperature and relative humidity, respectively for all varieties whereas Minimum temperature was negatively correlated with disease spread among the plant for all three varieties. Wind velocity was negatively associated with disease spread within the plant for all three varieties among the all weather parameters tested.
3. Maximum disease spread among the plant was found in genotype between 1st August to 10th August, while within the plants maximum progress of disease was found in genotype Monatta between 21st August to 30th August.
4. Among the tested antibiotics against *Xanthomonas axonopodis* pv. *glycines*, Tetracyclin was found to be most effective for inhibiting the growth of the pathogen after 72 hrs of incubation at 1000 ppm concentrations.
5. Regarding different tested strains of Fluorescent *Pseudomonas* bioagent, Pf 18 was found to be most significantly effective in inhibiting the growth of the pathogen after 24, 48 and 72 hrs of incubation period, respectively.
6. Copper oxychloride was found to be significantly superior against the test pathogen at 0.1% concentration whereas Copper sulphate was found to be most effective against the test pathogen at 0.3% level of concentration.
7. Growth promoting activities of local isolate of Fluorescent *Pseudomonas* on different plant parameters in soybean crop were evaluated. The best result all plant growth parameter showed by Pf 39, Pf 33 and Pf 34.

SUGGESTIONS FOR FUTURE RESEARCH WORK

This is an important pathogen of soybean causing wide spread and destructive disease, bacterial pustule. For management of disease there is need to extensive *in vitro* and field work on following issues;

1. Collection and identification virulence races of *Xanthomonas axonopodis* pv. *glycines* of different agroclimatic zone.
2. Effect of enviourmental parameter in disease progression and epidemic condition of disease to be studied.
3. Identification of resistance genes, there introgression and management.
4. Bio efficacy of bioagent against bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*) disease should be evaluated under field conditions with the IDM approaches.
5. Evaluation of potential antibiotics and chemicals and their phyto toxics effect on crop plants.

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Appendix B: **Correlation coefficient of disease spread within the plants and weather parameters in variety Punjab-1, Monatta and NRC-7**

	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5	Variable 6	Variable 7	Variable 8	Variable 9	Variable 10	Variable 11	Variable 12	Variable 13
Variable 1													
Variable 2	0.917^{NS}												
Variable 3	-0.895^{NS}	-0.985^{*}											
Variable 4	-0.967^{*}	-0.967^{*}	0.976^{*}										
Variable 5	-0.992^{**}	-0.938^{NS}	0.900^{NS}	0.958^{*}									
Variable 6	0.966^{*}	0.959^{*}	-0.908^{NS}	-0.942^{NS}	-0.990^{**}								
Variable 7	0.628^{NS}	0.271^{NS}	-0.222^{NS}	-0.428^{NS}	-0.584^{NS}	0.495^{NS}							
Variable 8	-0.968^{*}	-0.792^{NS}	0.782^{NS}	0.900^{NS}	0.935^{NS}	-0.876^{NS}	-0.777^{NS}						
Variable 9	0.816^{NS}	0.833^{NS}	-0.725^{NS}	-0.749^{NS}	-0.880^{NS}	0.926^{NS}	0.431^{NS}	-0.695^{NS}					
Variable 10	0.824^{NS}	0.931^{NS}	-0.856^{NS}	-0.831^{NS}	-0.886^{NS}	0.942^{NS}	0.238^{NS}	-0.665^{NS}	0.959^{*}				
Variable 11	0.923^{NS}	0.756^{NS}	-0.788^{NS}	-0.895^{NS}	-0.868^{NS}	0.793^{NS}	0.703^{NS}	-0.972^{*}	0.536^{NS}	0.550^{NS}			
Variable 12	0.903^{NS}	0.724^{NS}	-0.760^{NS}	-0.873^{NS}	-0.843^{NS}	0.762^{NS}	0.714^{NS}	-0.964^{*}	0.498^{NS}	0.508^{NS}	0.999^{**}		
Variable 13	0.917^{NS}	0.744^{NS}	-0.776^{NS}	-0.886^{NS}	-0.861^{NS}	0.784^{NS}	0.713^{NS}	-0.971^{*}	0.527^{NS}	0.537^{NS}	1.000^{**}	0.999^{**}	

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Membership of Professional Societies (If any)	:	Yes
Awards/Recognitions (If any)	:	No
Publications (If any)	:	Yes

Signature