CHEMICAL MANAGEMENT OF BULB ROT AND PURPLE BLOTCH OF ONION

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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for the Degree of

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IN
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By

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DECLARATION OF STUDENT

I here by declare that the experimental work and its interpretation of Thesis entitled **"CHEMICAL MANAGEMENT OF BULB ROT AND PURPLE BLOTCH OF ONION''** or part there of has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis or publication of University or Scientific Organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola **(GORE PRAKASH MAROTI)**

Date: / / 2019 Enrolment No. PP/3379

CERTIFICATE

This is to certify that the thesis entitled "**CHEMICAL MANAGEMENT OF BULB ROT AND PURPLE BLOTCH OF ONION"** submitted in partial fulfillment of the requirements for the degree of "**Master of science in Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafied research work carried out by **GORE PRAKASH MAROTI** under my guidance and supervision.

The subject of thesis has been approved by the student's Advisory Committee.

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Date : / / 2019 (Gore Prakash Maroti)

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(C) Abbreviations

@	-	At the rate
%.	-	Per cent
&	-	And
/	-	Per
°C	-	Degree celcius
RBD	-	Randomized Block Design
C.D.	-	Critical difference
cm	-	Centimetre
DAI	-	Days after incubation
DAP	-	Days after planting
Dr. PDKV	-	Dr. Panjabrao Deshmukh Krishi Vidyapeeth
e.g.	-	Exampli gratia (For example)
et. al.	-	Et alia (and others)
etc.	-	Et cetra
Fig.	-	Figure
gm	-	Gram
ha	-	Hactor
i.e.	-	That is
J.	-	Journal
ml	-	mililitre
mm	-	milimetre
PDI	-	Per Cent Disease Intensity
PGI	-	Per cent growth inhibition
PDA	-	Potato Dextrose Agar
NA	-	Nutrient Agar Media
ppm	-	Parts per million
Spp./sp	-	Species
SE (m) +	-	Standard error of mean
Sig.	-	Significant
viz.,	-	Videlicet (Namely)
pv.	-	Pathovar
No	-	Number

(E) THESIS ABSTRACT

a) Title of the thesis	**: CHEMICAL MANAGEMENT OF BULB ROT AND PURPLE BLOTCH OF ONION**
b) Full name of student	**: GORE PRAKASH MAROTI**
c) Name and address of Major Advisor	**: Dr. R. W. Ingle** Associate Professor, Department of Plant Pathology, PGI Dr. P.D.K.V, Akola - 444 104 (M.S.)
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ABSTRACT

India ranks first in area, but second in world production, and productivity is low (14.2 t/ha) as against the world productivity of 17.47 t/ha. Several factors have been identified for the low productivity of onion in India. Among several factors, diseases are the most important, especially numerous foliar, bulb and root pathogens that reduce the yield of onion. A present investigation entitled “Chemical management of bulb rot and purple

blotch of onion" was carried out at Department of Plant Pathology and Department of Vegetable Science, College of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

The bulb rot caused by *Erwinia carotovora* pv. *carotovora* and purple blotch caused by *Alternaria porri* are major important diseases of onion is difficult to control without application of bactericides and fungicides respectively. *Erwinia carotovora* pv. *carotovora* causes 40 to 80 % losses in onion where *Alternaria porri* reduces 5.0 to 96.5 per cent yield in onion. In present investigation studies were undertaken to manage bulb rot and purple blotch disease of onion through the use of bactericide and fungicides. The rotted bulb and purple blotch diseased leaf samples were collected from experiment field, for isolation of bacterial and fungal pathogen. The bacterial pathogen *Erwinia carotovora* pv. *carotovora* associated with the diseased bulbs and was found pathogenic and causing bulb rot of onion. The fungus pathogen *Alternaria porri* also found pathogenic and responsible for purple blotch disease in onion.

The isolate of *Erwinia carotovora* pv. *carotovora* showed negative reaction towards gram reaction and urease production test where as it shows positive reaction towards KOH test, catalase test, potato soft rot test, gelatin liquification test, growth in 5% Nacl, H_2S production test, indole production test, oxidase test and methyl red test. Based on the colony characters and morphological characters of mycelium, conidiophores and conidia, the fungus was identified as *Alternaria porri*. It produced septate mycelium with conidiophores arising singly or in small groups. The conidiophores was straight or flexuous or geniculate. They were brown in colour with septations.

Efficacy of different chemicals was tested by filter paper disc diffusion method against *Erwinia carotovora* pv. *carotovora.* Maximum growth inhibition of *Erwinia carotovora* pv. *carotovora* recorded in copper oxychloride @ 0.25% + streptomycin @ 200 ppm (23.00 mm), followed by streptomycin @ 200 ppm (18.00 mm). Under field condition, treatment (T8) bulb dip (copper oxychloride @ 0.25% + streptomycin sulphate @ 200 ppm) + spraying with (copper oxychloride @ 0.25%) was found most

effective treatment against bulb rot of onion as it recorded minimum disease incidence (19.67%) with maximum disease control (53.46%).

Poisoned food method was employed to test the efficacy of various chemicals against *Alternaria porri*. The mancozeb @ 0.25%, mancozeb @ 0.25% + carbendazim @ 0.10% and mancozeb @ 0.25% + streptomycin @ 200 ppm concentration inhibited the 100 per cent mycelial growth of *Alternaria porri*. The next best treatments was copper oxychloride @ 0.25% + mancozeb @ 0.25% exhibited 86.09 per cent growth inhibition of *A. porri*. In the field experiment, treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%) treatment recorded lowest per cent disease intensity i.e 15 per cent and it showed highest percent disease control i.e. 55.13 per cent.

Maximum seed yield obtained in treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin 200ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%) i.e 1022 kg/ha which was found significantly superior over rest of the treatments.

CHAPTER I

INTRODUCTION

1.1 Background of Information

Onion (*Allium cepa* L.) is one of the oldest bulb crops belongs to Amaryllidaceae family. The genus Allium comprises over 700 species which can be found throughout the tropical, temperate and sub-temperate regions of the world (Fritsch and Friesen, 2002). There are five important species of Allium of which the onion (*Allium cepa*) is the major cultivated species grown all over the world (Messiaen 1994). According to Vavilov (1951) the primary center of origin lies in central Asia.

Among vegetables, onion often called as “queen of kitchen” is one of the oldest known and an important crop. Onion a bulbous, biennial herb, is one of the most important vegetable crop grown throughout world and in India. As a vegetable and spice, it is used both as tender and mature bulb.

The world production of onion was 85.94 million tonnes in 4.44 million hectares area (Anonymous 2015). China, India, United States of America, Turkey, Iran and Pakistan are the major onion producing countries in the world. India is a traditional producer and assumes second global position in onion production with 19.40 million tonnes (mt) from 1.20 million hectares (mha) area (Anonymous 2015). It is cultivated round the year throughout the country. The major onion growing states in India, are Maharashtra (30%), Karnataka (11%), Gujarat (10%), Bihar (7%), Madhya Pradesh (15%), Andhra Pradesh (5%), Rajasthan (4%), Haryana (3%) and others (15%) (Anonymous 2015). Bangladesh, Malaysia, Sri Lanka, U.A.E, Pakistan and Nepal are the major market of Indian onions.

1.2 Importance of Study

Even though India ranks first in area, but second in world production, and productivity is low (14.2 t/ha) as against the world productivity of 17.47 t/ha (Anon, 2011). Several factors have been identified for the low productivity of onion in India. Among several factors, diseases

are the most important, especially numerous foliar, bulb and root pathogens that not only reduce the yield of onion. but also pose harmful effects during harvesting, post harvesting, processing and marketing stages, which lower the quality and export potential of the crop that significantly causes the economic loss.

In the world, onion is attacked by 66 diseases including 10 bacterial, 38 fungal, 6 nemic, 3 viral, 1 mycoplasmal, 1 parasitic plant and 7 miscellaneous diseases and disorders (Schwartz and Mohan, 2008, Schwartz, 2010). In india, several diseases have become widespread and serious enough to limit the production. The common diseases like purple leaf blotch (*Alternaria porri*), *Stemphylium* blight (*Stemphylium vesicarium)* downy mildew (*Peronospora destructor*) basal/stem rot (*Fusarium* sp., *Sclerotium* sp., *Rhizoctonia* sp.), damping off (*Fusarium sp., Pythium sp.*) and bulb rot (*Erwinia carotovora* pv. *carotovora*) etc, are the most destructive diseases that damage the crop and reduce the seed yield even up to 100% (Brewster, 2008).

Among the diseases, bulb rot (*Erwinia carotovora* pv. *carotovora*) and purple blotch (*Alternaria porri*) are the the most destructive diseases, commonly prevailing in almost all onion growing pockets of the India, which causes heavy loss in onions under field conditions as well as in storage. Now days these two diseases threaten to the onion seed and bulb production in india.

Bulb rot losses may be occur in field or after harvest, during transport, or marketing. *Erwinia carotovora* pv. *carotovora* reduces the yield upto 30% in vegetables (Agrios,2005). *Erwinia* caused 40 to 80 % losses in different crops such as onion, *Aloe Vera*, potato, carrot, ornamental and fruit crops etc. (Anonymous, 2006). The bacteria chiefly attack succulent, tender tissues of storage organ such as fleshy bulb, tubers, fruits, roots, corms & rhizomes as well as bud, stem, petiole & leaf, stalk tissues. Rot bacteria pose constant threat because of their extensive host range and wide spread distribution.

The purple blotch disease affects both aerial and underground parts in the field conditions (Ahmed and Hossain, 1985). It causes reduction in leaf production by 62-92% (Utikar and Padule, 1980), bulb yield by 59% (Gupta and Pathak, 1988) and seed yield by 97% (Lakra, 1999). The yield loss of onion in India due to this disease under favorable conditions varies from 5.0 to 96.5 per cent (Gupta and Pathak, 1988). Temperature and humidity are the most predominant factors for the development of purple blotch disease. The disease is favored by moderate temperature (24-30^{0}C) and high relative humidity (Gupta and Pathak, 1986; Evert and Locy, 1990 and Rodriguez et al., 1994)

All these factors have led to a new dimension in research management of onion diseases. With increase in production of onion in the country, the emphasis was laid on export of onion. Hence, to increase the further production and productivity of onion, the disease incidence has to be reduced. In this context, the present investigation was undertaken with the following objective:

1.3 Objectives of study

1. To find out the effective chemicals against bulb rot and purple blotch of onion

1.4 Hypothesis

Onion is one of the major vegetable crop of India, but little research and development work has been done to address the major constraints limiting its production and productivity.

Among the onion diseases bulb rot incited by *Erwinia carotovora* pv. *carotovora* and purple blotch incited by *Alternaria porri* cause quantitative and qualitative losses to onion seed and bulb production. Hence, proper bulb treatment and foliar application of antibiotics and fungicides may help in reduction of bulb rot and purple blotch under field condition.

1.5 Scope and Limitation

The onion bulb as well as seed production is a broadest economical sector which plays a significant role in the overall socio-economic development of India. However, productivity of bulb and seed is greatly affected due to infection of diseases like Stemphylium leaf blight, purple blotch, downy mildew, basal rot, neck rot, black mold, bulb rot, smudge etc. Among these diseases bacterial bulb rot of onion incited by *Erwinia carotovora* pv. *carotovora* and purple blotch incited by *Alternaria porri* are much more prevalent in tropical and subtropical regions of India. Bulb rot causes heavy loss in onions under field conditions as well as in storage. Whereas purple blotch attacks all aerial plant parts and reduces bulb and seed production. Effect of these two diseases reduce both the quality and quantity of crop yield which hampers the export of onion. Number of fungicides, combinations of fungicides and bactericides are available as option for farmers against the attack of these diseases. However, most of these chemicals are not target specific and could affect beneficial microorganism associated with the plant. Moreover, some of these chemical compounds get accumulated in the plant tissue which in turn causes several health-related problems is one of the major limitation of chemical management.

All these factors have led to a new dimension in research in order to find effective chemical tratments for the management of bulb rot and purple blotch of onion.

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CHAPTER II

REVIEW OF LITERATURE

The present investigation was undertaken entitled “Chemical management of bulb rot and purple blotch of onion”. The literature pertaining to research is reviewed and presented in this chapter with following sub heads-

2.1 BULB ROT

2.2 Occurrence

Perombelon and Kelman (1980) stated that pectinolytic *Erwinia* are enterobacteria that caused soft-rot disease in a wide range of plant species, including many crops of economic importance such as vegetables and ornamentals.

Goto and Matsumoto (1987) reported that *Erwinia carotovora* subsp. *carotovora* causes bacterial soft rot and blackleg diseases in Japanese Horse radish.

Babadoost (1990) stated that soft rot caused by several types of bacteria, but primarily subspecies and pathovars of *Erwinia carotovora* and *E. chrysanthemi*, is a widespread and destructive causing soft rot of fleshy fruits, vegetables and ornamentals throughout the world.

Fraaije *et al*. (1997) stated that pectolytic *Erwinias* are involved in soft rot diseases of various agricultural crops such as potato, sugar beet and chicory etc. *Erwinia* spp. associated with soft rot of potato have been studied extensively because of their economic importance.

Masukaat *et al*. (1998) reported that, in Zimbabwe *Pectobacterium carotovorum* subsp. *carotovorum* and *Pectobacterium atrosepticum* have been listed as the major pathogens causing blackleg and tuber soft rot diseases.

Toth *et al*. (2001) reported that *Pectobacterium carotovorum* subsp. *carotovorum* has a broad host range, causing soft rot disease in potato, carrot, capcicum, calla lily and aloevera.

Gracia-Garza *et al*. (2002) reported that soft rot caused by *Erwinia* spp. can be devastating for greenhouse grower of ornamental plants. In particular, *Erwinia carotovora* pv. *carotovora* cause severe losses in calla lilies.

Perombelon (2002) stated that *Pectobacterium* spp. almost exclusively infected potato and caused blackleg of the stem and tuber soft rot.

Garden *et al*. (2003) and Samson *et al*. (2005) stated that plant pathogen which macerated and decayed plant tissues, sometimes referred to as pectolytic *Erwinias* (formerly called *Erwinia) Erwinia caratovora* and *Erwinia chrysanthemi* were important plant pathogens found in this genus but had now been placed into two new genera viz., *Pectobacterium* and *Dickeya* spp. respectively.

Huang *et al*. (2003) stated that *Erwinia rhapontici* (millard) Burkholdar is an opportunistic bacterial pathogen causes two kinds of plant disease is pink seed and crown rot or soft rot on more than 15 species of plant and it has been reported to occur in North America, Europe, Japan, Korea and Israel.

Yanez-Morales *et al*. (2003) reported that severe soft rot of onion bulb in commercial field was detected in Southern Tamaulipas and Eastern San Luis Potosi States, Mexico in 1989.

Duarte *et al*. (2004) observed that *Erwinia carotovora* and *Erwinia chrysanthemi* are the most important pectolytic bacteria that cause maceration of plant tissues and diseases of many crop plants including potato.

Agricos (2005) recorded that *Pectobacterium* bacterial genus is considered as the main bacteria that caused the damage in USA during storage.

Mandal and Maiti (2005) reported that a new leaf soft rot disease of aloevera caused by *Pectobacterium chrysanthemi* was recorded for the first time in 2000, at National Research Centre for Medicinal and Aromatic Plants, India.

Bdliya and Dahiru (2006) reported soft rot of potato tubers is a major threat to potato production worldwide. This was especially true in developing countries, where in Nigeria there had accounted for about a 40% or more loss of tubers.

Hajamed *et al*. (2007) reported that bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* is one of the most important and widespread bacterial disease of wide variety plants either in the field or during storage. Losses due to bacterial soft rot have been seriously complained by potato grower in Egypt.

Mahmoudi *et al*. (2007) reported that pectolytic *Erwinia* which causes soft rot disease have a wide host range including potato, cabbage, tomato and ornamental plants.

Maiti *et al*. (2007) reported that *Pectobacterium chrysanthemi* caused leaf rot of some cultivated medicinal plants. This pathogen is widely distributed in lower Gangetic plains of Gujarat (India). The pathogen was isolated from the infected lesions and confirmed by pathogenecity test and disease symptoms.

Schwart and Gent (2007) reported that bacterial soft rot pathogens are commonly found and easily disseminated by irrigation water. *Erwinia* spp. survive between onion crops in soil, crop debris and pathogenically on another crop.

Sagar and Bakade (2009) stated that bacterial soft rot can cause significant loss of potato tubers at harvest, transport and during storage.

Bhat *et al*. (2010) reported that soft rot caused by *Erwinia carotovora* subsp. *carotovora* is one of the destructive diseases of vegetable and occurs worldwide wherever fleshy storage tissues of vegetable and ornamental are found. It causes greater loss of produce than any other bacterial disease and the disease can be found in the field, transport, storage and during marketing resulting in great economic loss.

Alvarado *et al*. (2011) reported that yield of Chinese cabbage can be limited by various diseases among which soft rot caused by pectiolytic bacteria are the most destructive worldwide.

Kumar *et al*. (2011) reported bacterial soft rot of aloevera caused by *Erwinia carotovora* from different part of the world.

Marquez-Villavicencio *et al*. (2011) stated that *Pectobacterium* causes wilt, soft rot and blackleg in potato and affected plant health during field production and storage.

Ismail *et al*. (2012) reported post harvest diseases caused by *Erwinia carotovora* affected quality and availability of fruit and vegetables.

Rahaman *et al*. (2012) reported that bacteria belonging to the *Pectobacterium* and *Dickeya* genera caused soft rot and blackleg in potato.

Al-zomor *et al*. (2013) stated that *Erwinia carotovora* a seed borne pathogen the causal agent of blackleg disease is one of the most important diseases attacking potato and drastically resulting in economic losses wherever it is grown.

Prajapat *et al*. (2013) reported that *Erwinia carotovora* is a soft rot disease causing bacteria and economically very harmful pathogen in terms of post harvest losses and a common cause of decay in stored fruits and vegetables.

Rashid *et al*. (2013) reported that *Erwinia carotovora* subsp. *carotovora* the causal agent of soft rot of potato is an economically important pathogen in terms of postharvest losses and decay in stored fruits and vegetables.

Ali *et al*. (2014) stated that blackleg and soft rot are important diseases of potato that cause heavy losses to potato crop not only in the field but also in the storage where the bacteria are transmitted from diseased to healthy tubers.

Nagadze and Icishahayo (2014) reported that *Erwinia chrysanthemi* is the causal agent of aerial stem rot and wilt disease in tomatoes.

2.3 Symptomology

Sherf and Macnab (1986) recorded symptoms of soft rot of potato in the form of mushy, disintegrated, depressed and discoloured tissues. The affected tissues within the region become creamy and slimy in colour and gradually become disintegrated into a mushy mass of disorganized cells. Sometimes the whole tuber turns into a soft, watery, decayed mass within 3 to 5 days which gave the confirmation of *Erwinia* spp.

Babadoost (1990) observed the symptoms of soft rot are similar on most of the plants. The disease initiates on leaves, stems and underground parts as small, watersoaked, translucent lesions. These rapidly enlarge in both diameter and depth. The host tissue get softened and becomes mushy or watery. Slimy masses of bacteria and cellular debris frequently ooze out from cracks in the tissues. Within 20 to 72 hours, entire fleshy fruits, roots, tubers, stems and rhizomes, bulbs, corms, buds, leafstalks and leaves may rot and collapse, sometimes leaving only the outer layer intact. Decaying tissue, which may be opaque, white, creamy colored, gray, brown or black frequently gave off a characteristically putrid odour.

Yanez-Morales *et al*. (2003) observed that *Erwinia carotovora* causing soft rot in onion bulb showing early infection in the inner part of the onion bulb in the neck region and the decay gradually invades the whole bulb, while the outer scale remains unaffected. At early stage of bulb infection, the older leaves become flaccid and yellow and later when the bulb becomes severely decayed, all the leaves die.

Agricos (2005) observed that crucifer and onion when infected by soft rot cause by *Erwinia* spp. always give repulsive odour.

Crowhurst (2006) recorded that *P. chrysanthemi* affected tuber tissues produce creamy to white coloured colonies and were soft and granular. Brown to black pigments often developed at the margins of decayed tissue.

Al-Jeboory and Al-Ani (2010) observed that *Erwinia chrysanthemi* infected potato plants were characterized by development of soft rot which produce black areas on the stem, beginning from the tuber, followed by yellowing, leaf rolling upward leading finally to wilting and death of the infected plants. The affected tissue becomes soft and watery, turning slimy with foul smell.

Bhat *et al*. (2010) observed that soft rot caused by *Erwinia carotovora* in vegetable showing symptoms as small water-soaked lesion, which enlarge rapidly, the affected area becomes slimy

Alvarado *et al*. (2011) observed the initial symptom of soft rot in Chinese cabbage as the maceration of leaf base tissues that are in contact with soil. The disease can progress quickly and symptoms can be observed in the main stem. The whole plant can collapse after a few days.

Pachupate and Pallavi Kininge (2012) observed *Erwinia carotovora* infected banana plant through leaves and pseudostem causing wilting or death of leaves before fruit has ripened, vascular discoloration and internal rot of the pseudostem accompanied by a characteristically foul odour.

Mohammad and Selman (2013) isolated *Erwinia* spp*.* from rotting potato tuber and stem with typical symptoms of soft rot. Infected tubers typically developed a watery soft rot accompanied by an offensive odour.

Prajapat *et al*. (2013) reported that *Erwinia carotovora* causing soft rot in potato showing symptoms both outside and inside the tuber. The potato skin look water soaked and dark blister forms. The bacteria enter through a weak point in the skin and rots out the centre of the potato.

Rashid *et al*. (2013) observed that potato tuber soft rot caused by *Erwinia carotovora* subsp*. carotovora* showing symptoms like small, water-soaked spots on the surface. These spots rapidly enlarged and the tissue was decomposed in a soft, blister-like area on the surface of the tuber.

2.4 Pathogenicity

Smith and Bartz (1990) proved the pathogenicity of different strains of *Erwinia carotovora* pv. *carotovora* and *E. chrysanthemi* on potato, pepper, tomato and tobacco and reported that all the strains caused lesions on pepper and tobacco fruit, potato tuber and young tomato plant but varied in pathogenicity in pseudostem of corm and in the stems of chrysanthemum, potato and tobacco.

Yanez-Morales *et al*. (2003) tested the pathogenicity of *E. Chrysanthemi* causing bulb rot of onion. The test were accomplished by stab inoculation of onion slice and onion bulbs and considered pathogenic when healthy tissue around and below the inoculation side had some kind of rot with or without tissue colouration bacteria was reisolated from rotten tissue.

Costa *et al*. (2006) tested the pathogenicity of different strains of *Erwinia carotovora* on tomato, potato and Chinese cabbage and evaluated the symptoms development up to 21 days after inoculation. The majority of strains were more virulent and pathogenic to tomato, Chinese cabbage and potato.

Mahmoudi *et al*. (2007) confirmed the koch's postulate of *Erwinia carotovora* pv. *carotovora* causing soft rot of crown imperial bulbs and observed severe rotting identical to that observed under natural condition.

Palacio-Bielsa *et al*. (2007) injected suspension of *pectobacterium chrysanthemi* (10^8 cell/ ml) on central leaves of onion plant, which were mentained at 28^0 C with uninoculated plant as control. Soft rot symptoms identical to those observed in field appeared in all inoculated plant within 1-2 days and the pathogen with identical characteristics was consistently reisolated from rotted tissue of inoculated plants.

Kumar *et al*. (2011) used different vegetables including onion bulb for pathogenicity of *Erwinia carotovora* and observation on symptoms development were recorded up to seven days of post inoculation.

Ismail *et al.* (2012) determined the pathogenicity of bacterial isolate associated with soft rot of girasole tuber in Egypt and observed that all bacterial isolates under investigation were able to infect girasole tubers and induced soft rot.

Rashid *et al*. (2013) confirmed the koch's postulate of *Erwinia carotovora* pv. *carotovora* the causal agent of soft rot of potato. Bacteria reisolated from diseased tuber and were reidentified.

Achbani *et al*. *(*2014) tested the pathogenicity of the culture of bacterial isolates, *Pectobacterium* and *Pseudomonas* obtained from rotted onion bulbs under greenhouse and laboratory condition and observed the symptoms after five days of incubation.

2.5 Morphological and biochemical properties of *Erwinia carotovora* pv. *carotovora*

Winslow *et al*. (1917) recorded observations on morphological and biochemical characters of *Erwinia* spp. the characters are rod shape with peritrichous flagella, nonspore forming, facultative anaerobic and gram negative.

Burkholder *et al*. *(*1953) recorded observation on morphological characteristics of *E. Chrysanthemi* as they are gram negative, straight rod with rounded ends and occur singly or in pairs.

Dickey (1979) reported that *Erwinia carotovora* pv. *carotovora* were gram negative, straight rod shape cell and showed initially cream coloured colonies and later yellow pigmentation.

Fahy and Hayward (1983) recorded that bacterium *E. chrysanthemi* produced creamy coloured colony.

Laat *et al*. (1994) reported that *Erwinia carotovora* pv. *carotovora* showed positive reaction to catalase and gelatine liquification test. However, negative reaction towards gram staining, starch hydrolysis and H_2S production test.

Schaad (1996) reported that bacterium *Erwinia carotovora* pv. *carotovora* produced round colonies with yellow pigmentation.

Yanez-morales *et al*. (2003) reported that *Erwinia* sp. showed negative reaction to gram staining. However, positive towards catalase and acid and gas production test.

Mandal and Maiti (2005) studied biochemical properties of *Pectobacterium chrysanthemi* and recorded positive reaction to catalase and gelatine liquification test. However, negative reactions to gram staining, starch hydrolysis and H_2S production test.

Samson *et al*. (2005) observed morphological and biochemical properties of *Erwinia carotovora* subsp. *carotovora* and reported that bacterium is a straight rod with rounded ends and showed positive reaction to catalase and gelatine liquification and negative for gram staining.

Rahman *et al*. (2012) observed that *Erwinia* sp. was gram negative and catalase test positive.

Mohammad and Selman (2013) studied the biochemical characteristics of *Erwinia carotovora* pv. *carotovora* showed negative reaction to gram staining and positive reaction to catalase, gelatine liquification and acid and gas production test.

Prajapat (2013**)** observed that *Erwinia carotovora* is rod shaped and gram negative.

Rashid *et al*. (2013) reported that *Erwinia carotovora* pv. *carotovora* showed negative reaction to gram staining, starch hydrolysis and KOH test. However positive reaction to catalase and gelatine liquification test

Ali *et al*. (2014) recorded that *Erwinia carotovora* pv. *carotovora* showed negative reaction to gram staining and positive reaction to catalase and acid production test.

Bhagat (2015) reported that *Erwinia carotovora* pv. *carotovora* causing bulb rot in onion showed negative reaction to gram staining, starch hydrolysis, KOH, H_2S production. Where as bacterium found positive towards the acid and gas production test, catalase test and gelatin liqification test.

EU and Asuquo (2016) found that *Erwinia carotovora* pv. *carotovora* causing soft rot in vegetables have ability to grow at 37^0C, production of reducing substances from sucrose; production of acid from maltose, α-methyl glucoside, lactose and trehalose; growth in 5% Nacl; production of indole, lecithinase and phosphatase; and sensitivity to erythromycin.

2.6 Efficacy of differents chemical treatments against *Erwinia carotovora* pv. *carotovora* by filter paper disc diffusion method

Gracia-Garza *et al*. (2002) tested the efficacy of copper oxychloride and streptomycin sulphate against *Erwinia* spp. and observed the inhibition.

Chung *et al*. (2003) observed that streptomycin, Bronopol (Bromo -2-Nitro propane 1,3 diol) and copper hydroxide significantly suppress the growth of *Erwinia carotovora* causing soft rot in Chinese cabbage.

Sendhilvel *et al*. (2005) reported that formalin two percent showed maximum inhibition zone of 1385.85 mm^2. Where as, the disinfectants viz. boric acid, bleaching powder and sodium hypochlorite were found less effective against *Erwinia carotovora var. carotovora* causing soft rot in onion.

Thammaiah *et al.* (2006) recorded maximum zone of inhibition with streptomycin sulphate 500 ppm and copper oxychloride 2000 ppm against E. *Chrysanthemi.*

Paresh *et al*. (2011) recorded the highest inhibition against *Erwinia carotovora* by copper oxychloride 500 ppm, and streptomycin sulphate 300 ppm showing 20.14% and 20.47% growth inhibition.

M. M. Rahman *et al.* (2012) evaluated the extracts from eleven different plant species such as jute (*Corchorus capsularis* L.), cheerota (*Swertia chiraita* Ham.), chatim (*Alstonia scholaris* L.), mander (*Erythrina variegata*), bael (*Aegle marmelos* L.), marigold (*Tagetes erecta*), onion (*Allium cepa*), garlic (*Allium sativum* L.), neem (*Azadiracta indica*), lime (*Citrus aurantifolia*), and turmeric (*Curcuma longa* L.) for antibacterial activity against potato soft rot bacteria, *E. carotovora* subsp. *carotovora (Ecc)* P-138, under *in vitro* and storage conditions. Out of the 11 different plant extracts, only extracts from dried jute leaves and cheerota significantly inhibited growth of *Ecc* P-138 *in vitro*.

Rahman *et al.* (2013) studied the bactericidal properties of eight chemicals against onion soft rot bacteria (*Burkholderia cepacia*). Among the chemicals, acetic acid, boric acid and bleaching powder showed bactericidal activity against onion soft rot bacteria (*B. cepacia)*.

Bhagat (2015) studied the management of bulb rot of onion caused by *Erwinia carotovora* pv. *carotovora* under *in-vitro* and *in-vivo* condition through the different fungicides and antibiotics such as copper oxychloride + streptomycin sulphate, streptomycin sulphate, bromo - 2 – nitro propane -1,3 diol (Bactericide Bactronol - 100), copper hydroxide, copper hydroxide + streptomycin sulphate. Among these chemicals combination of Copper hydroxide and streptomycin sulphate was found most effective chemical treatment for controlling bulb rot of onion.

2.7 Effect of different chemical treatments on bulb rot incidence of onion under field condition

Knauss and Miller (1972) recommended streptomycin to provide adequate control of *Erwinia carotovora* under field condition.

Kuehny *et al*. (1998) observed that streptomycin (200 ppm) dip of rhizome for 30 minutes can significantly reduce the infection of *Erwinia carotovora* pv *carotovora* in Calla rhizome.

Kapoor (1999) recommended steptocyclin in combination with copper oxychloride for control of soft rot diseases caused by *Erwinia carotovora* pv. *carotovora*

Gracia-Garza *et al*. (2002) observed that copper compound is effective against several plant pathogenic bacteria including *Erwinia* spp. This compound reduced the population of *Erwinia carotovora* pv. *carotovora* and found that post plant application of copper compound reduced the number of disease plants.

Chung *et al*. (2003) observed *in vitro* streptomycin, bronopol (bromo -2-nitro propane 1,3 diol) and copper hydroxide significantly suppress the growth of pathogenic bacteria *Erwinia carotovora* causing soft rot in Chinese cabbage.

Huang *et al*. (2003) reported that streptomycin possess the highest antibacterial activity against the soft rot diseases caused by *Erwinia* spp.

Kannan *et al*. (2006) reported that, soil drenching of streptomycin sulphate (500 ppm) performed well and reduced the rhizome rot disease incidence under field condition.

Palacio-Bielsa *et al*. (2007) observed that soft rot disease of onion caused by *Pectobacterium chrysanthemi* were sensitive to erythromycin.

Czajkowski *et al*. (2011) observed that streptomycin and chlorine-based compound bronopol (2-bromo-2-nitropropane-1, 3-diol) was promising for control of black leg and soft rot disease caused by *Erwinia carotovora* pv. *carotovora* in potato.

Marquez-Villavicencio *et al*. (2011) observed that copper spray may used to prevent infection of wounded plant stem and leaves due to *Erwinia carotovora.*

Pachupate and Kininge (2012) observed that streptomycin and copper compound with variable concentration were effective against *Pectobacterium* spp. in banana.

Rashid *et al*. (2013) observed that copper-based compound, copper oxychloride (0.2%) was highly effective to suppress the bacterial growth of *Erwinia carotovora* pv. *carotovora.*

Kenganal *et al.* (2017) studied different treatments on 25 plants in an orchard severely affected by soft rot. Among the treatments imposed, drenching and foliar spray of copper oxychloride 50WP at 3g/l + streptomycin sulphate 0.5g/l at 15 days interval, beginning from 15 days after planting and application of bleaching powder 25g/plant/month two inches away from pseudostem around the collar region upto four months was found most effective and recorded lowest soft rot disease incidence of 7.67 per cent during 2014 and 9.28 per cent during 2015. This treatment showed highest cost benefit ratio of 3.54 compared to control which had 1.6. Neither copper oxychloride nor the streptomycin sulphate alone or in combination could give better results as earlier reported. But their mixed application both by drenching and foliar spray had better impact and most effective when coupled with application of bleaching powder.

2.8 PURPLE BLOTCH

2.8.1 History and Occurance

Cook and Ellis (1879) first observed *Alternaria porri* fungus as *Macrosporium porri* on leaves of leek from New Jersey.

Ajrekar (1923) made first report on leaf spot and blight of onion threatening its cultivation in Bombay and designated the causal organism as the species of *Alternaria*.

Angell (1929) confirmed its identity with *Macrosporium porri* and named as ‘purple blotch’ being appropriate name due to the large size of lesions on the leaves and seed stalks.

Cifferi (1930) stated the binomial nomenclature for the causal organism as *Alternaria porri* on onion, it was described as *Macrosporium porri* (Ellis) in the literature.

Thirumalachar and Mishra (1953) studied purple blotch of onion and reported causal agent as *A. porri* (Ellis) Cif.

Rao (1964) reported that *Alternaria* blight of onion from Maharashtra caused by *Alternaria cepulicola.*

2.8.2 Symptomology

Chaput (1964) reported that warm temperature18-34^{0}C and wet period favoured the development of onion purple blotch disease, which exhibited small brown spots with purplish centres are the characteristic symptoms of the disease.

Utikar and Padulae (1980) reported that the disease in the field was characterized by dark purplish brown patches with paler outer zone which initially appear as small whitish sunken lesions about 1 mm in diameter towards the tip of the succulent leaves, later on extending downwards. The spots enlarge with age resulting in blight and wither-tip.

Verma and Sharma (1999) described that leaf blight symptoms of onion first appeared on leaves or inflorescence as small (2-3 mm in diameter) water-soaked lesions that quickly developed into white centers under favourable conditions. These lesions enlarged, coalesced, became zonate and turned brown to purple extending upwards and downwards. In moist weather, the surface of the lesions may be covered with black bodies of the fungus. The older leaves were more susceptible than younger leaves and were relatively more susceptible when they emerge close to bulb maturity. Similar lesions formed on seed stalks and as a result of this seeds either did not develop or they were shrivelled. The bulbs can also be affected at harvest when the fungus entered through the neck or injury causing storage rot.

Undhad (2009) described symtomps of purple blotch on naturally infected plants as whitish circular to irregular specks on leaves and flower stalks which are less than 1 mm in diameter which developed into whitish sunken lesions and increasesd in size and shape and converted into oval to irregular. The purplish spots were developed in the center of lesions. Later, on the purplish areas assumed the shape of blotch surrounded by chlorotic margins. Dark purple to black zones were developed within the purple blotch lesion in the advanced stages of

disease. The black zones in the blotch representing the sporulating mycelium. The purple blotch lesions gridled leaves in severe form resulting in drooping of leaves.

Vijayalakshmi *et al*. (2012) reported that symptoms of purple blotch on infected leaves of onion as small, sunken, oval to foot-ball shaped lesions. The lesions were brown to purple at the center surrounded by brown area. Concentric light and dark zones also observed on infected leaves. Blotches were enlarged up to 4 inches long and were covered with conidia. Brown lesions with reddish-purple margins resembling bull's-eye were also noticed.

2.8.3 Isolation and proving pathogenicity

Nolla (1927) observed *Alternaria alli* to cause the disease of onion by conducting some inoculation experiments in the glasshouse. Six healthy plants growing in clay pots were sprayed with spore-suspension of the fungus. The pots were then covered with bell jars lined inside with moist filter paper. Two days later the first symptoms of the disease appeared as small, whitish necrotic areas. The second set of six plants was sprayed with suspension of bits of mycelium from six days old culture and covered with bell jars. The symptom of the disease appeared on second and third day. A third set of plants was inoculated by transferring spores of the fungus to needle pricks made on the surface of the leaves. A rapid dying of the adjacent tissues was observed on the second day. Check plants wounded but not inoculated showed no further evidences of injury.

Ayyangar (1928) observed the infection by *A. palandui* when the fungus was inoculated on plants.

Skiles (1950) reported that *A. porri* was most pathogenic on mechanically injured leaves.

Boisson and Renard (1967) described the symptoms of onion disease caused by *A. porri* as well as the morphology of the pathogen from Ivory Coast.

Ponnappa (1974) evaluated some inoculation techniques and reported that the symptoms appeared much earlier on the plants sprayed

with suspension of spores and mycelium, following pricks made by needle. Further he reported that *A. cepulae* produced symptoms on the leaf with a circular to oval water-soaked areas which later on, as the disease progressed, became oblong. As the fungus advanced and ramified within the host tissue, a fresh zone of discoloured tissue was formed around the spots. Initially spots were white, but later turned pinkish or purple. The change in colour started from the center and gradually progressed towards the periphery, where it changed into light purplish. The transition of colour was marked by concentric rings clearly visible to the naked eye. The lesions on the stalk of the inflorescence axes caused girdling and, in most cases, resulted in the destruction of the stalk. In older spots, the spores were produced abundantly on tufts of conidiophores, which appeared as large black patches over the diseased area measuring 6-7 x 3-5 cm.

Castellanos-Linares *et al*. (1986) proved the pathogenicity of *Alternaria alternata* by keeping the plants after inoculation in moist chambers for 72 hours. Symptoms very similar to those manifested as a result of infection by *Alternaria porri* developed.

Patil and Patil (1992) proved the pathogenicity of *Alternaria* state of Pleospora infectoria on 75 days old plants. The disease appeared on the leaves 12 days after artificial inoculation.

Docampo and Conci (1996) proved the pathogenicity of *Alternaria alternata* and *Alternaria porri* on garlic (*Allium sativum*). Three weeks after inoculation, there was evidence of brown blotch on inoculated areas of leaves.

Koike and Hinderson (1998) proved the pathogenicity of *A. porri* by spraying conidial suspension (1.0 x 104/ ml) on 2 months old leek plants. After 14 days the leaf spots similar to the original symptoms developed on inoculated plants.

Verma and Sharma (1999) leaf blight symptoms on onion first appeared on leaves or inflorescence as small (2-3 mm in diameter) water-soaked lesions that quickly developed into white centers under favourable conditions. These lesions enlarged, coalesced, became zonate and turned

brown to purple extending upwards and downwards. In moist weather, the surface of the lesions may be covered with black bodies of the fungus. The older leaves were more susceptible than younger leaves especially at the time of bulb maturity. When the lesions were formed on seed stalks, seeds either did not develop or they were shrivelled. The bulbs were affected at harvest when the fungus entered through the neck or any injury at the neck region causing storage rot.

Tripathi *et al*. (2008) isolated and purified *A. porri* (Ellis) Cif. from infected plant of onion collected from different locations.

Undhad (2009) isolated *A. porri* (Ellis) Cif. by tissue isolation technique.

Madhavi *et al*. (2012) established the pathogenicity of *Alternaria porri* by following Koch's postulate, on onion leaves.

2.8.4 Morphological characters of *Alternaria porri*

Cifferi (1930) described morphological characteristics of *Alternaria porri* as conidiophore arised singly or in group and are straight or flexouse sometimes geniculate, pale septate and pale to mid brown, upto 120 µm with one or several well defined conidials scars. Conidia usually occured singly, straight or curved and tapered to beak.

Ellis (1971) reported that conidiophores arising singly or in groups, straight or flexouse, sometimes geniculate, pale septate and pale to mid brown, upto 120 µm long, 5-10 µm thick, with one or several well defined conidials scars. Conidia were generally solitary, straight or curved, obclavate or with the body of conidium ellipsoidal tapering to the beak which was commonly about the same length as the body but may be shorter or longer usually 100-300 µm, 15-20 µm thick in the broadest part with 8-12 transverse and zero to several longitudinal or oblique septa, beak flexuous, pale 2-4 µm thick, tapering.

Koike and Hinderson (1998) noted that the conidia obtained from leaves were obclavate in shape with slender, unbranched beaks extending from the narrow end of the spore body. Spores bodies had 6-9 transverse septa and occasionally one longitudinal septum.

Agale *et al*. (2015) recorded that hyphae of *Alternaria porri* were septate and irregular, branched, conidiophores arising singly or in groups, straight and pale to brown, measured 90-100 x 9-12 µm. Conidia were generally solitary, straight or curved, obclavate and measured 52.7-126 x 9.26 - 16.20 µm and long beak with 6-11 transverse and 0-3 logitudinal septa. It exhibited fluzzy, aerial and greenish grey to black colonies on oat meal agar medium.

2.8.5 Evaluation of fungicides under *In-Vitro* condition;

Ponnappa (1974) studied *in-vitro* efficacy of fungicides against leaf blight on onion caused by *A. cepulae* and reported that mancozeb (Dithane M-45), Aureofungin and Duter showed complete inhibition at 0.2% concentration.

Gupta *et al*. (1981) found that 10 fungicides inhibited fungal growth at all tested concentrations but Dithane M-45 (Mancozeb) at 1000 ppm was most effective.

Gupta (1987) found bavistin superior to other fungicides both under in vitro as well as in field trials. This was followed by difoltan, ziram and berstan in descending order of their efficacy against *A. porri*.

Srivastava *et al.* (1991) evaluated four fungicides, copper oxychloride, mancozeb, carbendazim and thiram on purple blotch of onion caused by *Alternaria porri.* Mancozeb gave the highest efficacy in controlling the disease.

Sastrahidayat (1994) reported that difenconazole (0.8 ml/liter) inhibited the growth of *Alternaria porri* under laboratory condition.

Huq *et al.* (1994) evaluated four fungicides, iprodone 0.2% (Rovral) mancozeb 0.2%, cuprovit 0.2%, copper oxychloride and propineb 0.2% (Antracol) for inhibition of growth of *Alternaria porri.* They reported that rovral gave the best inhibition of growth followed by mancozeb.

Sachin and Sharma (2007) reported that metalaxil (Ridomil MZ) was most effective in controlling the purple blotch disease of onion caused by *Alternaria porri* followed by hexaconazol, penconazol, difenconazole and mancozeb.

Deshmukh *et al*. (2008) tested seven fungicides viz., mancozeb, thiram, copper-hydroxide, copper oxychloride, chlorothalonil, zineb, sulphur for their effect on growth of *Alternaria porri* using poisoned food technique. The most and least effective fungicides were mancozeb (83.8%) and sulphur (24.8%), respectively. The next best fungicide was thiram, which exhibited mean inhibition of 81.3 per cent within the treatments. Mancozeb, thiram, copper hydroxide, copper oxychloride and chlorothalonil inhibited more than 50 per cent fungal growth even at lower (500 ppm) concentration.

Chethana *et al*. (2011) evaluated different systemic and non-systemic fungicides against *Alternaria porri* causing purple blotch of onion under *in-vitro* conditions. Among the systemic fungicides tested difenconazole was most effective in inhibiting the growth of test pathogen at 0.1% with per cent inhibition of 98.85% followed by kitazin and propiconazole with 96.70% and 86.15% respectively. While among the non-systemic fungicides tested mancozeb (0.3%) was most effective in inhibiting the fungal growth by recording 100 per cent inhibition followed by iprodione (0.3%) and copper oxychloride (0.3%) with of 91.50 per cent inhibition and 88.80% respectively.

Madhavi *et al*. (2012) studied the efficacy of five fungicides in inhibiting the growth of *A. porri* by culturing the pathogen in Czapek-Dox broths prepared with different concentrations of fungicides. Out of the five fungicides tested, mancozeb was highly effective at all concentrations followed by blitox and benlate. Results also revealed that all the fungicides recorded a gradual reduction in fungal growth as the concentration was increased from 10 to 500 ppm.

Mishra and Gupta (2012) studied the effect of systemic and non-systemic fungicides on mycelial growth of *A. porri* and *S. vesicarium*. All the fungicides tested showed significant differences in their efficacy to inhibit the growth of both the pathogens. Mancozeb at 0.2% recorded maximum mean per cent inhibition (98.94%) of *A. porri* and 100% of *S. vesicarium* followed by companion at 0.2% (98.40 and 97.13%) as compared to other treatments. Among the systemic fungicides,

Azoxystrobin (0.1%) gave maximum mycelial inhibition (94.40% and 94.23%), followed by propiconazole at 0.1% (89.10% and 87.14%).

Aujla *et al*. (2013) studied the efficacy of nine fungicides against purple blotch of onion under in vitro conditions. All the fungicides significantly inhibited mycelial growth of the pathogen. Score 25 EC (difenconazole) and Nativo-75WG (trifloxystrobin 25% + tebuconazole 50%) were found to be most effective as these completely inhibited the mycelial growth at 0.1 per cent.

Yadav *et al*. (2013) reported that hexaconazole was the most effective fungicide among six systemic fungicides tested *in-vitro* inhibiting mycelial growth of A. porri followed by propiconazole, difenconazole, tebuconazole, thiophanate methyl and carbendazim. Hexaconazole completely inhibited (100%) the growth of the test fungus at higher concentration of 250 and 500 ppm, propiconazole remained second in inhibiting fungal growth completely at 500 ppm. Carbendazim was least effective with a mean inhibition per cent of 10.97 within the treatments.

Wanggikar *et al.* (2014) were evaluated different fungicides *in-vitro and in-vivo* against *Alternaria porri* and recorded significant inhibition of test pathogen over untreated control. Result revealed that in hexaconazole cent per cent (100.00%) inhibition was observed, followed by difenozole (83.91%), mancozeb (63.58%), *P. florescene* (58.94%) and *T. viridae* (54.45%). The minimum per cent inhibition was observed in chlorothalonil (31.40%) followed by the plant extract NSKE (43.92%), copper oxychloride (46.87%) and carbendazim (47.11%).

Jhala Priyanka *et al.* (2017) evaluated different fungicides and botanicals against *Alternaria porri*. Among different fungicides difenaconazole completely inhibited the mycelial growth of *A. porri* at concentration of 250, 500, 1000 and 2000 ppm, followed by tebuconazole at 1000 and 2000 ppm. Neem formulations azadirachtin was found effective *in vitro* followed by garlic extract and neem oil proved to be least inhibitor. Under pot culture study, combination of difenaconazole and azadirachtin was found most effective when applied as foliar spray.

Yadav Ritesh Kumar *et al.* (2017) reported six systemic fungicides *viz.*, Iprobenfos, Propiconazole, Tebuconazole, Difenoconazole, Amistar Top 325 SC (azoxystrobin 18.2% + difenoconazole 11.4%) and Nativo 75 WG (trifloxystrobin25% + tebuconazole 50%), and two non-systemic fungicides *viz.*, Mancozeb and Copper hydroxide, were evaluated under *in vitro* conditions for their efficacy to manage purple blotch complex of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. The triazole fungicides, tebuconazole and difenoconazole proved superior in inhibiting growth of *A. porri* and *S. vesicarium* under *in vitro* conditions, respectively.

2.8.6 Evaluation of fungicides under *In-Vivo* condition

Sharma (1986) evaluated the efficacy of different fungicidal sprays under field conditions against purple blotch of onion and found Dithane M-45 (0.2%) as most effective followed by Difolatan (0.3%), Dithane Z-78 (2%) and Daconil (0.1%).

Gupta *et al*. (1986) reported purple blotch to be a serious disease prevalent all over the country. They found Dithane M-45 as the most effective fungicide for control of the disease.

Kolte and Dhawale (1989) assessed the efficacy of nine fungicides as 4 sprays- against purple blotch. The treatment-with 'Dithane M-45 was -found most promising, which reduced the PDI to 1.02%.

Mishra *et al*. (1989) conducted field trials at Chiplima and Keonjhalin in Orissa with fungicides for control of purple blotch of onion. They found maximum disease control with the application of mancozeb (0.2%) followed by carbendazim. The treatment had also reduced the disease intensity and increased the bulb yield by 25.73 and 17.60 per cent, respectively.

Gupta *et al*. (1991) found that 4 sprays of Mancozeb @ 0.25% at fortnightly intervals were quite effective in controlling the disease.

Srivastava *et al*. (1991) evaluated 4 fungicides viz. Copper oxychloride, Mancozeb, Carbendazin and Thiram against *Alternaria porri* and all the fungicides significantly reduced the disease incidence.

Kolte *et al*. (1993) reported Dithane M-45 as foliar sprayings at 40 DAT, 61 DAT and 82 DAT to bemost effective in controlling the disease.

Maheshwari *et al*. (1993) evaluated five treatments of fungicides and malathion applied as foliar spray@ 0.2%; maximum disease control (77.68%) was achieved with M-45 + malathion.

Sokhi (1994) reported purple blotch (*Alternaria porri*) is the most damaging disease both for bulb and seed crop. Mancozeb or diflotan spray gives protection against the disease.

Borkar and Patil (1995) tested five fungicides for the control of this disease in Kharif onion and found that mancozeb @ 0.2% reduced the disease intensity by 6% and increased the yield by 10.99%.

Sugha (1995) reported that three foliar sprays of ipredione @ 01% alone or in combination with copper oxychloride 0.1% and mancozeb 0.1% at 15 days interval resulted in 53.5 to .62% protection to the crop from blight.

Upadhaya and Tripathi (1995) determine the effect of Bavistin (Carbendazin), Blitox (copper oxychloride), Calixin (tridemorph), Captafol, Dithane M-45 (Mancozeb), Dithane Z-78 (Zineb), Jkstein (methyl), Karathane EC (dinocap) and Topsin M-70 (thiophanate-methyl) on control of *Alternaria porri* on onions (*Allium cepa*). All treatments significantly reduced disease intensity and gave increased yields over the control. The best results, however, were obtained with captafol.

Maheshwari *et al*. (1996) obeserved that copper oxychloride to be the most effective fungicide, controlling the disease by 68%, followed by mancozeb, dodine and ziram. Though the differences in disease controlling potential of these fungicides were marginal yet differences in the yield were quite pronounced, as sprays with copper oxychloride resulted in greater yield increase than with mancozeb. The disease index for all the foliar sprays differed significantly from the unsprayed control. Again, disease control both with zineb and captafol was similar, i.e. 54%, but the increase in bulb yield was greater in the latter.

Raja *et al*. (2000) reported copper oxychloride to be most effective against leaf spot disease of onion.

Khambete *et al*. (2001) tested newer and safer formulations of Mancozeb- Mancozeb-flowable and Cuprous oxide 75% WP against purple blotch and found them quite effective in controlling the disease.

Memane *et al*. (2001) found a combination of Mancozeb @ 0.3% and Monocrotophos, @ 0.05% or Cypermethrin 0.1% along with sticker Sandovit @ 0.1 %. sprayed alternately starting from 15 days after transplanting of the crop at 15 days interval to be most effective, and it reduced thrips population by 87%, leaf blight by 68% and increased yield by 69% over control.

Ghure *et al*. (2001) reported that treatment Rovral (ipridon) 0.2% as most effective in controlling disease by recording minimum disease intensity (22.53%).

Champawat and Sharma (2003) evaluated fungicides as seed dresser @ 0.25% and soil drenching @ 0.3% 15 days after planting. Captan proved distinctly superior over rest of the treatments with neem cake as the next best treatment.

Vijaya and Rahman (2004) observed that four sprays of Mancozeb @ 0.3% with Monocrotophos @ 0.05% proved as the best to reduce the disease incidence.

Rahman (2004) observed the effect of three fungicides viz., Ridomil, Rovral and Tilt 250 EC (0.2%) comprising 13 treatments in field experiment. Eight sprays of Rovral or Ridomil at 7 days interval minimized disease incidence and increased yield. Rovral 0.2% sprayed at 7 days interval was the best, which gave the highest reduction in disease incidence and severity of leaf blotch and eventually increased the yield of onion.

Mathur and Sharma (2006) found that mancozeb and copper oxychloride to be most effective in reducing purple blotch intensity and increasing the yield of onion bulbs.

Akter (2007) conducted a field experiment to study the management of purple blotch of onion through chemicals and plant extracts. Eleven treatments comprising Dithane M-45, Rovral 50WP, Bavistin 50WP, Cupravit 50WP, Proud 250EC, Champion, Tilt 250EC, Ridomill Gold, Neem leaf extract, Allamanda leaf extract and Control were explored in the experiment. The highest bulb yield (8.767 t/ha) was obtained with Rovral 50WP treated plot. The percent plant infection, percent leaf infection, percent Leaf Area Diseased (% LAD) and Percent Disease Index (PDI) were the lowest in foliar spray with Rovral 50WP and the highest in control treatment. Neem extract performed better than Allamanda extract.

Deshmukh *et al.* (2007) studied eight different fungicides were tested on highly susceptible variety Pilipatti under net house conditions. Maximum disease control (79.58%) was recorded in foliar application of a mixture of hexaconazole (0.005%) + mancozeb (0.3 %) followed by difenoconazole (0.025 %) + mancozeb (0.3 %) in reducing disease intensity (78.58 % and 70.72 %) as well as avoiding yield loss by 26.16 and 22.67 per cent, respectively over control.

Beig *et al* (2008) evaluated nine fungicides *viz.*, hexaconazole, difenoconazole, metalaxy, mancozeb, propineb, ziram, captan, zineb and carbendazim against PLB of onion under field conditions and reported that hexaconazole and difenoconazole were superior over all other fungicides tested, which were at par with each other, and restrained the disease incidence and severity to the least value. However, carbendazim proved least effective against the disease.

Aujla *et al*. (2013) studied the efficacy of nine fungicides against purple blotch of onion. Out of these nine fungicides, highest disease control (85.0%) and seed yield were recorded in foliar application of Nativo–75 WG followed by Folicur 250 EC (tebuconazole) and Tilt 25 EC (propiconazole).

Wanggikar *et al.* (2014) were evaluated different fungicides against *Alternaria porri*. Result revealed that hexaconazole (0.1%) was found most effective and recorded significantly mean disease incidence

(6.03%) and intensity (13.33%) with corresponding significantly increased bulb yield (438.00 q/ha, followed by mancozeb (@ 0.2%) and copper oxychloride (0.25%) which recorded significantly mean disease incidence of 6.83 and 8.53 per cent and intensity,15.00 and 20.00 per cent, respectively and gave correspondingly bulb yield, respectively of 375.00 and 429.00 q/ha. The botanical tested, *A. indica* @ 5% was found antifungal against *A. porri* was recorded significantly disease incidence (7.96%) and intensity (27.00%).

Rao A. S. *et al*. (2015) observed Cymoxanil 8% + Mancozeb 64% WP @ 2500 ppm and Mancozeb 70% WP @ 2500 ppm were effective in reducing the disease severity by 54.86 and 52.88 % over untreated control. However, Mancozeb 70% WP and Cymoxanil 8% + Mancozeb 64% WP recorded the maximum yield of 39.71 and 37.06 t/ha and obtained benefit cost ratio of 33.85 and 20.67. Thus, these two fungicides can be recommended for the effective and economical management of the disease.

Umme Sarifun Akter *et al.* (2015) study the management of purple blotch of onion through some chemicals and plant extracts such as Dithane M-45, Rovral, Bavistin, Cupravit, Proud, champion, Tilt, Ridomil, Neem extract and Alamanda extract. Among these chemicals Dithene M-45 were found most effective to minimize disease severty as well as increase of yield. Between two plant extract Neem extract performed better than Alamanda extract

Behera *et al.* (2017) reported that fungicides applied as foliar sprays along with sticker were Difenaconazole 25 EC (0.025%), Difenaconazole 25 EC (0.05%), Difenaconazole 25 EC (0.1%), Chlorothalonil (0.2%), Mancozeb 75 WP (0.25%), Copper oxychlororide (0.3%) and untreated control. Though the differences in disease controlling potential of these fungicides are marginal yet differences in the yield were quite pronounced, as sprays with Difenaconazole 25 EC (0.1%) resulted in greater yield increase than the other fungicides. Difenaconazole had earlier been reported to be quite effective in controlling purple blotch disease of onion and increase the bulb yield by Sastrahidayat. However, in the

present study Difenaconazole 25 EC (0.1%) proved to be more promising in management of disease as well as increasing bulb yield

Yadav Ritesh Kumar *et al.* (2017) reported six systemic fungicides *viz.*, Iprobenfos, Propiconazole, Tebuconazole, Difenoconazole, Amistar Top 325 SC (azoxystrobin 18.2% + difenoconazole 11.4%) and Nativo 75 WG (trifloxystrobin25% + tebuconazole 50%), and two non-systemic fungicides *viz.*, Mancozeb and Copper hydroxide, were evaluated to manage purple blotch complex of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Field efficacy of the fungicides at different concentrations were determined in controlling the purple blotch complex of onion under artificial epiphytotic conditions on bulb and seed crop. Foliar sprays (3 for bulb crop and 4 for seed crop) of tebuconazole 25 EC (Folicur) @ 0.1 per cent at fortnightly interval most effectively managed purple blotch complex of onion under field conditions with highest Benefit: Cost ratio (8.75:1 and 88.7:1) in bulb and seed crop, respectively.

2.9 Effect of different chemical treatments on seed yield of onion

Tomar and Negi (2002) recorded highest onion seed yield 576.80 Kg/ha with low incidence of diseases and better seed quality.

Anisuzzaman *et al*. (2009) recorded onion seed yield 529.06 Kg/ha with better seed quality in Bangladesh.

Karim and Ibrahim (2013) observed that 402.28 Kg/ha seed yield of onion.

Ali *et al*. (2015) recorded medium size bulb yielded 370-520 Kg/ha onion seed yield.

Selim Ahmed *et al*. (2018) reported highest onion seed yield i.e 580 kg/ha against purple blotch of onion.

Zakirul islam (2013) observed maximum seed yield (649.40 kg/ha) with low incidence and intensity of purple blotch of onion.

CHAPTER III

MATERIAL AND METHODS

The present investigations on “Chemical management of bulb rot and purple blotch of onion” were conducted during 2018-2019 at the Department of Plant Pathology and Department of Vegetable Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The materials used and methods adopted during the present investigation are described in this chapter.

3.1 Materials

Following materials were used for experimentation.

3.1.1 Glassware

The glassware used during the course of research work were of Borosil make. The glassware *viz.* Petri-plates, test tubes, conical flasks of 250 ml, 500 ml and 1000 ml, funnel, beakers, pipettes, measuring cylinder, slides, cover slips and glass rods were used.

3.1.2 Equipments used

Standard laboratory equipments *viz*., autoclave, BOD incubator, laminar air flow, research microscope, stereoscopic binocular microscope, refrigerator, hot air oven, digital weighing balance, bunsen burner, water distillation unit etc. were used.

3.1.3 Onion bulb

The bulbs of onion were received from Department of Vegetable Science, College of Horticulture Dr. PDKV Akola.

3.1.4 Other materials used

Blotter paper, non-absorbent cotton, muslin cloth, polythene bags, cork borer, inoculation needle, bacterial loop, dissection needle, forceps, spirit lamp, paper bags, pencil, permanent marker, cello tape, whatman No. 1 filter paper, test tube stand, tray, knapsack spray, potato, rubber band, etc. were used during research programme.

3.2 Methods

3.2.1 Sterilization of glassware's and other materials

The cleaned and dried glassware sterilized in hot air oven at 180^0C for 1 hr. Culture media and distilled water was sterilized in autoclave at 1.05 kg cm^{-2}pressure for 15 min at 121.6^0C. Soil sterilization was done with the help of 10% formalin solution. Soil was mixed with 10% formalin solution and then covered with the polythene for 5 days.

3.2.2. Collection of disease sample

Naturally infected onion showing typical well-developed symptoms of bulb rot and purple blotch were collected from the experiment field, located at Department of Vegetable Science, College of Horticulture Dr. PDKV Akola.

3.2.3. Preparation of Nutrient Agar (NA) medium and Potato Dextrose Agar (PDA) medium.

3.2.3.1 NA medium

The nutrient agar (NA) medium was used as basal medium for the *in-vitro* stuides and maintenance of pure culture of *Erwinia carotovora* pv. *carotovora* in slants.

Composition of NA medium:

Beef extract - 3 gm

Peptone - 5 gm

Agar - 20 gm

Nacl - 5 gm

Water - 1000 ml.

Suspend the beef extract, peptone, agar, and Nacl in 1 litre of distilled water. Heat this mixture while stirring to fully dissolve all components. The medium was distributed in flasks and tubes and sterilized in autoclave at 1.05 kg cm^{-2}pressure for 15 minutes at 121.6^0C. The slants were used for maintenance of culture and the medium in flask for isolation of bacteria.

3.2.3.2 PDA medium

The potato dextrose agar (PDA) medium was used as basal medium for *in-vitro* studies and for maintenance of pure culture of *Alternaria porri* in slants.

Composition of PDA medium:

Peeled potatoes	-	200 gm
Agar-agar	-	20 gm
Dextrose	-	20 gm
Distilled water	-	1000 ml

200 g peeled potatoes were sliced into pieces and boiled in 500 ml distilled water till properly cooked. The extract was strained through muslin cloth and measured. In the remaining water after dissolving 20 g agar and 20 g dextrose, potato extract was added and the volume was made to one litre. The medium was distributed in flasks and tubes and sterilized in autoclave at 1.05 kg cm^{-2} pressure for 15 min at 121.6^0C. The slants were used for maintenance of culture and the medium in flask for isolation of fungus.

3.3 Isolation of pathogens

3.3.1 Isolation of *Erwinia carotovora* pv. *carotovora*

Isolation of bacterial pathogen was made from diseased bulb collected from field by streaking method.

1) Diseased bulbs were washed under running water to remove excess soil but avoided breaking the skin.
2) Braked or cut the skin was removed and a small tissue from the rotting front of the lesion with healthy portion was transfered into sterile Petri-plate using a sterile scalpel containing sodium hypochlorite solution for surface disinfection.
3) Then pieces were transfered to sterilized water and washed with 3 changes to remove the traces of sodium hypochlorite.

4) Cut and teased the tissue in sterile water (2ml.) in a Petri-plate. Leaved for 5 min to allow the bacteria to diffuse out of the tissue.

5) Approximately 20ml autoclaved NA media was poured in each sterilized petridish and allowed to solidify.

6) Streaked loopful of liquid sample suspension with a sterile inoculating loop.

7) NA plates were incubated at 27^0C for 48 hr. at upside down position.

Growth of organism was observed regularly and maintained on NA slants.

3.3.2 Isolation of *Alternaria porri*

Isolation of the fungus was done by tissue isolation technique.

1) Diseased leaves were washed under running water to remove excess dust but avoided breaking the leaves.

2) Typical diseased spots on leaves were selected and cut into small bits with the help of a sterilized blade.

3) These bits of diseased tissues were washed with sterilized distilled water and disinfected with 0.1 per cent sodium hypochlorite solution for 30 to 60 seconds.

4) These disinfected bits were immediately washed thrice with sterilized distilled water to remove the excess of sodium hypochlorite.

5) Then these bits were placed on the surface of Petri-plates containing potato dextrose agar (PDA) and incubated at 27 ± 1^0C for 10 days.

6) Growth of organism was observed regularly and after 10 days subculture to obtain the pure culture.

All these operations were performed under the aseptic condition.

3.3.3 Purification and maintenance of the culture:

Resulting bacterial colonies were selected from each NA medium plate and re-streaked the bacteria on to a fresh NA plate and these plates were incubated at 28 $\pm$ 2^0C for 24 hr. The single colonies developed, were transferred in NA medium slants and the pure cultures so obtained were stored in refrigerator at 4^0C for further studies.

The resulting fungus culture was purified by hyphal tip technique in PDA slant. The pathogen was sub cultured on PDA slants and allowed to grow at 27±1^{0}C for 10 days and such slants were preserved in a refrigerator at 4^{0}C and renewed once in 30 days.

3.4 Identification of pathogens

The isolate of *Erwinia carotovora* pv. *carotovora* were identified on the basis of their morphological and biochemical properties.

3.4.1 Morphological properties of *Erwinia carotovora* pv. *carotovora*

The confirmations of the *Erwinia carotovora* pv. *carotovora* isolate were performed with the following studies. Pure culture of bacteria was streaked on NA medium petri-plate separately for colony development. The individual colonies were examined for colony colour, shape, size and pigmentation.

3.4.2 Biochemical properties of *Erwinia carotovora* pv. *carotovora*

Twenty-four hours-old cultures, of the obtained bacterial isolate, were biochemically and physiologically characterized, identified and subjected to the following tests: Gram reaction, KOH test, catalase test, potato soft rot test, gelatin liquification test, urease production test, growth in 5% Nacl test, H_2S production test, indole production test, oxidase test and Methyl red test, were carried out for biochemical confirmation of *Erwinia carotovora* pv. *carotovora.*

3.4.2.1 Gram reaction

First a smear was prepared of bacterial cells by holding a clean slide by grapping at edges. A loopful of bacterial suspension was transferred in the center of slide, with the help of wire loop. The drop was smeared over slide and air dried. Then dried smear was fixed by passing the slide 3-4 times rapidly over the flame. The smear was flooded with crystal violet for 30 seconds, washed in the tap water. Then the smear was immersed in potassium iodide/ Lugol's iodine solution for 30 second's washed in tap water then decolorized with 95% alcohol and rinsed with water. Counterstained with saffranin for 10 second, again washed with tap

water and air dried. Drop of cedar wood oil was placed on the slide and examined the smear under oil immersion lense.

3.4.2.2 KOH (3%) test

Fresh solution of 3% potassium hydroxide (KOH) was prepared and then a drop of this solution was placed on regular microscopic slide. A 24-hour old bacterial culture was placed in this drop and mixed for 10 seconds. Bacterial suspension making thread when lifted up by bacterial loop were considered gram negative. Production of a watery suspension (no viscous strands when lifted up) indicates gram positive result.

3.4.2.3 Catalase test

A small amount of culture from isolated bacteria colony was placed on the microscopic slide. Using a dropper or pasteur pipette, 1 drop of 3% H_2O_2 was placed onto the organism on the microscope slide. Immediately observed for bubble formation (O_2 + water = bubbles). Positive reactions were evident by immediate bubble formation.

3.4.2.4 Potato soft rot test

The bacterial isolate originated from single colonies were tested for their ability to cause soft rot on potato tubers. Briefly, potato tubers were sterilized with 70% ethyl alcohol, rinsed with sterile distilled water, and aseptically cut into slices (1 cm). The potato slices were put in Petridishes containing sterilized filter paper impregnated with 2 ml of sterile distilled water. The potato slices were inoculated with needle pricking method. The inoculated slices were maintained in moistened Petri-plates and incubated at 30^0C for 2 to 3 days. The bacterial cultures that produced characteristic symptoms of soft rot on potato slices were selected and preserved in test tubes containing NA media overlayed with sterile liquid paraffin at 4^0C in a refrigerator for further studies.

3.4.2.5 Gelatin liquification test

The test was performed to check the ability of the microorganism to produce the enzyme gelatinase. Prepared the medium according to the given composition and sterilized after pouring into the test

tubes. Inoculated the test organism into the sterilized test tube and left one uninocculated as control. Incubated both the tubes at 37^0C for 24-48 hrs. After incubation kept the tubes in freeze for 30 min. The results were recorded depending on the condition wheather gelatin is in the liquid state (Possitive reaction) or in the solid form (Negative reaction).

3.4.2.6 Urease production test

The urease test is used to determine the ability of an organism to split urea, through the production of enzyme urease. For this test media was prepared according to the given composition and sterilize after pouring into the test tubes. The test organisms were inoculated into the tubes and one was left uninoculated as control. The tubes were inoculated at 35-37^0C for 7 days and the observations were recorded as development of pink colour–possitive test where, no colour change – negative test.

3.4.2.7 Growth in 5% NaCl

For this test, nutrient agar medium was prepared with 5% NaCl. The medium was inoculated with bacterial isolates, and the inoculated test tubes were incubated at 27^0C for 24 hours. Growth of isolates in this medium was considered as salt tolerance. In test tube used for control there was no growth.

3.4.2.8 Hydrogen sulphide (H_2S) production

The peptone water medium was used which comprised of Peptone 10 g, NaCl 5.0 g, water 1000 ml and pH 7.0. The medium was dispensed in 5 ml quantities in tubes and autoclaved. To detect H_2S, the lead acetate test strips were prepared as follows. Whatman No. 1 filter paper was cut into 5 × 50 mm strips which are then soaked in warm saturated solution of lead acetate. The strips were then dried, autoclaved and again dried at 60^0C. The medium in each tube was inoculated with a loopful of 48 hours slant growth of the test bacterium. After inoculation, the test strip was inserted in between the plug and inner wall of the tube, so that it hangs just above the broth. The tubes are incubated at 25^0C and the observations were recorded at regular intervals upto 14 days. The blackening of test strip indicates liberation of H_2S.

3.4.2.9 Indole production test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Trypton broth was prepared according to the given composition. The broth was dispensed into the tubes and sterilized. The test organisms were inoculated into the tubes and one was left uninoculated as control. The tubes were inoculated at 37^0C for 48 hours After incubation 1ml of KOVAC's reagent was added to all the tubes including control. The tubes were shaken gently and allowed to stand for 1-2 minute. The tubes were observed for formation of cherry red ring.

3.4.2.10 Oxidase test

The oxidase disc was added to 48 hours. culture grown on NA medium. The oxidase reaction was carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction was observed within 5-10 seconds at 25-30^0C (blue colouration). While the no change at all was considered a negative reaction.

3.4.2.11 Methyl red test

Methyl red (MR) test is performed with the bacterial isolates to detect the ability of organism to produce stable acid end products (Mixed acid fermentation) from supplied glucose. Methyl red broth was prepared according to the given composition. The broth was dispensed into the tubes and sterilized. The test organisms were inoculated into the tubes and one was left uninoculated as control. The tubes were inoculated at 35-37^0C for 48 hours. After incubation 3-4 drops of methyl red reagent was added to the tubes including control. The observations were recorded as development of red colour results as positive test where, no colour change declares negative test.

3.4.2.12 Morphological characters and identification of the *Alternaria porri*

The study was undertaken to confirm the identity of the isolated pathogen. Identification of the fungus was made after examining conidia under microscope (under 40x) from mature pure culture of the

fungus obtained from infected leaves of onion. Stage and ocular micrometer were used to measure the length, breadth, beak length and number of septa of the fungus. The average length and breadth of the conidial body, beak and septal number were recorded. These observations were compared with those of the standard measurements given by Ellis (1971) to identify the pathogen.

3.5 Pathogenicity test

3.5.1 Pathogenicity test of *Erwinia carotovora* pv. *carotovora*

Bulb and soil inoculation methods were carried to test the pathogenicity of *Erwinia carotovora* pv. *carotovora.* Plastic pots having 1 Kg capacity were disinfected with the help of denatured spirit. Sterilized potting mixture with bacterial culture suspension (3 X 10^{-6} CFU/ml) of test pathogen 100 ml was filled in the pot. The onion bulbs (cultivar Akola safed) were inoculated by dipping in bacterial suspension of *Erwinia carotovora* pv. *carotovora* and these inoculated bulbs were planted in the pot. The uninoculated pot and bulb served as control. The pots were watered as a when required and observation was recorded an appearance of disease symptoms on growing plant.

3.5.2 Pathogenicity test of *Alternaria porri*

Seven days old culture of the organism was used for proving the pathogenicity by applying Koch's postulates. For this purpose, bulbs of onion cultivar Akola safed which were used on sown in the plastic pots filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Healthy growing onion seedlings were maintained, watered regularly and kept in the shade net green house for further development. The spore suspension was prepared and filtered through two layers of sterile muslin cloth to remove residual mycelia. Filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 1 x 10^6 spores per ml. The seedlings firstly injured with carborandom powder then were inoculated with seven days old fungus. Un-inoculated seedlings of the same age sprayed with sterilized water served as control. After inoculation, the seedlings (both inoculated and un-inoculated) were incubated in the green

house, where relative humidity (80-90%) and optimum temperature (27±1^{0}C) were maintained for further development of purple blotch symptoms.

Symptoms observed at every 24 hours interval. Disease symptoms appeared four days after inoculation in both injured and uninjured plants as minute water-soaked discoloration on the older leaves. These water-soaked lesions rapidly increased in size and elongated with purplish center eventually turning to purplish blotch. Dark zones were noticed 7 to 10 days after inoculation of fungus, whereas plants under controlled condition remained healthy throughout the periods of experiment. It can be seen from results that injured leaves produced purple lesions whereas uninjured leaves did not show clear symptoms.

3.5.3 Re-isolation

The reisolation was made to confirm the identity of pathogen associated with disease symptoms so as to prove the Koch's postulate. The both test pathogen was reisolated from artificially diseased onion plants, on NA and PDA medium and morphological and cultural characteristics studied were found similar to that of the test pathogen isolated from naturally infected onion plants.

3.6. In-*vitro* evaluation of chemicals against *Erwinia carotovora* pv. *carotovora*

The sensitivity of different antibiotics and fungicides against *Erwinia carotovora* pv. *carotovora* was studied using Inhibition zone technique. The bacterium was multiplied by inoculating the culture into 50 ml of nutrient broth taken in flask. The inoculated flasks were incubated at 30^{0}C for 72 hours. The bacterial suspension was then added to the lukewarm nutrient agar medium (1000 ml). The added medium was poured into the sterilized Petri-plates and plates were allowed to solidify. The bactericides were prepared at different concentrations. Filter paper disc of five mm diameter were cut, placed in Petri-plate and autoclaved. Then filter paper discs were soaked in the respective chemical concentrations for 15 min and transferred onto the surface of medium in the Petri-plates. Four discs in each plate, the paper disc without chemical (sterilized distilled

water) served as a control. The plates were then incubated at 30^0C for 72 hours. The observations for the production of inhibition zone around the filter paper discs were recorded. The results obtained were analyzed statistically.

3.6.1 In-*vitro* evaluation of chemicals against *Alternaria porri*.

Different chemicals were tested for their effect on growth of *Alternaria porri* using poisoned food technique (Sinclair and Dhingra, 1985). The technique involves cultivation of test organism on a medium containing the test chemical. In all experiments, PDA was used as basal medium. The required quantity of each chemical was incorporated aseptically in 100 ml of PDA in 250 ml flasks at the time of pouring the media in Petri-plates. The medium shaken well to give uniform dispersal of the chemical and then in each Petri-plate 20 ml of medium was poured aseptically and allowed to solidify.

The Petri-plates were inoculated with 4 mm diameter mycelial disc cut from the periphery of 10 days old fungus cultures. The mycelial disc was placed in the center of the plates in an inverted position to make a direct contact with the poisoned medium and incubated at 27 ± 1^0C. Each treatment was replicated thrice. Simultaneously a suitable control was also maintained by growing the fungus on chemical free PDA. Observations on radial mycelial growth diameter were recorded on 3^{rd}, 5^{th} and 7^{th} days after inoculation. The per cent inhibition of growth of the fungus in each treatment was calculated by using following formula (Bliss, 1934).

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

Where,

I = Per cent inhibition,

C = Colony diameter in control (mm),

T = Colony diameter in respective treatment (mm).

Table 1. List of chemicals used.

Sr. No.	Chemical name	Trade name	Company	Conc. (%)
1	Mancozeb 75 % WP	Indofil M-45	Indofil Industries Ltd.	0.25
2	Carbendazim 50 %WP	Bavistin	BASF, India Ltd.	0.10
3	Copper oxychloride 50 % WP	Blitox 50	Syngenta India Ltd.,	0.25
4	Streptomycin	Ambistryn-s	Abbott Healthcare Pvt. Ltd	200 ppm

3.7. Experimental details: -

- Location : Department of Horticulture, Dr.P.D.K.V.Akola
- Soil type : Vertisol
- Design : RBD
- No. of replication : 03
- No. of treatments : 10
- Total no. of plots : 30
- Plot size : 2.70 x 3.00 mt
- Spacing : 45 x 30 cm
- No. of plants : 60
- Crop : Onion
- Variety : Akola *safed*
- Planting season : Rabi
- Date of planting : 25/10/2018
- Fertilizer dose : 100:50:50 N.P.K / ha

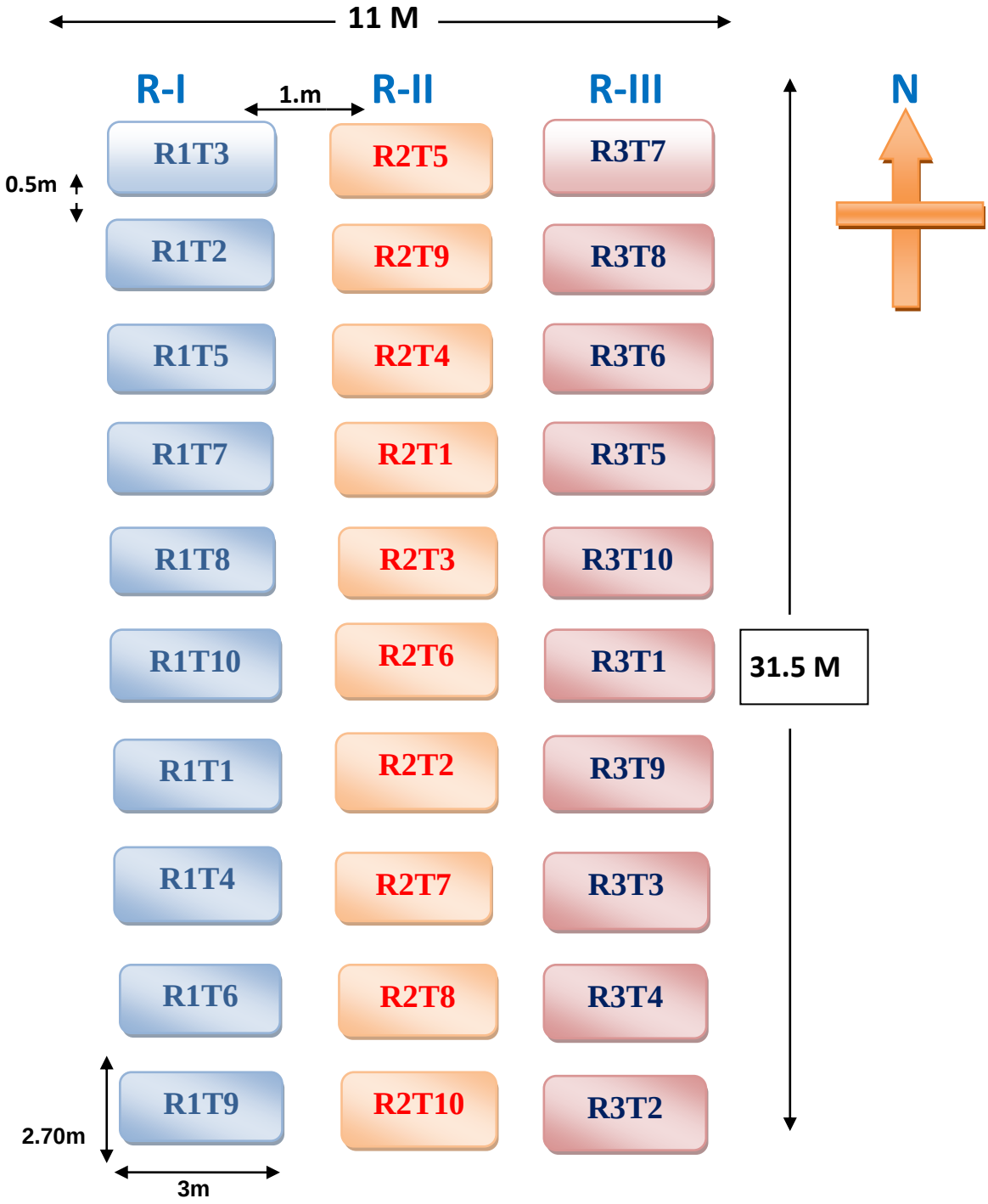


Fig. 1 Plan of field layout

3.7.2 Treatment details :-

Table 2: *In-Vivo* Treatments

Bulb dip treatments for bulb rot -

Tre. No.	Treatment Name	Conc.
T_1	Copper oxychloride	0.25%
T_2	Streptomycin	200 ppm
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm

Spraying treatments for purple blotch -

Tre. No.	Treatment Name	Conc.
T_4	Mancozeb + Carbendazim	0.25% + 0.10%
T_5	Copper oxychloride	0.25%
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%

Bulb dip + spraying treatments for bulb rot and purple blotch -

Tre. No.	Treatment Name	Conc.
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%
T_{10}	Control	-

Table 3: *In-Vitro* Treatments

Tre. No.	Treatment Name	Conc.
T_1	Copper oxychloride	0.25%
T_2	Streptomycin	200ppm
T_3	Copper oxychloride + Streptomycin	0.25% + 200ppm
T_4	Copper oxychloride + Carbendazim	0.25% + 0.10%
T_5	Copper oxychloride + Mancozeb	0.25% + 0.25%
T_6	Carbendazim	0.10%
T_7	Mancozeb	0.25%
T_8	Carbendazim + Mancozeb	0.10% + 0.25%
T_9	Mancozeb + Streptomycin	0.25% + 200ppm
T_{10}	Carbendazim + Streptomycin	0.10% + 200ppm
T_{11}	Control	-

3.7.3 Per cent incidence of disease

Observations of disease incidence were recorded at 15 days intervals from date of planting up to harvesting**.** The per cent disease incidence was calculated according to the formula:

$$\text{Per cent incidence} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total no. of plants observed}} \times 100$$

3.7.4 Per cent disease intensity

The observation on leaf spot infection were recorded at 90 DAP and continue upto harvesting at 15 days interval by selecting two leaves each from top, middle and lower portion of the plant. The observations were recorded on the basis of 0-5 scale (Sharma, 1986).

Table 4: Disease rating scale

Grade	Description
0	No infection
1	A few spots towards tip covering 10 per cent leaf area.
2	Several dark purplish brown patches covering up to 20 per cent leaf area.
3	Several patches with paler outer zone covering up to 40 per cent leaf area.
4	Leaf streaks covering up to 75 per cent leaf area or breaking of the leaves from center.
5	Complete drying of the leaves or breaking of the leaves from center.

The above rating scales or grades are utilized for the calculation of PDI using the following formula (Wheeler,1969).

$$\text{Percent Disease Intensity (PDI)} = \frac{\sum \text{ of all numerical ratings}}{\text{Total number of leaves examined} \times \text{Maximum ratings}} \times 100$$

3.7.5 Seed yield (kg/plot)

Seed yield was recorded from each plot. Seed weighed properly and converted to kg/ha.

3.7.6 Statistical anaylsis

The data obtained from all the experiments were statistically analyzed following the standard methods (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

The present studies on “Chemical management of bulb rot and purple blotch of onion” was conducted at Department of Plant Pathology and Department of Vegetable Science, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2018-2019 (Plate1) and observatios recorded on different parameters during the studies are presented below-

4.1 Collection of diseased samples

Naturally infected diseased samples of bulb rot and purple blotch were collected from experiment field which is located at Department of Vegetable Science, College of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the study. *Erwinia carotovora* pv*. carotovora and Alternaria porri* was obtained from the diseased samples were used for further studies.

4.2 Isolation and identification of pathogen

4.2.1 Isolation and identification of *Erwinia carotovora* pv*. carotovora*

The causal organism *Erwinia carotovora* pv. *carotovora* was isolated from infected bulb showing typical symptoms. Isolation was done by streaking method, as explained in material and methods on NA medium. Well separated single colony was picked and streaked on NA medium at 27^0C for 48 hours. The pure colonies obtained were again streaked in slants. The culture so obtained were stored in the refrigerator at 4^0C, which served as a stock culture for further studies. The culture was renewed by sub-culturing once in a fortnight nutrient agar slants**.**

4.2.2 Morphological and biochemical characteristics of *Erwinia carotovora* pv*. carotovora*

The results of the various morphological characters of *Erwinia carotovora* pv*. carotovora* are given in table 5. The bacterium is a rod shaped facultatively anaerobic, gram negative and peritrichously flagellated.

Plate 1. Experimental Field View

At seedling stage

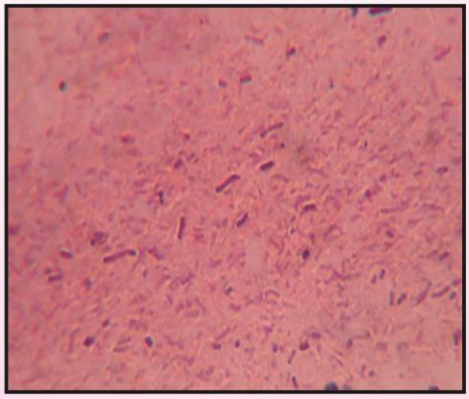
At Vegetative stage

At flowering stage | At maturity stage | Healthy plant

Plate 2 Symptoms of bulb rot at different growth stages of onion in field

Plate 3 Pure culture of *Erwinia carotovora* pv. *carotovora*

Plate 4 Pathogenicity of *Erwinia carotovora* pv. *carotovora* on onion plant *in vitro*



Gram reaction

Plate 5(a) Biochemical properties of *Erwinia carotovora* pv. *carotovora*

Table 5: Morphological characteristics of *Erwinia carotovora* pv. *carotovora*

Sr. No.	Properties	Morphological characters
1	Colony shape	Round and convex
2	Colony colour	White-Creamy
3	Pigmentation	Yellow
4	Cell shape	Straight rod
5	Arrangement	Single

Dickey (1979) and Fahy and Hayward (1983) reported that *Erwinia carotovora* pv*. carotovora* were gram –ve, straight rod-shaped cell and showed initially creamy coloured colonies and later yellow pigmentation. Ali *et al*. (2014), Prajapat (2013) and Rashid *et al*. (2013) reported rod shape of the bacterium where as Mohammad and Selman (2013) recorded rod shape and having smooth, convex, white circular creamy colonies of the bacteria. In present investigation also, the similar morphological properties of the bacteria were recorded, thus confirmed the findings.

4.2.3 Biochemical properties of *Erwinia carotovora* pv. *carotovora*

The isolate of *Erwinia carotovora* pv*. carotovora* showed negative reaction for gram reaction and urease production test. However, positive towards the KOH test, catalase test, potato soft rot test, gelatin liquification test, growth in 5% Nacl test, H_2S production test, indole production test, methyl red test and oxidase test. Table 6 (Plate 5a, 5b & 5c).

Table 6: Biochemical properties of *Erwinia carotovora* pv. *carotovora*

Sr.No.	Biochemical properties	Reaction of isolate
1	Gram reaction	-ve
2	KOH test	+ve
3	Catalase test	+ve
4	Potato soft rot test	+ve
5	Gelatin liquification test	+ve
6	Urease production test	-ve
7	Growth in 5% Nacl test	+ve
8	H_2S production test	+ve
9	Indole production test	+ve
10	Oxidase test	+ve
11	Methyl red test	+ve

Positive reaction = +ve

Negative reaction = - ve

4.2.3.1 Gram reaction

The bacterium *Erwinia carotovora* pv. *carotovora* showed gram negative reaction.

4.2.3.2 KOH test

In this test the drop of 3% potassium hydroxide was mixed with the 24-hour old bacterial culture to make the suspension. When this suspension lifted by bacterial loop it forms the thread like structure which indicates that bacteria is gram negative. Because, gram negative bacteria have relatively fragile cell walls, bounded by an outer membrane. This is readily disrupted by exposure to 3 % KOH releasing the viscous DNA. The bacterium showed positive reaction for this test.

4.2.3.3 Catalase test

After covering with few drops of hydrogen peroxide to a slide smeared by a loopful of test bacteria, there was production of gas bubbles. It happens because, catalase enzyme breakdown the hydrogen peroxide (H_2O_2) into oxygen and water indicating the positive reaction for the test.

4.2.3.4 Potato soft rot test

This test is performed to determine the ability of *Erwinia carotovora* pv. *carotovora* to cause the soft rot in potato. In this test potato slices were inoculated *Erwinia carotovora* pv. *carotovora.* The inoculated slices were maintained in moistened Petriplates and incubated at 30^0C for 2-3 days. After incubation potato slices produce the characteristic symptoms of soft rot. The bacterium showed positive reaction for this test.

4.2.3.5 Gelatin liquification test

The test is performed to check the ability of the *Erwinia carotovora* pv. *carotovora* to produce the enzyme gelatinase. The bacterial isolates were added to the media and incubated for 24 - 48 hours at 37^0C; after that test tubes were kept in freeze for 30 min. The tubes containing bacterial culture remain in liquid state. It means that bacterium produces the enzyme gelatinase.The bacterium showed positive reaction for this test.

4.2.3.6 Urease production test

The urease test is used to determine the ability of an organism to split urea, through the production of enzyme urease. The bacterial isolates were added to the broth and incubated for 7 days at 35-37^0C. After incubation the medium did not change the colour into pink colour. The bacterium showed negative reaction for this test.

4.2.3.7 Growth in 5% Nacl test

The test is performed to check the salt tolerance of the *Erwinia carotovora* pv. *carotovora*. In this test nutrient broth is prepared with 5% Nacl. The broth inoculated with bacteria and incubated for 24 hours at 27^0C. After incubation bacterium grew in broth which was a positive result for *Erwinia carotovora* pv. *carotovora*.

4.2.3.8 H_2S production

The activity of bacterium on sulphur containing amino acids frequently results in production and liberation of H_2S gas. It was observed that bacterium produced the gas which reacted with the lead acetate strips and gave black colour of test strips indicated liberation of H_2S. The bacterium showed positive reaction to this test.

4.2.3.9 Indole production test

This test was performed to determine the ability of organism to split indole from the amino acid tryptophan by the intracellular enzyme tryptophanase produced by *Erwinia carotovora* pv. *carotovora*. The bacterial isolate were added to the broth and incubated for 48 hours at 37^0C; after that, the Kovac's reagents were added to the medium which developed a cherry red color in the medium which was a positive result for *Erwinia carotovora* pv. *carotovora.*

4.2.3.10 Oxidase Test

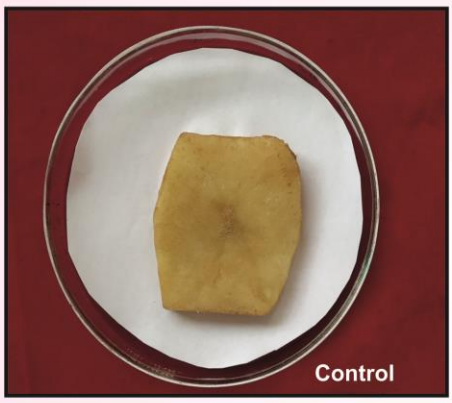
The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) purplish blue colour end product. When the enzyme is not present, the reagent remains reduced and is colorless. After adding of 24-hour old culture over the oxidase disc. There is the formation of blue colour over the disc. The result is positive test.

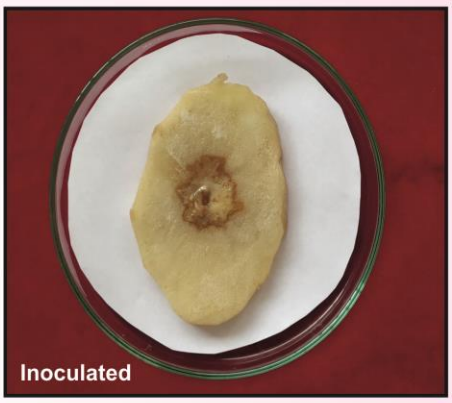
4.2.3.11 Methyl Red Test

Methyl red (MR) test is performed with the *Erwinia carotovora* pv*. carotovora* to detect the its ability to produce stable acid end products (Mixed acid fermentation) from supplied glucose. The bacterial isolate were added to the sterile media and incubated at 35-37^0C for 48 hours. After incubation 3-4 drops of methyl red reagent were added into the media which developed the red colour immediately which was a positive result for *Erwinia carotovora* pv. *carotovora.*

KOH test

Catalase test

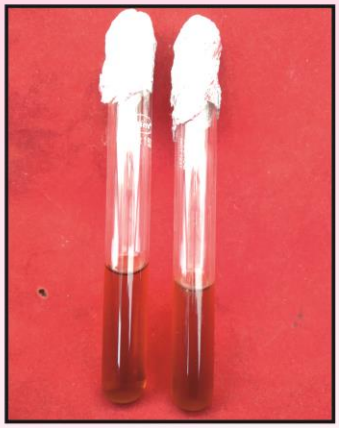




Potato soft rot test

Gelatin liquification test

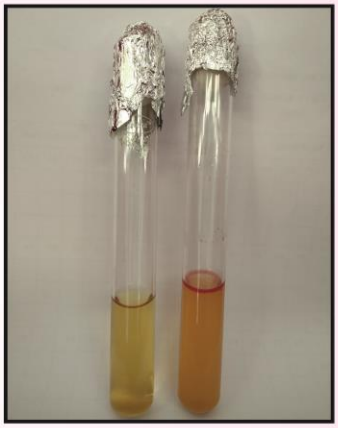
Plate 5(b) Biochemical properties of *Erwinia carotovora* pv. *carotovora*



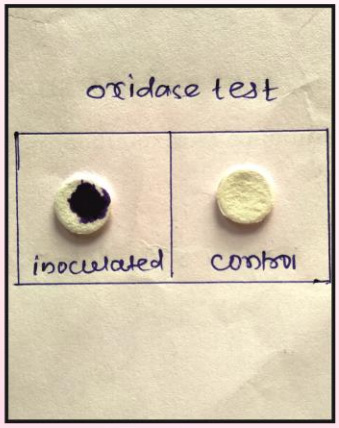
Urease production test

Growth in 5% Nacl

H2s production test



Indole production test



Oxidase test

Methyl red test

Plate 5(c) Biochemical properties of *Erwinia carotovora* pv. *carotovora*

The present results of respected biochemical test of *Erwinia carotovora* pv. *carotovora.* are similar to Rahman *et al*. (2017) who recorded negative result for gram staining and positive reaction for catalase, potato soft rot, gelatin liquification, methyl red, indole production, growth in 5% NaCl, The –ve gram reaction and positive biochemical test of catalase, gelatin liquification and acid and gas production in respect of *Erwinia carotovora* was also reported by Mohammad and Selman (2013), Rahman (2012), Ali *et al*. (2014) and Yanez-Morales (2003).

Costa *et al*. (2006) recorded that *Erwinia carotovora* positive to catalase activity and gelatin liquification test. Alvarado *et al*. (2011) recorded that *Erwinia carotovora* is gram negative and catalase positive. Achbani *et al*. (2014) and Mahmoudi (2007) reported that *Erwinia carotovora* were identified by biochemical test including gram staining, catalase, gelatin liquification test, and H_2S production test.

4.2.4 Isolation and identification of *Alternaria porri*.

Infected onion plants showing the typical symptoms of onion purple blotch disease were collected from the experiment field. The causal organism was isolated from the infected leaves on sterilized potato dextrose agar medium by standard tissue isolation methods and purified by hypal tip method as described under “Materials and Methods”.

In culture, the fungal colony was observed to be white, cottony with profuse aerial mycelium which gradually turned greenish grey. Aged culture appeared completely black with no aerial mycelium. Microscopic examination revealed the production of conidiophores either singly or in small clusters. The conidiophores were observed to be straight or flexuous, some time geniculate, septate, pale or mid brown in colour and measured upto 120 µm long and 6 to 10 µm thick, with one or several conidial scars. A mature conidiophore usually produced a solitary conidium, but occasionally it also produced conidia in short chains. Conidia were observed to be straight or curved, rostrate, beak generally equal to the length of the body of the conidium, pale brown to mid golden brown in colour. Overall length of conidia ranged from 100 to 300 µm, 15 to 20 µm wide in the broadest part with 7 to 12 transverse and zero to several

Healthy plant

Infected plant

Infected plant

Infected plant

Plate 6 Symptoms of purple blotch on onion in field

Plate 7 Disease rating scale for purple blotch of onion

Plate 8 Pure culture of *Alternaria porri*

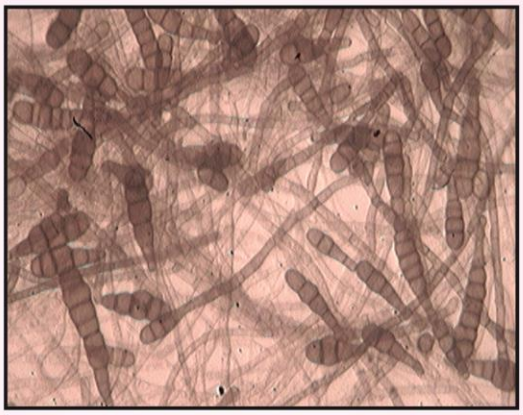


Plate 9 Microscopic view of *Alternaria porri*

Plate 10 Pathogenicity of *Alternaria porri* on onion plant *in vitro*

longitudinal septa. Conidia showed flexuous beak, pale in colour, 2 to 4 µm thick and tapering.

Based on the colony characters and morphological characters of mycelium, conidiophores and conidia, the fungus was identified as *Alternaria porri* (Ellis) Cif*.* The description of the fungus, *Alternaria porri* was found comparable with standard measurements given by Ellis (1971), "CMI Descriptions of Pathogenic Fungi and Bacteria" (1952) and "Novel Dematiaceous Hyphomycetes" (Simmons, 2004) to identify the *Alternaria porri*.

4.3 Pathogenicity test and symptoms

4.3.1 Pathogenicity *Erwinia carotovora* pv*. carotovora*

The bulb rot of onion caused by *Erwinia carotovora* pv. *carotovora* was isolated from diseased bulb of onion collected from experiment field. The identification of the pathogen was confirmed based on biochemical properties and comparing to the published literature and pathogenicity were proved under artificial inoculation condition (Plate 4).

Pathogenicity test were conducted by using onion bulbs. *Erwinia carotovora* pv. *carotovora* was observed to be pathogenic to, healthy onion plant by artificial inoculation. The initial small watery lesions on leaf base were observed after 2-3 days of inoculation. The bulb rotting symptoms were observed on leaves after 5 to 7 days in the form of yellowing of leaves. Reisolation from infected bulb/ plant yielded the same pathogen where as control plants remained healthy. Thus, *Erwinia carotovora* pv. *carotovora* was found pathogenic and caused bulb rot of onion confirming the Koch's postulates. In advanced stage, rotting of whole plants lead to death of plants after 15-17 days.

The pathogenic nature of *Erwinia carotovora* were observed by Achbani *et al*. (2014), Palacio-Bielsa (2007) and Yanez-Morales (2003) in the form of development of bulb rot symptoms after five days of incubation of onion bulbs by *Pectobacterium* spp. Pathogenicity test of soft rot pathogen in other crop have also been reported by many earlier

workers, Kumar *et al*. (2011), Mandal and Maiti (2005), Duarte *et al*. (2004), Smith and Birtz (1990) and Rashid *et al*. (2013).

4.3.2 Symptoms of bulb rot

Symptoms were observed on bulb and leaf (Plate 2). The above ground portion of infected plants appeared weak, thrifty with pale yellow lustreless leaves with marginal necrosis or scorching in older leaves. The plants appeared dwarf and such plants when uprooted along with bulb, showed rotting, tissue becoming soft.

Bulb rot symptoms were noticed at the base of central leaves of onion plant in field condition, longitudinal sections revealed that lesions developed downward and the inner layers of bulb also appeared macerated. Onion bulb exhibited water soaking or yellowish-brown rot symptoms. (Achbani *et al*., 2014 and Palacia-Bielsa, 2007). Infection has been observed at the inner part of the onion bulb in the neck region and the decay gradually invades the whole bulb and affected part become slimy and gives offensive odour. In the early state of bulb infection, the older leaves become severaly decayed and all the leaves die (Yanez-Morales 2003).

Symptoms on the same line due to *Erwinia* spp. have been reported earlier by Maitham and Selman (2013), Agricos (2005), Mohammad and Selman (2013), Pachupate and Kininge (2012) and Bhat *et al*. (2010) in Potato, Banana and other tuber crops where soft rot disease caused by Erwinia *carotovora* pv*. carotovora* showed small water-soaked lesions, which enlarge rapidly, affected part become slimy and soft rot accompanied by a characteristically offensive odour.

4.3.3 Pathogenicity of *Alternaria porri*

The pathogenicity of the fungus was established by following Koch's postulates (Plate10). The pathogenicity test was conducted in the cage house as described under materials and methods. The onion variety Akola Safed susceptible to purple blotch disease was raised in pots and standard mycelial spore suspension was spray inoculated on six-week-old

onion plants and then covered with polythene covers. Initial symptoms were observed on 4th day of inoculation.

The symptoms of the disease first appeared as small whitish sunken lesions which later turned brown in colour. The lesions enlarged turned purplish in colour and were surrounded by a yellow halo. Verma and Sharma (1999) observed small water-soaked lesions on leaves 2-3 mm in diameter that quickly developed into white centers. These lesions enlarged, coalesced, became zonate and turned brown to purple extending upwards and downwards.

The pathogen was reisolated from the typical symptoms of purplish zonation on to PDA medium and the morphological characters were compared with the original culture which was similar in all aspects. Hence, the causal agent of the disease was confirmed as *Alternaria porri*. Koike and Hinderson (1998) proved the pathogenicity of *A. porri* by spraying conidial suspension on 2-month-old leek plants. After 14 days the leaf spots similar to the original symptoms developed on inoculated plants.

4.3.4 Symptoms of purple blotch

Symptoms of onion purple blotch disease were studied on infected onion plants in experiment field. The symptoms appeared on leaves, flowering stalk, inflorescence and also on bulbs (Plate 6). The disease initially appeared in the field as whitish circular to irregular specks on the leaves. These specks developed into whitish sunken lesions, increased in size, became oval to irregular in shape and developed into dark purple/ brownish patches with yellow halo. Mostly, these symptoms first appeared on older leaves.

At initial stages, leaves were with circular to oval water-soaked areas which later on, as the disease progressed, became oblong and a fresh zone of discoloured tissue was formed around the spots. Initially spots were white, but later turned pinkish or purple. The change in colour started from the center and gradually progressed towards the periphery, where it changed into light purplish. The transition of colour was marked by concentric rings clearly visible to the naked eye. The concentric

zones in the spot of leaves were the most outstanding and diagnostic symptoms of the disease. These symptoms were more prominent towards tip of the leaves. In some cases, such leaves started drying downwards. In severe cases, some of the leaves were found girdled, dried and remained hanging on the plant.

Nolla (1927) and Pandotra (1964), observed white irregular spots less than 1 mm in diameter gradually deepen to dark violet or reddish purple colour on onion leaves. Skiles (1950), Mandal *et al.* (1970), Verma and Sharma (1999), Patil and Patil (1992) and Madhavi *et al*. (2012) have also reported similar symptoms.

4.4.1 *In-vitro* efficacy of different chemical treatments against *Erwinia carotovora* pv. *carotovora* by filter paper disc diffusion method

The filter paper disc diffusion method was employed to test the efficacy of different chemicals against *Erwnia carotovora* pv. *carotovora.* Result are presented in Table 7, Fig 2 and Plate 11.

The significant differences in zone of inhibition was recorded among various treatments. Maximum zone of inhibition was recorded by copper oxychloride @ 0.25% + streptomycin @ 200 ppm (23.00 mm) followed by streptomycin @ 200 ppm (18.00 mm), copper oxychloride @ 0.25% (16.25 mm), mancozeb @ 0.25% + streptomycin @ 200 ppm (11.45 mm), carbendazim @ 0.1% + streptomycin @ 200 ppm (10.50 mm) and copper oxychloride @ 0.25% + mancozeb @ 0.25% (9.70 mm). Where as treatments having only fungicide or combination of two fungicide viz. mancozeb @ 0.25%, carbendazim @ 0.10%, mancozeb @ 0.25% + carbendazim @ 0.10% and copper oxychloride @ 0.25% + carbendazim @ 0.10% showed no zone of inhibition against *Erwnia carotovora* pv. *carotovora.*

Among the fungicides and antibiotic evaluated by filter paper disc method, copper oxychloride @ 0.25% + streptomycin @ 200 ppm were found significantly superior over remaining chemicals for inhibiting the bacteria.

Table 7: *In-vitro* efficacy of different chemical treatments against *Erwinia carotovora* pv. *carotovora* by filter paper disc diffusion method

Tre. No.	Treatment Name	Conc.	Mean inhibition zone (mm)
T_1	Copper oxychloride	0.25%	16.25 (4.09)*
T_2	Streptomycin	200 ppm	18.00 (4.30)
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	23.00 (4.85)
T_4	Copper oxychloride + Carbendazim	0.25% + 0.10%	0.00 (0.70)
T_5	Copper oxychloride + Mancozeb	0.25% + 0.25%	9.70 (3.19)
T_6	Carbendazim	0.10%	0.00 (0.70)
T_7	Mancozeb	0.25%	0.00 (0.70)
T_8	Carbendazim + Mancozeb	0.10% + 0.25%	0.00 (0.70)
T_9	Mancozeb+ Streptomycin	0.25% + 200 ppm	11.45 (3.46)
T_{10}	Carbendazim + Streptomycin	0.10% + 200 ppm	10.50 (3.32)
T_{11}	Control	-	0.00 (0.70)
		F' test	Sig.
		SE(m)±	0.33
		CD(P=0.01)	1.27

*Mean of four replications. (Figure in parentheses are corresponding values of square root transformation).

The highest efficacy might be due to diffusion of chemical in media resulting the restriction of bacterial growth. These result support the finding of Paresh *et al*. (2011) who observed streptomycin (300 ppm) and copper oxychloride (500 ppm) was effective antimicrobial compound against *Erwnia carotovora* inhibiting 20.47% and 20.14% growth

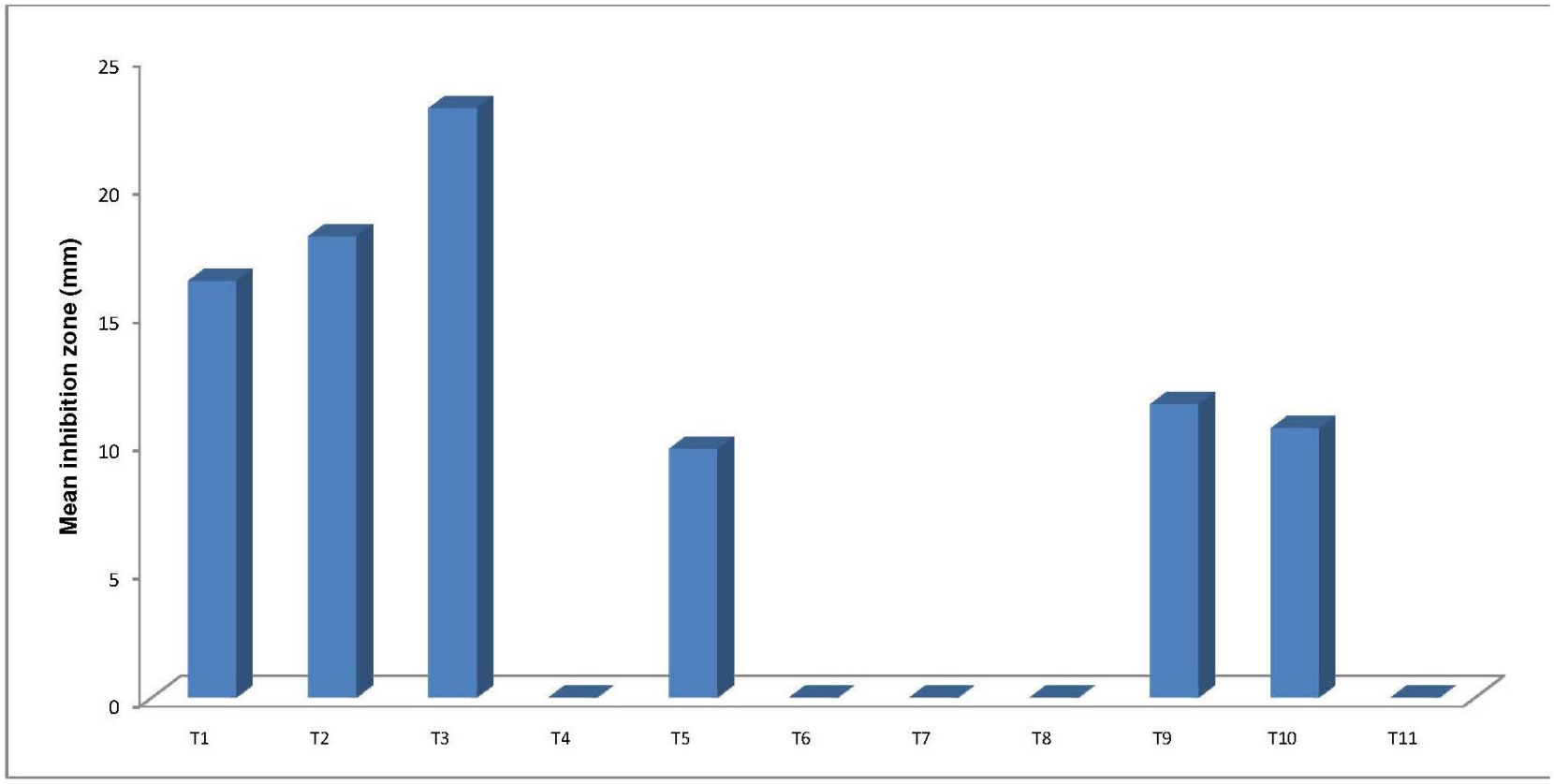


Fig. 2 *In vitro* efficacy of different chemical treatments against *Erwinia carotovora* pv. *carotovora* by filter paper disc diffusion method

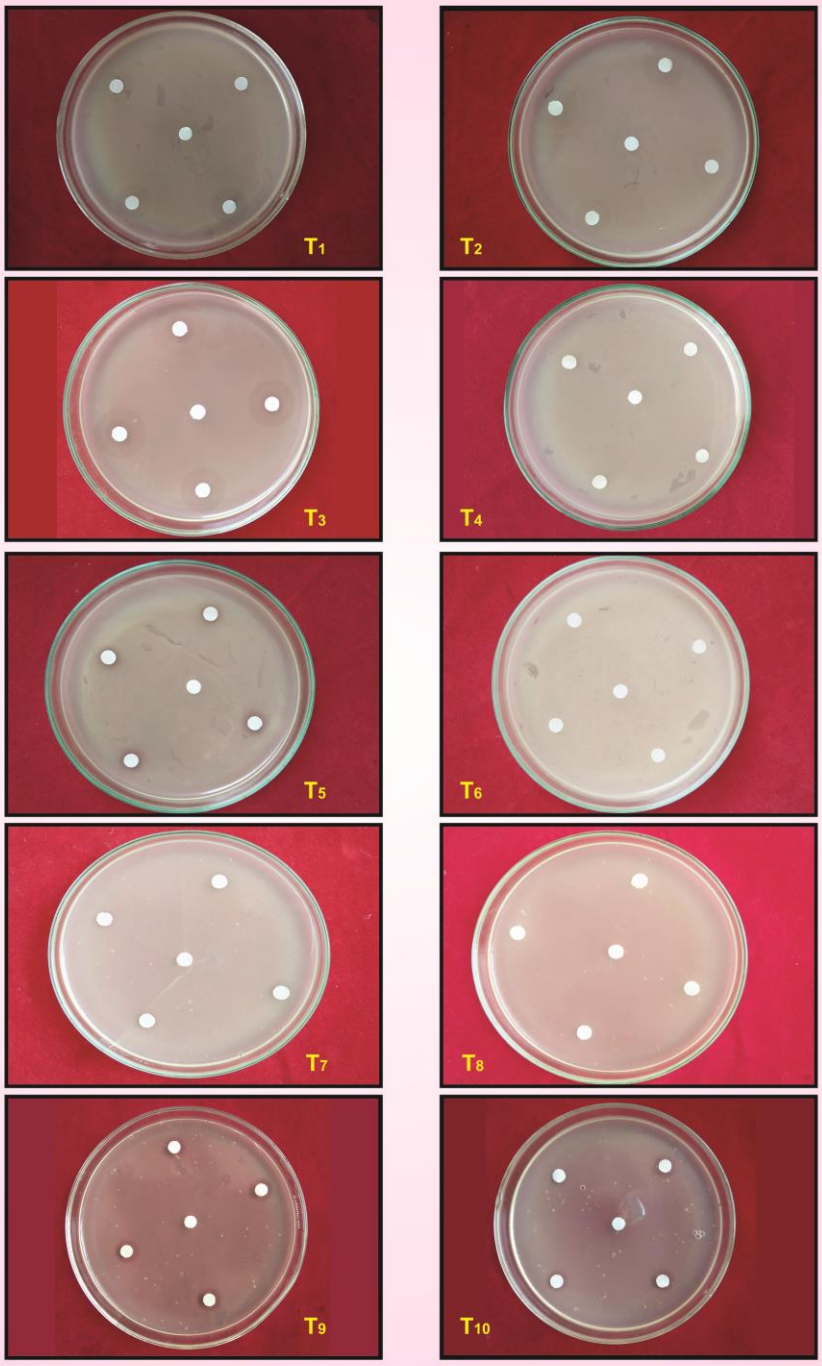


Plate 11 *In vitro* evaluation of chemicals against the *Erwinia carotovora* pv. *carotovora* by paper disc method

respectively. Bhagat (2015) also found that copper oxychloride + streptomycin sulphate is highly effective against *Erwinia carotovora* pv. *carotovora.* Rashid *et al*. (2013) observed that copper-based compound, copper oxychloride (0.2%) was highly effective to suppress the bacterial growth of *Erwinia carotovora* pv. *carotovora.*

The present results are also in confirmation with earlier workers, Thammaiah *et al*. (2006) and Gracia-Garza *et al*. (2002) who reported positive activity of streptomycin sulphate and copper compounds against *Erwnia* spp. in calla lilies.

4.4.2 Effect of different chemical treatments on bulb rot incidence of onion under field condition

The effect of different chemicals in controlling the bulb rot of onion were evaluated under field conditions, during *rabi* 2018-19. Different chemicals were applied as treatments. Chemicals where applied as per the dose described under materials and methods. The treatment remains untreated served as control. The bulb dip treatment was done by dipping onion bulbs in chemical solution for 10 min prior to planting. Where as spraying was done at 15 DAP. Per cent disease incidence was recorded for each treatment from date of planting up to the harvesting at 15 days interval. Data on disease incidence is presented in Table 8a, 8b, 8c,8d, 8e, and fig 3. All the chemicals recorded significantly low disease incidence over control.

Disease incidence after planting was found significant over control and ranged from 19.67% to 30.03%. The most effective treatment was (T8) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (copper oxychloride @ 0.25%) recorded minimum disease incidence i.e 19.67 per cent and showed maximum disease control (53.46 per cent) which was at par with treatments i.e (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%), (T3) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm), and (T7) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%)

that showed 20.10, 20.77, 21.80 per cent disease incidence and 52.44, 50.86, 48.42 per cent disease control respectively. These treatments followed by (T2) streptomycin @ 200 ppm and (T3) copper oxychloride @ 0.25% which showed 24.17, 30.03 per cent disease incidence and 42.81, 28.95 per cent disease control respectively. The treatments i.e. (T4) mancozeb @ 0.25% + carbendazim @ 0.10%, (T5) copper oxychloride @ 0.25% and (T6) mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25% sprayed for purple blotch at 90 DAP did not show considerable effect on bulb rot incidence. Where as highest incidence was recorded in control plot i.e 42.27%.

The result obtained under field condition correlates with the finding of Kenganal *et al.* (2017) reported that drenching and foliar spray of copper oxychloride 50WP at 3g/l + streptomycin sulphate 0.5g/l at 15 days interval, beginning from 15 days after planting was found most effective and recorded lowest soft rot disease incidence. Pachupate and Kininge (2012) observed that streptomycin and copper compound with variable concentration effective against *Pectobacterium* spp. in banana. Kapoor (1999**)** recommended steptocyclin in combination with copper oxychloride for control of soft rot diseases caused by *Erwinia carotovora* pv. *carotovora*. Knauss and Miller (1972) recommended streptomycin to provide adequate control of *Erwinia carotovora* under field condition. Rashid *et al*. (2013) observed that copper-based compound, copper oxychloride (0.2%) was highly effective to suppress the bacterial growth of *Erwinia carotovora* pv. *carotovora.*

Antagonistic activity of streptomycin, copper oxychloride, soft rot of pathogen in another crop have been earlier reported Kuehny *et al*. (1998), Huang *et al*. (2003), Kannan *et al*. (2006) and Gracia-Garza *et al*. (2002).

Table 8a: Effect of different chemical treatments on bulb rot incidence of onion under field condition.

Tre. No.	Treatment Name	Conc.	15 days after planting		30 days after planting	
			Per cent disease incidence	Per cent disease control	Per cent disease incidence	Per cent disease control
	Bulb dip for soft rot					
T_1	Copper oxychloride	0.25%	7.50 (2.83) *	46.54	11.50 (3.46)	42.98
T_2	Streptomycin	200 ppm	3.33 (1.95)	76.26	8.43 (2.98)	58.20
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	2.50 (1.17)	82.18	6.23 (2.59)	69.11
	Spraying for purple blotch					
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	13.43 (3.73)	4.27	19.43 (4.45)	3.66
T_5	Copper oxychloride	0.25%	14.00 (3.81)	0.21	20.13 (4.54)	0.19
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	13.23 (3.70)	5.70	19.20 (4.44)	4.80
	Bulb dip + Spraying for soft rot and purple blotch					
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	3.00 (1.87)	78.61	6.67 (2.67)	66.93
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	2.83 (1.82)	79.82	6.03 (2.55)	70.10
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	2.17 (1.63)	84.53	7.17 (2.77)	64.45
T_{10}	Control	-	14.03 (3.81)	-	20.17 (4.54)	-
		F test	Sig.		Sig.	
		SE(m)±	0.08		0.12	
		CD @ 5%	0.26		0.37	

*Mean of three replications. Figures in parenthesis are square root values.

Table 8b: Effect of different chemical treatments on bulb rot incidence of onion under field condition

Tre. No.	Treatment Name	Conc.	45 days after planting		60 days after planting	
			Per cent disease incidence	Per cent disease control	Per cent disease incidence	Per cent disease control
	Bulb dip for bulb rot					
T_1	Copper oxychloride	0.25%	17.00 (24.34) *	33.85	21.10 (27.34)	32.43
T_2	Streptomycin	200 ppm	12.03 (20.29)	53.19	16.00 (23.57)	48.76
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	9.33 (17.72)	63.69	13.50 (21.53)	56.77
	Spraying for purple blotch					
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	24.00 (29.33)	6.61	31.03 (33.85)	0.64
T_5	Copper oxychloride	0.25%	25.07 (30.04)	2.45	28.40 (32.18)	9.06
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	23.47 (28.97)	8.67	30.07 (33.25)	3.71
	Bulb dip + Spraying for bulb rot and purple blotch					
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	10.00 (18.43)	61.08	13.97 (21.90)	55.26
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	8.33 (16.74)	67.58	11.7 (19.43)	62.53
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	9.17 (17.62)	64.31	12.30 (20.53)	60.61
T_{10}	Control	-	25.70 (30.45)	-	31.23 (33.98)	-
		F test	Sig.		Sig.	
		SE(m)±	0.60		0.62	
		CD @ 5%	1.78		1.85	

*Mean of three replications. Figures in parenthesis are arc sine values.

Table 8c: Effect of different chemical treatments on bulb rot incidence of onion under field condition

Tre. No.	Treatment Name	Conc.	75 days after planting		90 days after planting	
			Per cent disease incidence	Per cent disease control	Per cent disease incidence	Per cent disease control
	Bulb dip for bulb rot					
T_1	Copper oxychloride	0.25%	25.57 (30.37) *	28.23	28.23 (32.10)	29.47
T_2	Streptomycin	200 ppm	19.67 (26.31)	44.79	23.00 (28.65)	42.54
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	16.77 (24.15)	52.93	19.33 (26.05)	51.71
	Spraying for purple blotch					
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	34.17 (35.77)	4.09	38.57 (38.39)	3.64
T_5	Copper oxychloride	0.25%	35.03 (36.29)	1.68	39.17 (38.74)	2.14
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	33.03 (35.08)	7.29	37.33 (37.66)	6.74
	Bulb dip + Spraying for bulb rot and purple blotch					
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	17.67 (24.82)	50.40	20.13 (26.60)	49.71
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	14.00 (21.97)	60.70	17.83 (24.97)	56.30
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	16.27 (23.18)	54.33	18.97 (25.81)	52.61
T_{10}	Control	-	35.63 (36.65)	-	40.03 (39.25)	-
		F test	Sig.		Sig.	
		SE(m)±	0.54		0.74	
		CD @ 5%	1.62		2.21	

*Mean of three replications. Figures in parenthesis are arc sine values.

Table 8d: Effect of different chemical treatments on bulb rot incidence of onion under field condition

Tre. No.	Treatment Name	Conc.	105 days after planting		120 days after planting	
			Per cent disease incidence	Per cent disease control	Per cent disease incidence	Per cent disease control
	Bulb dip for bulb rot					
T_1	Copper oxychloride	0.25%	30.03 (33.23)*	27.63	30.03 (33.23)	28.95
T_2	Streptomycin	200 ppm	24.17 (29.44)	41.75	24.17 (29.44)	42.81
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	20.77 (27.10)	49.95	20.77 (27.10)	50.86
	Spraying for purple blotch					
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	40.20 (39.35)	3.13	40.20 (39.35)	4.89
T_5	Copper oxychloride	0.25%	41.20 (39.93)	0.72	41.20 (39.93)	2.53
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	38.20 (38.17)	7.95	39.20 (38.76)	7.26
	Bulb dip + Spraying for bulb rot and purple blotch					
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	21.80 (27.74)	47.46	21.80 (27.74)	48.42
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	19.67 (26.32)	52.60	19.67 (26.32)	53.46
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	20.10 (26.63)	51.56	20.10 (26.63)	52.44
T_{10}	Control	-	41.50 (40.10)	-	42.27 (40.55)	-
		F test	Sig.		Sig.	
		SE(m)±	0.72		0.70	
		CD @ 5%	2.14		2.08	

*Mean of three replications. Figures in parenthesis are arc sine values.

Table 8e: Effect of different chemical treatments on bulb rot incidence of onion under field condition

Tre. No.	Treatment Name	Conc.	At harvesting time	
			Per cent disease incidence	Per cent disease control
	Bulb dip for bulb rot			
T_1	Copper oxychloride	0.25%	30.03 (33.23)*	28.95
T_2	Streptomycin	200 ppm	24.17 (29.44)	42.81
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	20.77 (27.10)	50.86
	Spraying for purple blotch			
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	40.20 (39.35)	4.89
T_5	Copper oxychloride	0.25%	41.20 (39.93)	2.53
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	39.20 (38.76)	7.26
	Bulb dip + Spraying for bulb rot and purple blotch			
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	21.80 (27.74)	48.42
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	19.67 (26.32)	53.46
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	20.10 (26.63)	52.44
T_{10}	Control	-	42.27 (40.55)	-
		F test	Sig.	
		SE(m)±	0.70	
		CD @ 5%	2.08	

*Mean of three replications. Figures in parenthesis are arc sine values.

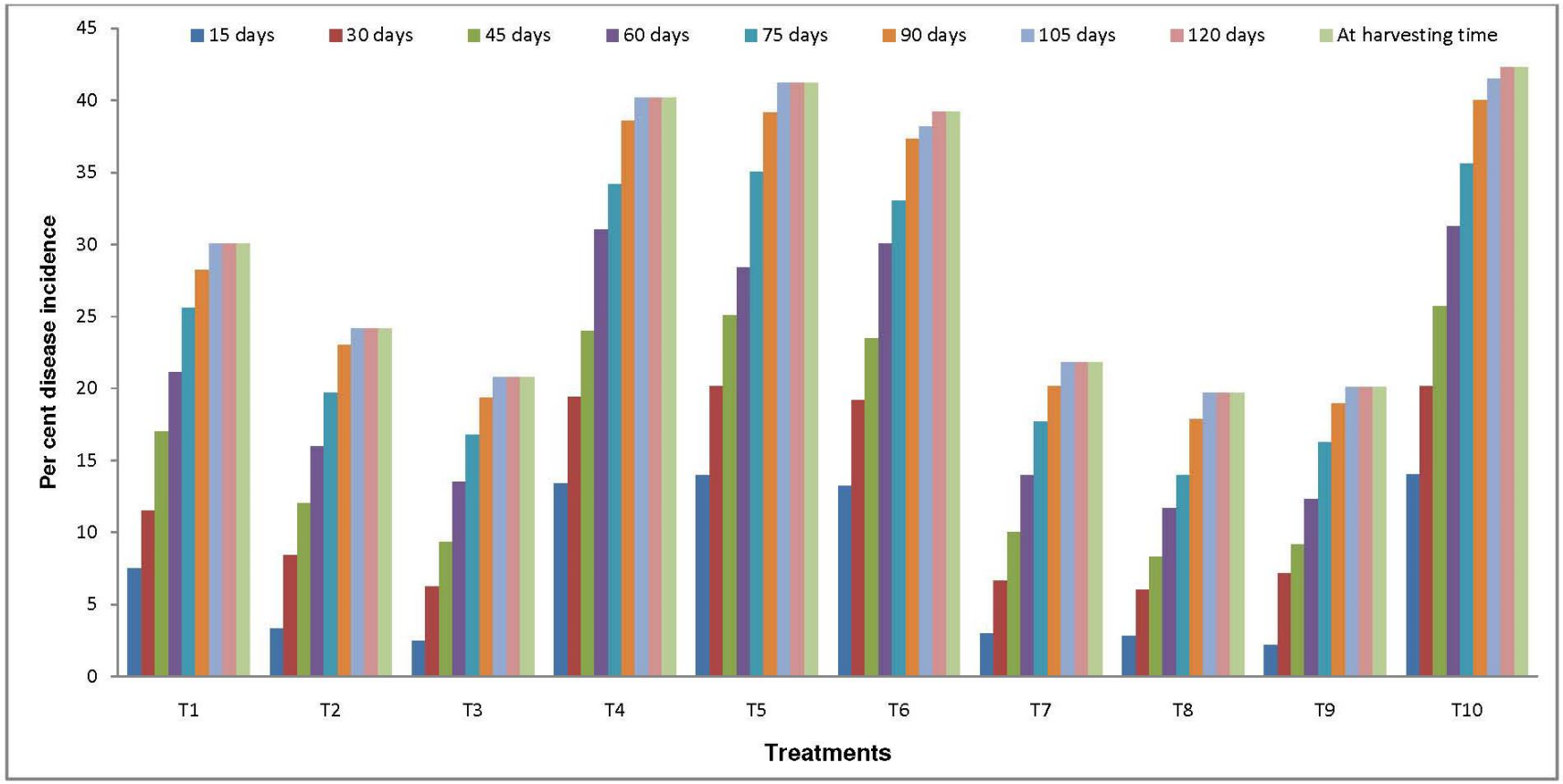


Fig. 3 Effect of different chemical treatments on bulb rot incidence of onion under field condition

4.4.3 *In-vitro* efficacy of different chemicals against *Alternaria porri* by poisoned food technique

The result presented in Table 9, Fig 4 and Plate 12 showed that different chemicals are evaluated *in vitro* against the *A. porri* to check their efficacy.

Table 9: *In vitro* efficacy of different chemical treatments against *Alternaria porri by* poisoned food technique

Tre. No.	Treatment Name	Conc.	Mean radial mycelial growth (mm) 7 DAI	Mycelial inhibition (%)
T_1	Copper oxychloride	0.25%	18.83 (4.39)*	73.35
T_2	Streptomycin	200 ppm	69.67 (8.37)	1.41
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	19.17 (4.43)	72.87
T_4	Copper oxychloride + Carbendazim	0.25% + 0.10%	21.00 (4.63)	70.28
T_5	Copper oxychloride + Mancozeb	0.25% + 0.25%	9.83 (3.21)	86.09
T_6	Carbendazim	0.10%	55.33 (7.47)	21.7
T_7	Mancozeb	0.25%	0.00 (0.70)	100
T_8	Carbendazim + Mancozeb	0.10% + 0.25%	0.00 (0.70)	100
T_9	Mancozeb+ Streptomycin	0.25% + 200 ppm	0.00 (0.70)	100
T_{10}	Carbendazim + Streptomycin	0.10% + 200 ppm	59.0 (7.71)	16.51
T_{11}	Control	-	70.67 (8.43)	-
		F ‘ test	Sig.	
		SE(m)±	0.33	
		CD(P=0.01)	1.34	

DAI- Day after inoculation. *Mean of three replications. Figure in parentheses are corresponding values of square root transformation.

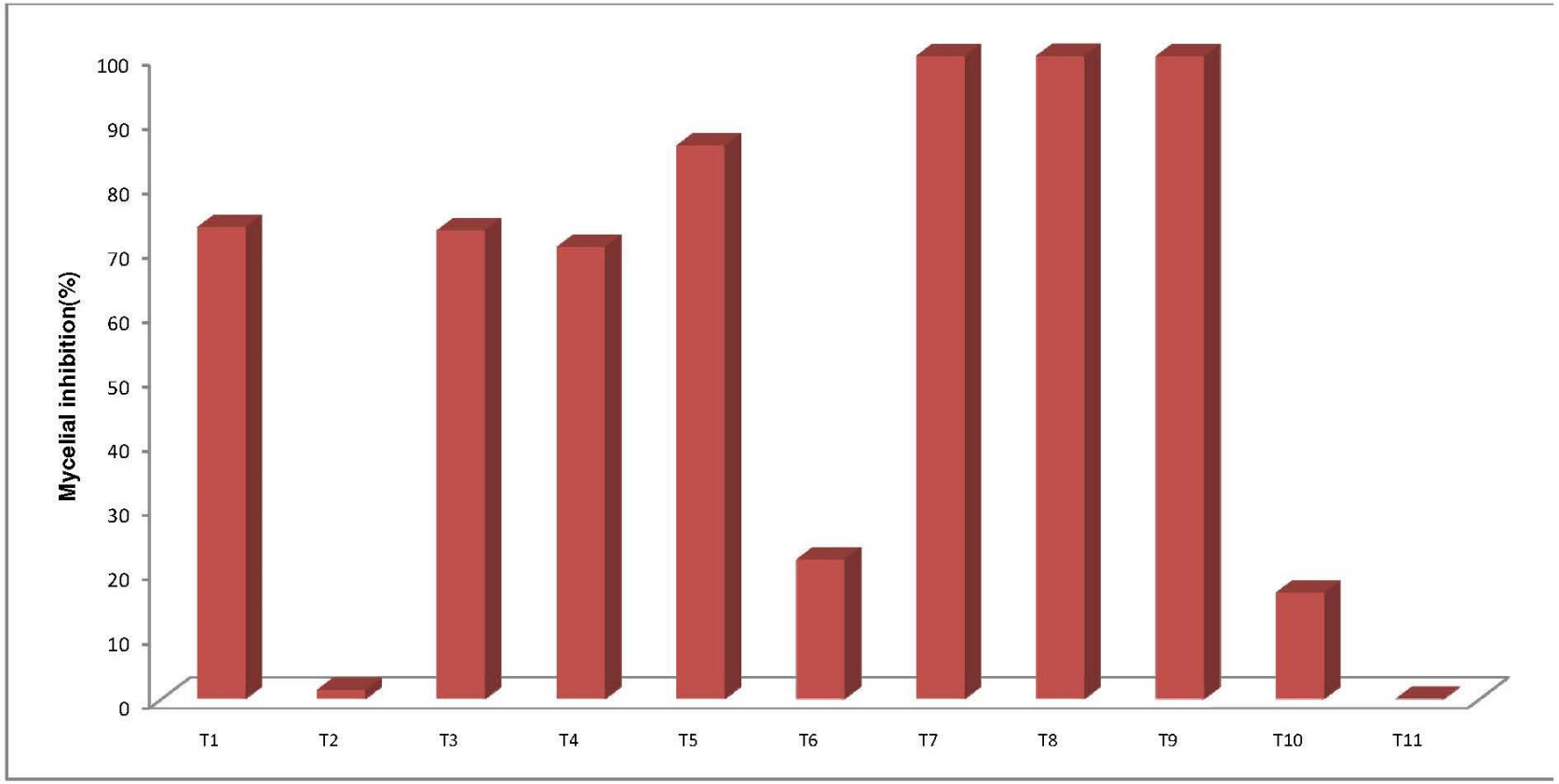
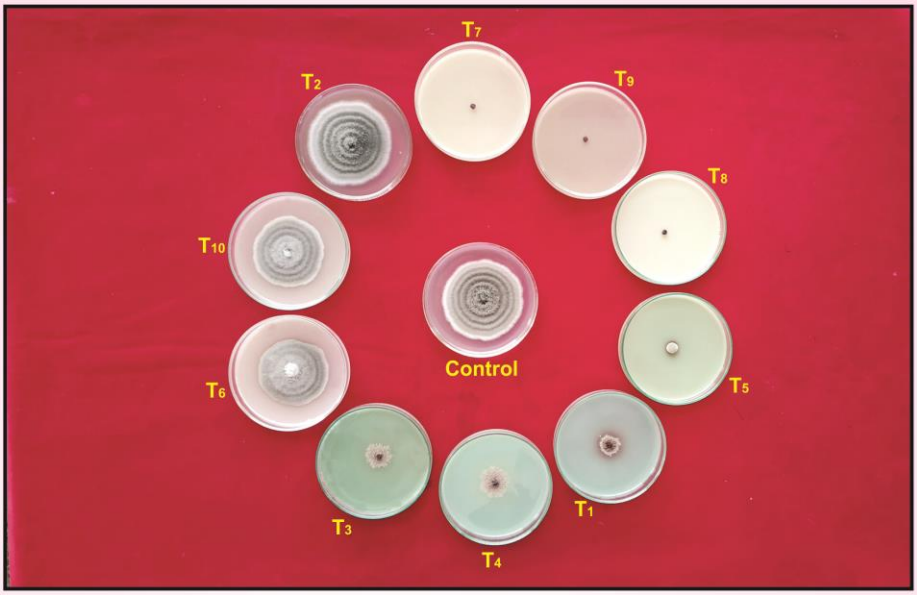


Fig. 4 In vitro efficacy of different chemical treatments against *Alternaria porri by* poisoned food technique



T_1 Copper oxychloride (0.25%)

T_2 Streptomycin (200ppm)

T_3 Copper oxychloride (0.25%) + Streptomycin (200ppm)

T_4 Copper oxychloride (0.25%) +Carbendazim(0.10%)

T_5 Copper oxychloride (0.25%)+ Mancozeb (0.25%)

T_6 Carbendazim(0.10%)

T_7 Mancozeb(0.25%)

T_8 Carbendazim (0.10%) +Mancozeb(0.25%)

T_9 Mancozeb (0.25%) + Streptomycin (200ppm)

T_{10} Carbendazim(0.10%)+ Streptomycin (200ppm)

T_{11} Control

Plate 12 *In vitro* evaluation of chemicals against the *Alternaria porri* by poisoned food technique

It was evident from the data presented in table 9 that mancozeb @ 0.25%, mancozeb @ 0.25% + carbendazim @ 0.10% and mancozeb @ 0.25% + streptomycin @ 200 ppm concentration inhibited the 100 percent mycelial growth of *A. porri* and these treatments was significantly superior over rest of the treatments. The next best treatment was copper oxychloride @ 0.25% + mancozeb @ 0.25% which exhibited 86.09% growth inhibition of *A. porri* followed by copper oxychloride @ 0.25%, copper oxychloride @ 0.25 + streptomycin @ 200 ppm, and copper oxychloride @ 0.25 + carbendazim @ 0.10% which exhibited 73.35, 72.87 and 70.28 growth inhibition. The least percent inhibition of mycelial growth was recorded in streptomycin @ 200 ppm i.e. 1.41 per cent. The radial mycelial growth 70.67 mm was recorded in control.

These results were in close conformity with the findings of Gupta *et al*. (1981) reported that dithane M-45 as most effective in inhibition growth of *A. porri* under in vitro condition. Chethana *et al*. (2011) reported that mancozeb is the best fungicide in inhibiting the growth of *A. porri* with 100 percent inhibition and also copper oxychloride with 88.80 percent inhibition.

Deshmukh *et al*. (2008) also reported that mancozeb (83.0 percent inhibition) is most effective against *A. porri* and also showed that copper oxychloride inhibited more than 50 per cent fungal growth even at lower (500 ppm) concentration. Mishra and Gupta (2012) reported that mancozeb found most effective in inhibiting the growth of *A. porri.* Archana S Rao *et al*. (2015). reported that 100 percent mycelial inhibition of A. *porri* were recorded at 2500 ppm concentration of mancozeb. Madhavi *et al*. (2012) reported that mancozeb is most effective against *A. porri* at all concentration followed by copper oxychloride. Srivastava *et al*. (1991) and Ponnappa (1970) also reported that mancozeb is most effective against *A. porri.* Yadav *et al*. (2013) reported that carbendazim was least effective with a mean inhibition per cent of 10.97 within the treatments.

4.4.4 Effect of different chemical treatments on purple blotch intensity of onion under field condition.

The performance of different chemicals in controlling the purple blotch of onion were evaluated along with untreated control under natural field conditions, against purple blotch of onion during *rabi* 2018-19. Different chemicals were used as treatments. Chemicals were applied as per the dose described under materials and methods. The spraying was done immediately after initiation of disease symptoms i.e at 90 DAP. Per cent disease intensity was recorded for each treatment after spray at 15 days interval up to the harvesting and workout the per cent disease intensity over the control.

The data presented in Table 10a,10b and Fig 5 indicated that treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%) recorded lowest per cent disease intensity i.e 15 per cent and it showed highest percent disease control i.e. 55.13 per cent. Which was at par with the treatments viz. (T6) spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%), (T7) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%) and (T4) spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%) showed 15.10%, 16.03%, 16.57% disease intensity and 54.83%, 52.04%, 50.43% per cent disease control respectively. Followed by (T8) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (copper oxychloride @ 0.25%) and (T5) spraying of (copper oxychloride @ 0.25%) recorded 18.00%, 19.67% disease intensity and 46.15%, 41.16% disease control respectively. The treatments (T1) copper oxychloride @ 0.25%, (T2) streptomycin @ 200 ppm and (T3) copper oxychloride @ 0.25% + streptomycin @ 200 ppm have only bulb dip treatment at the time of planting did not show considerable effect on intensity of purple blotch. The highest intensity 33.43% was recorded in control.

The results are in accordance with findings of Mishra *et al*. (1989) found maximum disease control with the application of mancozeb

(0.2%) followed by carbendazim. Borkar and Patil (1995) found that Mancozeb @ 0.2% reduced the disease intensity by 6% and increased the yield by 10.99%. Mathur and Sharma (2006) found that mancozeb and copper oxychloride to be most effective in reducing purple blotch intensity and increasing the yield of onion bulbs. Archana S. Rao *et al*. (2015) recorded that Mancozeb 70% WP @ 2500 ppm was effective in reducing the disease severity by 52.88 % over untreated control. Umme Sarifun Akter *et al.* (2015) recorded Dithene M-45 was found most effective to minimize disease severty as well as increase of yield. Wanggikar *et al.* (2014) recorded mancozeb (@ 0.2%) and copper oxychloride (0.25%) which recorded significantly mean disease incidence of 6.83 and 8.53 per cent and intensity,15.00 and 20.00 per cent, respectively.

Table 10a: Effect of different chemical treatments on purple blotch intensity of onion under field condition

Tre. No.	Treatment Name	Conc.	Before spraying	15 days after spraying	
			Per cent disease intensity at 90 DAP	Per cent disease intensity at 105 DAP	Per cent disease control
	Bulb dip for bulb rot.				
T_1	Copper oxychloride	0.25%	6.30 (2.61)*	17.00 (4.18)	0.00
T_2	Streptomycin	200 ppm	5.00 (2.34)	15.77 (4.03)	7.23
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	6.20 (2.59)	16.37 (4.11)	3.70
	Spraying for purple blotch				
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	6.33 (2.61)	9.17 (3.11)	46.05
T_5	Copper oxychloride	0.25%	5.50 (2.45)	10.63 (3.34)	37.47
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	5.73 (2.50)	8.20 (2.95)	51.76
	Bulb dip + Spraying for bulb rot and purple blotch				
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	5.57 (2.46)	9.03 (3.09)	46.88
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	6.03 (2.55)	10.93 (3.38)	35.70
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	5.27 (2.40)	8.13 (2.94)	52.17
T_{10}	Control	-	6.03 (2.55)	17.00 (4.18)	-
		F test	Non-Sig.	Sig.	
		SE(m)±	0.075	0.069	
		CD @ 5%	-	0.20	

*Mean of three replications. Figures in parenthesis are square root values. DAP - Days After Planting

Table 10b: Effect of different chemical treatments on purple blotch intensity of onion under field condition

Tre. No.	Treatment Name	Conc.	30 days after spraying		At harvesting time	
			Per cent disease intensity at 120 DAP	Per cent disease control	Per cent disease intensity at 135 DAP	Per cent disease control
	Bulb dip for bulb rot.					
T_1	Copper oxychloride	0.25%	24.10 (29.39)*	5.49	30.60 (33.58)	8.46
T_2	Streptomycin	200 ppm	25.17 (30.11)	1.29	32.00 (34.44)	4.27
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	24.07 (29.37)	5.06	31.67 (34.24)	5.26
	Spraying for purple blotch.					
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	13.03 (21.16)	48.90	16.57 (24.02)	50.43
T_5	Copper oxychloride	0.25%	15.67 (23.31)	38.54	19.67 (26.32)	41.16
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	11.50 (19.81)	54.90	15.10 (22.85)	54.83
	Bulb dip + Spraying for bulb rot and purple blotch.					
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	12.20 (20.44)	52.15	16.03 (23.60)	52.04
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	14.20 (22.13)	44.31	18.00 (25.09)	46.15
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	11.33 (19.66)	55.56	15.00 (22.77)	55.13
T_{10}	Control	-	25.50 (30.33)	-	33.43 (35.32)	-
		F test	Sig.		Sig.	
		SE(m)±	0.46		0.55	
		CD @ 5%	1.38		1.63	

*Mean of three replications. Figures in parenthesis are arc sine values. DAP - Days After Planting.

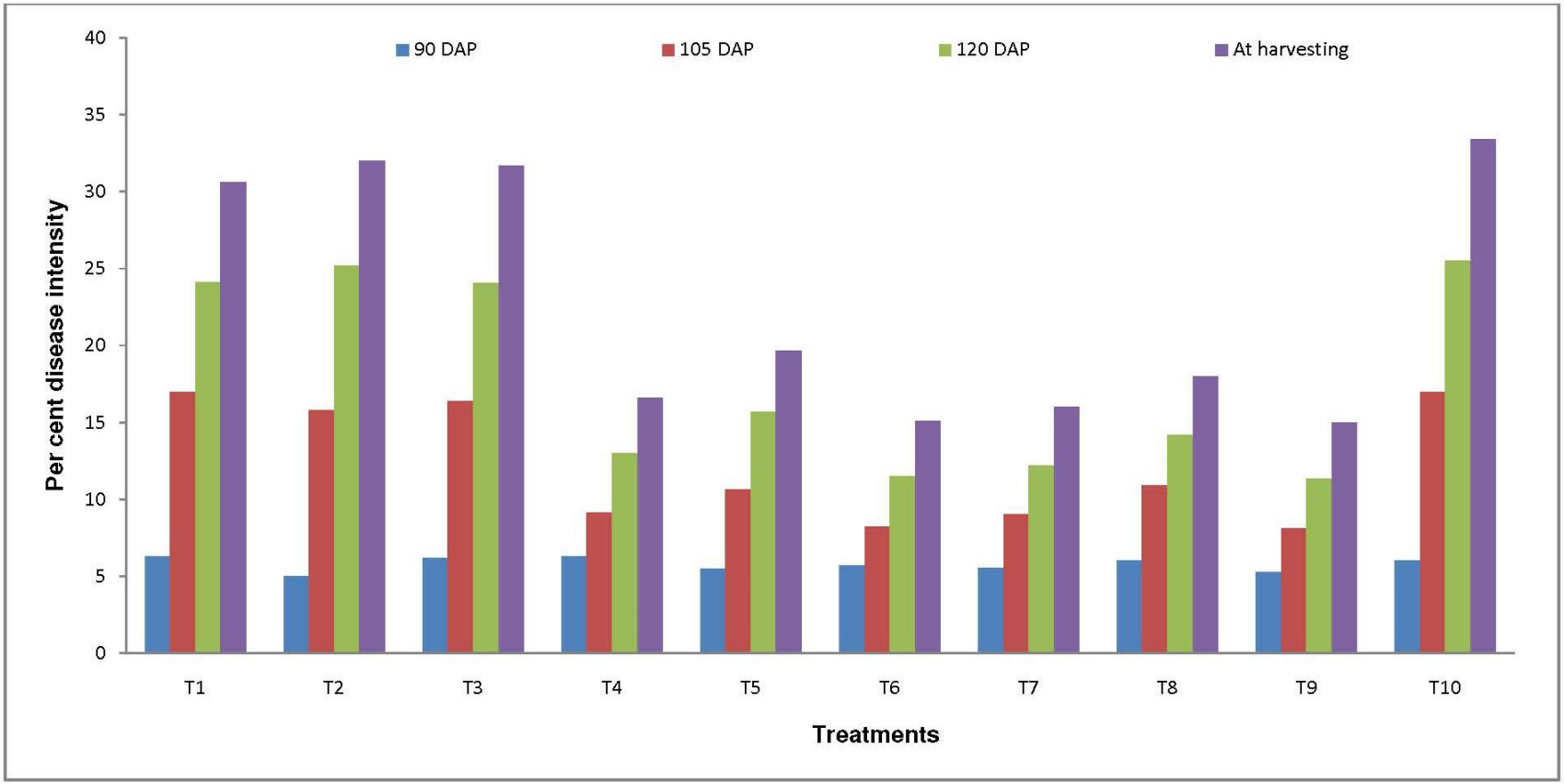


Fig. 5 Effect of different chemical treatments on purple blotch intensity of onion under field condition

The present results are also in confirmation with earlier workers, Srivastava *et al*. (1991) and Upadhaya and Tripathi (1995) who reported that copper oxychloride, mancozeb, and carbendazin against *Alternaria porri* and all the fungicides significantly reduced the disease incidence and intensity and gave increased yields over the control.

4.4.5 Effect of different chemical treatments on seed yield of onion

Data on onion seed yield is presented in Table 11 and Fig 6. Result of different chemical treatments on the seed yield onion was found significant over control and was ranged from 432 to 1022 kg/ha as against 317 kg/ha seed yield in control plot.

The bulb dip (T9) (copper oxychloride @ 0.25% + streptomycin 200ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%) was found significantly superior over rest of the treatment in which the maximum seed yield of onion 1022 kg/ha was obtained and followed by (T7) bulb dip (Copper oxychloride @ 0.25% + streptomycin 200ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%) 1003 kg/ha. Different chemical treatments effectively controlled the onion bulb rot incidence and purple blotch with increased seed yield over control in the range of 26.62% to 68.98%.

The present investigation was confirmed by the result obtain by Bhagat S. P (2015) reported that maximum seed yield of onion 546 kg/ha against bulb rot of onion. Karim *et al*. (2013) and Ali *et al*. (2015) recorded that seed yield of onion in the ranged of 370 – 500 Kg/ha. Selim Ahmed *et al*. (2018) reported highest onion seed yield i.e 580 kg/ha against purple blotch of onion. Zakirul islam (2013) observed maximum seed yield (649.40 kg/ha) with low incidence and intensity of purple blotch of onion.

Table 11: Effect of different chemical treatments on seed yield of onion

Tre. No.	Treatment Name	Conc.	Seed yield gm/plot	Seed yield kg/ ha	Per cent increased seed yield over control
	Bulb dip for bulb rot.				
T_1	Copper oxychloride	0.25%	350*	432	26.62
T_2	Streptomycin	200 ppm	455	561	43.49
T_3	Copper oxychloride + Streptomycin	0.25% + 200 ppm	520	641	50.54
	Spraying for purple blotch.				
T_4	Mancozeb + Carbendazim	0.25% + 0.10%	445	549	42.25
T_5	Copper oxychloride	0.25%	365	450	29.55
T_6	Mancozeb + Carbendazim + Copper oxychloride	0.25% + 0.10% + 0.25%	463	571	44.48
	Bulb dip + Spraying for bulb rot and purple blotch.				
T_7	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim)	0.25% + 200 ppm + 0.25% + 0.10%	813	1003	68.39
T_8	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Copper oxychloride)	0.25% + 200 ppm + 0.25%	745	919	65.50
T_9	Bulb dip (Copper oxychloride + Streptomycin) + Spraying (Mancozeb + Carbendazim + Copper oxychloride)	0.25% + 200 ppm + 0.25% + 0.10% + 0.25%	828	1022	68.98
T_{10}	Control	-	257	317	-
		F test	Sig.	-	-
		SE(m)±	6.79	-	-
		CD @ 5%	20.17	-	-

*****Mean of three replications.

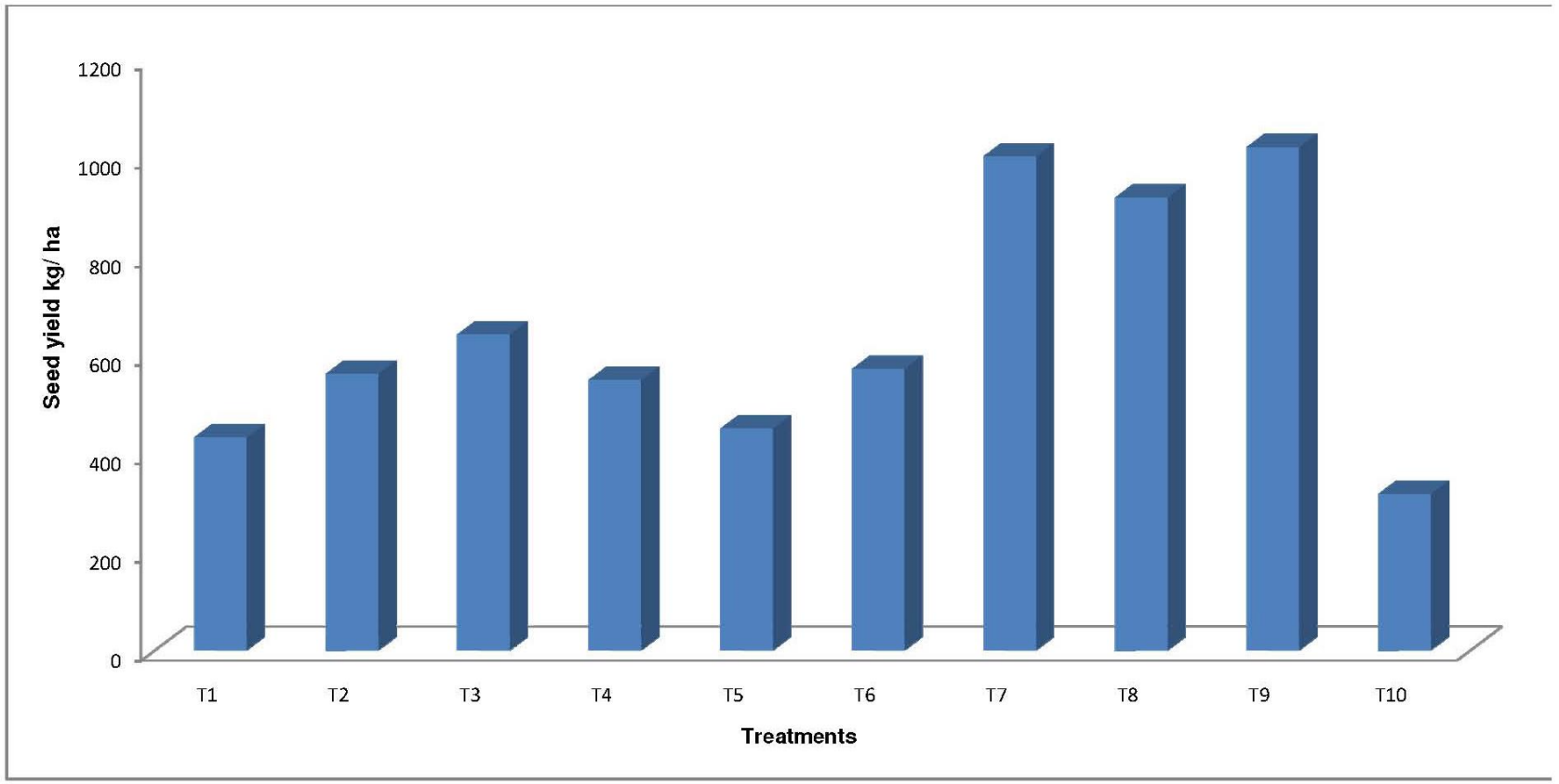


Fig. 6 Effect of different chemical treatments on seed yield of onion

CHAPTER V

SUMMARY AND CONCLUSIONS

Onion (*Allium cepa* L.) is one of the oldest bulb crops belongs to Amaryllidaceae family. Among vegetables, onion often called as “queen of kitchen” is one of the oldest known and an important commercial vegetable crop. India is a traditional producer and assumes second global position in onion production with 19.40 million tonnes (mt) from 1.20 million hectares (mha) area.

In India, several diseases of onion have become widespread and serious enough to limit the production. The common diseases like purple leaf blotch, *Stemphylum* blight, downy mildew basal/stem rot, damping off and bulb rot etc, are the most destructive diseases that damage the crop and reduce the seed yield even up to 100%. Among these diseases, bulb rot (*Erwinia carotovora* pv. *carotovora*) and purple blotch (*Alternaria porri*) are the the most destructive diseases, commonly prevailing in almost all onion growing pockets of the India, which causes heavy loss in onions under field conditions as well as in storage. *Erwinia carotovora* pv. *carotovora* causes 40 to 80 % losses in onion where *Alternaria porri* reduces 5.0 to 96.5 per cent yield in onion. Now days these two diseases threaten to the onion seed and bulb production in India.

Bulb rot symptoms were noticed at the base of central leaves of onion plant in field condition, longitudinal sections revealed that lesions developed downward and the inner layers of bulb appeared macerated. Infection observed at the inner part of the onion bulb in the neck region, and the decay gradually invades the whole bulb, while the outer scale remains unaffected. In the early stage of bulb infection, the older leaves become severally decayed and all the leaves die.

The purple blotch disease affects both aerial and underground parts in the field conditions. The purple blotch disease is characterized with small water-soaked lesions initially produce on leaves and seed stalk that quickly develop white centers. As lesions enlarge, they

become zonate, brown to purple, surrounded by a yellow zone and extend upward and downward for some distance. Under humid condition, the surface of the lesion may be covered with brown to dark gray structure of fungus. A few large lesions have been formed in a leaf or seed stalk which may coalesce and girdle the leaf or seed stalks. Usually the affected leaves or seed stalks break down and die within 4 weeks if the environment favors the disease.

Naturally infected onion showing typical well-developed symptoms of bulb rot and purple blotch was collected from the experiment field, located at Department of Vegetable Science, College of Horticulture Dr. PDKV Akola, for isolation of bacterial and fungal pathogen.

The bacterial pathogen *Erwinia carotovora* pv. *carotovora* and fungal pathogen *Alternaria porri* found to be associated with the diseased samples and isolated from infected bulb and leaf of onion by streaking and tissue isolation method on NA and PDA medium respectively.

Pathogenicity test of bacterial pathogen was carried out by bulb and soil inoculation method. The isolate of *Erwinia carotovora* pv. *carotovora* proved to be pathogenic causing bulb rot of onion. Where as pathogenicity of fungal pathogen was carried out by spore suspension method. The fungus *A. porri* proved to be pathogenic and responsible for purple blotch disease of onion.

The isolate of *Erwinia carotovora* pv. *carotovora* showed negative reaction towards gram reaction and urease production test where as it shows positive reaction towards KOH test, catalase test, potato soft rot test, gelatin liquification test, growth in 5% Nacl test, H_2S production test, indole production test, oxidase test and methyl red test. Based on the colony characters and morphological characters of mycelium, conidiophores and conidia, the fungus was identified as *Alternaria porri.*

Efficacy of different chemicals were tested by filter paper disc diffusion method against *Erwinia carotovora* pv. *carotovora.* Maximum growth inhibition of *Erwinia carotovora* pv. *carotovora* recorded in copper

oxychloride @ 0.25% + streptomycin @ 200 ppm (23.00 mm), followed by streptomycin @ 200 ppm (18.00 mm).

Under field condition, treatment (T8) bulb dip (copper oxychloride @ 0.25% + streptomycin sulphate @ 200 ppm) + spraying with (copper oxychloride @ 0.25%) was found most effective treatment against bulb rot of onion as it recorded minimum disease incidence (19.67%) with maximum disease control (53.46%). Which was at par with treatments i.e (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%), (T3) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm), and (T7) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%) that showed 20.10, 20.77, 21.80 per cent disease incidence and 52.44, 50.86, 48.42 percent disease control respectively. The highest disease incidence (42.27%) was recorded in control plot.

Poisoned food method was employed to test the efficacy of various chemicals against *Alternaria porri*. The mancozeb @ 0.25%, mancozeb @ 0.25%+ carbendazim @ 0.1% and mancozeb @ 0.25% + streptomycin @ 200 ppm concentration inhibited the 100 per cent mycelial growth of *A. porri*. The next best treatments was copper oxychloride @ 0.25% + mancozeb @ 0.25% exhibited 86.09 per cent growth inhibition of *A. porri* followed by copper oxychloride at 0.25% exhibited 73.35 per cent growth inhibition.

In the field experiment, all treatments showed significant effect in reducing disease intensity. Among all treatments, treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%) treatment recorded lowest per cent disease intensity i.e 15 per cent and it showed highest percent disease control i.e. 55.13 per cent which was at par with treatments i.e (T6) spraying with (mancozeb @ 0.25% + carbendazim @ 0.10% + copper oxychloride @ 0.25%), (T7) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.10%) and (T4) spraying with

(mancozeb @ 0.25% + carbendazim @ 0.10%) that showed 15.10, 16.03, 16.57 per cent disease intensity and 54.83, 52.04, 50.43 per cent disease control respectively. The highest disease intensity (33.43%) was recorded in control plot.

The application of different chemicals significantly influenced seed yield of onion. seed yield was ranged from 432 to 1022 kg/ha as against 317 kg/ha seed yield in control plot. The highest seed yield was recorded in treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.1% + copper oxychloride @ 0.25%) i.e 1022 kg/ha as compared to control.

Conclusions:

1. The bacterium *Erwinia carotovora* pv. *carotovora* found pathogenic and was responsible for bulb rot of onion.
2. The bacterium is straight rod and produced round and convex, white creamy colony with yellow pigmentation on NA medium.
3. The isolate of *Erwinia carotovora* pv. *carotovora* showed negative reaction to gram reaction and urease production test. However, it positive towards KOH test, catalase test, potato soft rot test, gelatine liquification test. growth in 5% Nacl, H_2S production test, indole production test, oxidase test and methyl red test.
4. *In vitro* study, copper oxychloride (0.25%) + streptomycin (200 ppm) was found effective against *Erwinia carotovora* pv. *carotovora* as it is recorded maximum (23.00 mm) zone of inhibition.
5. Under field condition, treatment (T8) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying of (copper oxychloride @ 0.25%) recorded minimum bulb rot incidence with maximum disease control.
6. In the field, *Alternaria porri* attacks above ground plant parts of onion. It showed different types of symptoms of purple blotch disease like small water-soaked lesions or white flecks, white zonate spots, purple

coloured zonate spots, spots with black spore mass and drying and breaking of leaves.

7. Fungus *Alternaria porri* found pathogenic and was responsible for purple blotch of onion.
8. The fungus was identified as *Alternaria porri* based on the morphological characters and standard measurements. It produced septate mycelium with conidiophores arising singly or in small groups. The conidiophores were straight or flexuous or geniculate. They were brown in colour with septations.
9. In the laboratory study, all the tested chemicals significantly reduced mycelial growth of *Alternaria porri*. Among the chemicals, mancozeb @ 0.25%, mancozeb @ 0.25% + carbendazim @ 0.1% and mancozeb @ 0.25% + streptomycin @ 200 ppm concentration inhibit 100 per cent radial mycelial growth of *Alternaria porri.*
10. Field experiment conducted for evaluation of different chemicals revealed that, the lowest disease intensity at 15% was observed in treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm) + spraying with (mancozeb @ 0.25% + carbendazim @ 0.1% + copper oxychloride @ 0.25%) with highest disease control (55.13%).
11. The highest seed yield was recorded in treatment (T9) bulb dip (copper oxychloride @ 0.25% + streptomycin @ 200 ppm + spraying of (mancozeb @ 0.25% + carbendazim @ 0.1% + copper oxychloride @ 0.25%) i.e 1022 kg/ha as compared to control.

CHAPTER VI

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Date: / / 2019

(GORE PRAKASH MAROTI**)**

APPENDIX- I

Composition of potato dextrose agar (PDA)

Peeled potato	200 g
Agar- agar	20 g
Dextrose	20 g
Distilled water	1000 ml

Composition of nutrient agar (NA)

Beef extract	3 g
peptone	5 g
Nacl	5 g
Agar	20 g
Distilled water	1000 ml

APPENDIX - II

List of Biochemical tests and chemicals used

I) Gram staining

i)	Crystal violet	Primmary strain
ii)	Gram iodine	Mordant
iii)	95 %Ethyl alcohol	Decolouring agent
iv)	Saffranin	Counter strain

II) KOH Solubility test

i)	KOH	3.0 gm
ii)	Glycerol	3.0 ml
iii)	D.W	100 ml

III) H2S Production

i)	Peptone	10.0gm
ii)	Nacl	5.00gm
iii)	D.W	1000 ml
iv)	pH	7.0
v)	Lead acetate	-

IV) Gelatin liquification

i)	Peptone	10.0gm
ii)	Beef Extract	5.0 gm
iii)	Gelatin	20 gm
v)	D.W	1000 ml
vi)	PH	7.0

V) Catalase test

i)	Hydrogen peroxide	3%

VI) Oxidase Test

i)	N, N, N,' N,'-tetramethyl-p-phenylenediamine dihydrochloride solution.	1%

VII) Indole Production Test

i)	Trypton	10 g
ii)	L-tryptophan	1 g
iii)	Distilled Water	1000 ml

vIII) Growth in to 5% NaCl

i)	Nutrient broth	95 ml
ii)	Nacl	5 g

Ix) Urease test

i)	$NH_4H_2PO_4$	0.5 g
ii)	K_2HPO_4	0.5 g
iii)	$MgSo_4 \times 7H_2O$	0.2 g
iv)	NaCl	5 g
v)	Yeast extract	1 g
vi)	Cresol red	0.016
vii)	Water	800 ml

x) Methyi red test

i)	Buffered peptone	7 g
ii)	Glucose	5 g
iii)	Dipotassium phosphate	1000 ml