

**STUDIES ON EARLY BLIGHT OF POTATO CAUSED  
BY *Alternaria solani* (Ellis and Martin) Jones and Grout**

**RANGANATHA, R. N.**

**PALB 3263**

**DEPARTMENT OF PLANT PATHOLOGY  
UNIVERSITY OF AGRICULTURAL SCIENCES  
BENGALURU**

**2015**

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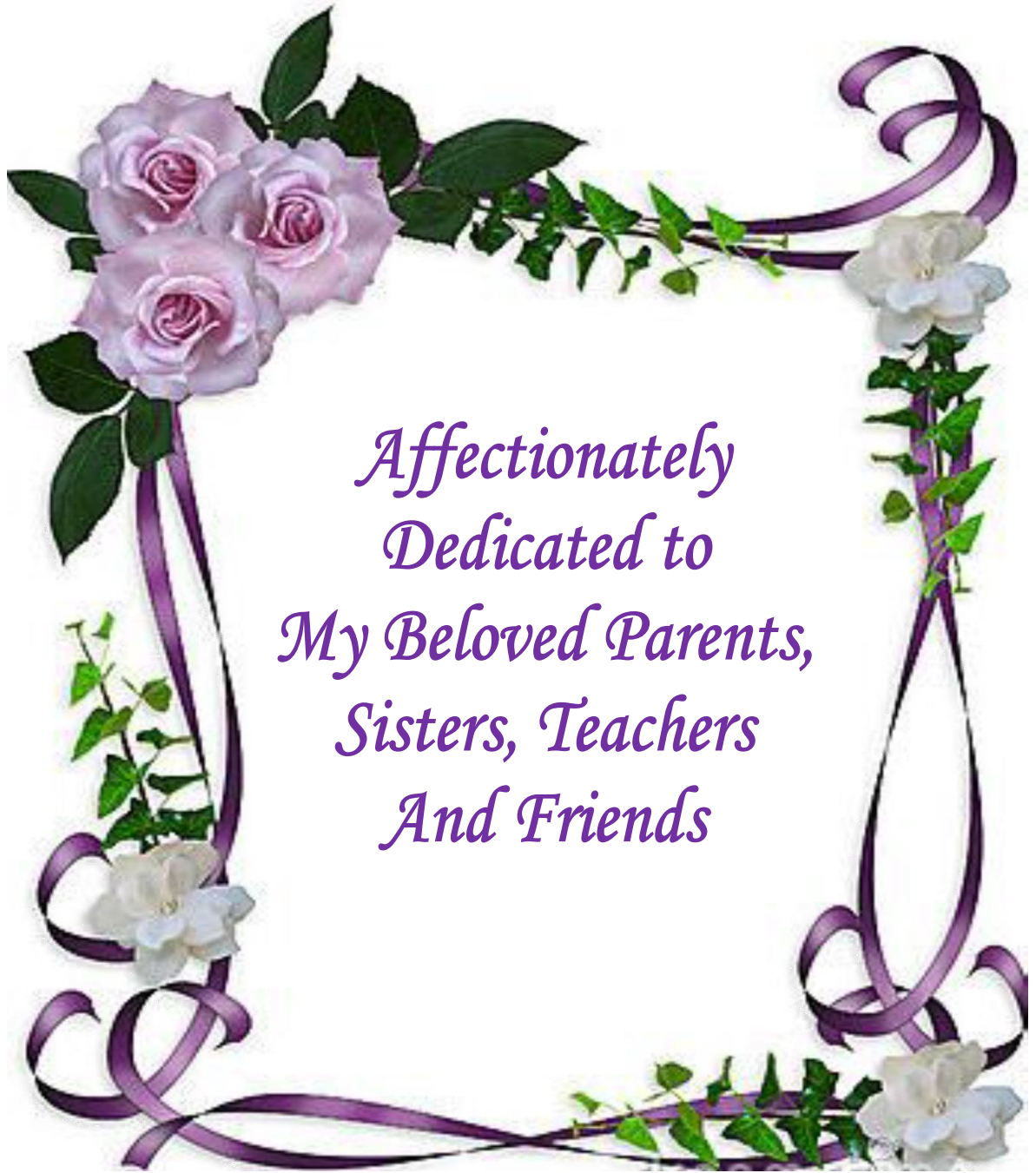
***MASTER OF SCIENCE (Agriculture)***

*in*

**PLANT PATHOLOGY**

BENGALURU

DECEMBER, 2015



*Affectionately  
Dedicated to  
My Beloved Parents,  
Sisters, Teachers  
And Friends*


**DEPARTMENT OF PLANT PATHOLOGY  
UNIVERSITY OF AGRICULTURAL SCIENCES  
GKVK, BANGALORE-560065**

**CERTIFICATE**

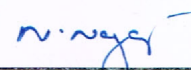
This is to certify that the thesis entitled “STUDIES ON EARLY BLIGHT OF POTATO CAUSED BY *Alternaria solani* (Ellis and Martin) Jones and Grout” Submitted by Mr. RANGANATHA, R. N., I. D. No. PALB 3263 in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (Agriculture) in PLANT PATHOLOGY to the University of Agricultural Sciences, Bangalore, is a record of *bona-fide* research work done by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associate ship, fellowship or other similar titles.


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Major Advisor

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**STUDIES ON EARLY BLIGHT OF POTATO CAUSED BY  
*Alternaria solani* (Ellis and Martin) Jones and Grout**

**RANGANATHA, R. N.**

**Thesis Abstract**

Potato is one of the most important staple food crops, ranking fifth and fourth place in area and production in the world, respectively. Among various diseases, *Alternaria* leaf spot is one of the most destructive disease on potato. Cultural studies revealed that, the growth of the pathogen was maximum on solid Potato Dextrose Agar medium (78.11 mm) and maximum fungal dry matter (188 mg) was observed in Potato Dextrose broth medium. The favourable temperature for the growth of the fungus was range between 30 °C to 35 °C. The maximum mycelial dry weight obtained at pH 6.0 (157 mg) to 6.5 (152 mg). *In vitro* evaluation of fungicides revealed that, the fungicide Tebuconazole (100 %), Hexaconazole+Zineb (95.18 %) and Trifloxystrobin+Tebuconazole (88.15 %) inhibited pathogen growth at 1000 ppm concentrations. *T.viride* IIHR-21 (69.63 %) and *T.viride* IIHR-22 (66.30 %) were found effective against *A. solani*. The crude leaf extracts from nine plants (Calotropis, Lantana, and Lemon grass, Nagadhale, Neem, Pongamia, Simarouba, Subabul and Tulasi) were tested against *A. solani* and found that Simarouba leaf extract (54.44 % at 1:1 dilution ) showed maximum inhibition of the pathogen. Under glass house condition Tebuconazole (48.33 % at 90 days) reduced disease severity and increased yield in Potato. The field evaluation offungicides, bioagents and sea weed extracts indicated that Mancozeb @ 0.2 % > Hexaconazole+Zineb @ 0.2% > Tebuconazole @ 0.1 % > Metalaxyl+ Mancozeb @ 0.2% > Tebuconazole+ Trifloxystrobin @ 0.1 % > Fenamidone+Mancozeb @ 0.2 % most effective in reducing severity (36.63 % at 90 days) of the early blight disease and increasing tuber yield (61.33 Kg/plot). In untreated check the disease severity was 83.03 per cent with plot yield of 32.66 kg. The foliar spray of fungicide combination is useful in the better management of early blight of potato.

December, 2015

Department of Plant Pathology  
UAS, GKVK, Bengaluru

**(Y.M. SOMASEKHARA)**  
Major Advisor

## ಆಲೂಗಡ್ಡೆಯ ಆಲ್ಪರ್ನೇರಿಯಾ ಎಲೆಚುಕ್ಕೆ ರೋಗದ ಅಧ್ಯಯನ

ರಂಗನಾಥ, ಆರ್. ಎನ್.

### ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಆಲೂಗಡ್ಡೆಯೂ ಅತಿ ಮುಖ್ಯವಾದ ಪ್ರಧಾನ ಆಹಾರ ಬೆಳೆಗಳಲ್ಲಿ ಒಂದಾಗಿದೆ. ಆಲೂಗಡ್ಡೆ ವಿಶ್ವದ ಪ್ರದೇಶ ಮತ್ತು ಉತ್ಪಾದನೆಯಲ್ಲಿ ಕ್ರಮವಾಗಿ ಐದನೇ ಮತ್ತು ನಾಲ್ಕನೇ ಸ್ಥಾನದಲ್ಲಿದೆ. ಆಲೂಗಡ್ಡೆಯು ಆಲ್ಪರ್ನೇರಿಯಾ ಎಲೆಚುಕ್ಕೆ ರೋಗವು ಪ್ರಮುಖ ಮತ್ತು ವಿನಾಶಕಾರಿಯಾದದ್ದು. ರೋಗಾಣುವಿನ ಸಾಂಸ್ಕೃತಿಕ ಅಧ್ಯಯನವನ್ನು ನಡೆಸಿದಾಗ ಘನ ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಲೋಸ್ ಅಗರ್ ಮಾಧ್ಯಮದಲ್ಲಿ (೭೮.೦೧ ಮಿಮೀ) ಮತ್ತು ದ್ರವ ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಲೋಸ್ ಸಾರು (೧೮೮ ಮಿಗ್ರಾಂ) ಮಾಧ್ಯಮದಲ್ಲಿ ರೋಗಾಣುವಿನ ಬೆಳವಣಿಗೆಯು ಗರಿಷ್ಠ ದಾಖಲಾಗಿದೆ. ಶಿಲೀಂಧ್ರ ಬೆಳವಣಿಗೆಗೆ ಅನುಕೂಲಕರ ತಾಪಮಾನವು ೩೦ ರಿಂದ ೩೫ ಡಿ ಸೆಂ ಮತ್ತು ಪಿಎಚ್ ೬ (೧೫೭ ಮಿಗ್ರಾಂ) ರಿಂದ ೬.೫ (೧೫೨ ಮಿಗ್ರಾಂ) ಉತ್ತಮವೆಂದು ಕಂಡುಬಂದಿದೆ. ಶಿಲೀಂಧ್ರನಾಶಕಗಳ ಪ್ರನಾಳೀಯ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಟೆಬುಕೊನಿಫೋಲ್ (ಶೇ ೧೦೦), ಹೆಕ್ಸಾಕೋನಾಜೋಲ್+ ಜೈನೆಬ್ (ಶೇ ೯೫.೧೮), ಟೆಬುಕೊನಿಫೋಲ್+ ಟ್ರೈಫ್ಲಾಕ್ಸಿಸ್ಟ್ರಾಬಿನ್ (ಶೇ ೮೮.೧೫) ೧೦೦೦ ಸಾಂದ್ರತೆಯಲ್ಲಿ ರೋಗಾಣುವಿನ ಬೆಳವಣಿಗೆಯನ್ನು ಕ್ರಮವಾಗಿ ಪ್ರತಿಬಂಧಿಸಿದೆ. ಜೈವಿಕಸೂಕ್ಷ್ಮಜೀವಿಗಳಾದ ಟ್ರೈಕೋಡರ್ಮಾ -ಐಐಎಚ್‌ಆರ್ - ೨೧ (ಶೇ ೬೯.೬೩) ಮತ್ತು ಟ್ರೈಕೋಡರ್ಮಾ -ಐಐಎಚ್‌ಆರ್ - ೨೨ (ಶೇ ೬೬.೩೦) ಗಳನ್ನು ಪ್ರಯೋಗಾಲಯದಲ್ಲಿ ಪರಿಶೀಲಿಸಿದಾಗ ರೋಗಾಣುವಿನ ಬೆಳವಣಿಗೆಯನ್ನು ಕುಂಡಿತಗೊಳಿಸುವಲ್ಲಿ ಉತ್ತಮವೆಂದು ಕಂಡುಬಂದಿದೆ. ಎಕ್ಸಿಗಿಡ, ಸಿಟ್ರೋನೆಲ್ಲ, ಬೇವು, ಹೊಂಗೆ, ತುಳಸಿ, ಸಿಮರುಬ ಹಾಗೂ ಇತರೆ ಕಚ್ಚಾವಲೆ ಉದ್ಧರಣವನ್ನು ಆಲ್ಪರ್ನೇರಿಯಾ ಸೊಲಾನಿ ಹತೋಟಿಯ ಬಗ್ಗೆ ಪರಿಶೀಲಿಸಿದಾಗ ಸಿಮರುಬ ಎಲೆಯ ರಸ ಸಾರವು (ಶೇ ೫೪.೪೪) ೧:೧ ದ್ರವೀಕರಣದಲ್ಲಿ ರೋಗಾಣುವಿನ ಬೆಳವಣಿಗೆಯನ್ನು ಕಡಿತಗೊಳಿಸಿರುವುದು ಕಂಡುಬಂದಿದೆ. ಗಾಜಿನ ಮನೆಯ ಸ್ಥಿತಿಯಲ್ಲಿ ಟೆಬುಕೊನಿಫೋಲ್ (ಶೇ. ೦.೧) ರೋಗದ ತೀವ್ರತೆ (ಶೇ ೪೮.೩೩) ಕಡಿಮೆ ಮತ್ತು ಬೆಳವಣಿಯ ನಿಯತಾಂಕಗಳು ಮತ್ತು ಆಲೂಗಡ್ಡೆಯ ಇಳುವರಿಯು ಹೆಚ್ಚಾಗಿ ಕಂಡು ಬಂದಿರುತ್ತದೆ. ಶಿಲೀಂಧ್ರನಾಶಕಗಳು, ಜೈವಿಕ ಸೂಕ್ಷ್ಮಜೀವಿಗಳು ಮತ್ತು ಸಮುದ್ರ ಕಳೆ ಉದ್ಧರಣಗಳನ್ನು ಕ್ಷೇತ್ರಪರೀಕ್ಷೆಗಳಲ್ಲಿ ಪರಿಶೀಲಿಸಿದಾಗ ಮ್ಯಾಂಕೊಜೈಬ್ (ಶೇ ೦. ೨) > ಹೆಕ್ಸಾಕೋನಾಜೋಲ್+ ಜೈನೆಬ್ (ಶೇ ೦.೨) > ಟೆಬುಕೊನಿಫೋಲ್ (ಶೇ. ೦.೧) > ಮೆಟಾಲಾ ಕ್ಲಿಲ್+ ಮ್ಯಾಂಕೊಜೈಬ್ (ಶೇ ೦. ೨) > ಟೆಬುಕೊನಿಫೋಲ್+ ಟ್ರೈಫ್ಲಾಕ್ಸಿಸ್ಟ್ರಾಬಿನ್ (ಶೇ. ೦.೧) ಮತ್ತು ಫೇನಮಿಡೋನ್ + ಮ್ಯಾಂಕೊಜೈಬ್ (ಶೇ ೦. ೨) ಕಡಿಮೆ ರೋಗದ ತೀವ್ರತೆ (ಶೇ. ೩೬.೬೩) ಹಾಗೂ ಗೆಡ್ಡೆಗಳ ಇಳುವರಿಯು (೬೧.೩೩ ಕೆಜಿ /ತಾಕು) ಹೆಚ್ಚಾಗಿ ಕಂಡು ಬಂದಿರುತ್ತದೆ. ವಿವಿಧ ಶಿಲೀಂಧ್ರನಾಶಕಗಳನ್ನು ಹಂತ ಹಂತವಾಗಿ ಸಿಂಪರಣೆ ಮಾಡುವುದರಿಂದ ಎಲೆಚುಕ್ಕೆ ರೋಗವನ್ನು ಹತೋಟಿ ಮಾಡಬಹುದು

ಡಿಸೆಂಬರ್, ೨೦೧೫

ಸಸ್ಯರೋಗಶಾಸ್ತ್ರವಿಭಾಗ

ಕೃಷಿವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಜಿ. ಕೆ.ವಿ.ಕೆ., ಬೆಂಗಳೂರು-೬೫

(ವೈ. ಎಂ. ಸೋಮಶೇಖರ)

ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು

# In-vitro and in vivo evaluation of fungicides, bio-agents and plant extracts against early blight (*Alternaria solani*) of Potato (*Solanum tuberosum*)



R.N. Ranganatha and Y.M.Somasekhara

Department of Plant Pathology, UAS, GKVK, Bengaluru

## Introduction

- ❖ Potato is one of the most important staple food crops, ranking fourth in production and fifth in area in the world.
- ❖ It is rich in potassium and phosphorus (Shekhawat *et al.*, 1992). Tubers contain at least 12 essential vitamins and are a good source of vitamin 'C'.
- ❖ Among the fungal diseases, early blight and late blight are most catastrophic diseases.
- ❖ The disease causing loss from 50 to 86 per cent in tuber yield (Mathur and Shekhawat, 1986).

## Objectives

1. Evaluation of new molecules of fungicides, plant extracts, bio-agents against early blight of potato *in vitro*.
2. Integrated management of blight disease of potato in field condition.

## Material and Methods

### 1. Evaluation of new molecules of fungicides and plant extract *in vitro*.

- ❖ The efficacy of eight fungicides at different concentrations (100, 250, 500, 1000ppm) were assessed against *Alternaria solani* in laboratory by adopting "Poison food technique".
- ❖ Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelium discs of 5 mm size from seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control.
- ❖ Three replications were maintained for each concentration. Such plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates.
- ❖ Nine plant extracts (calotropis, lantana, lemon grass, neem, simarouba, tulsi, nagadhale, pongamia, subabul) were tested at 10, 20 and 30 per cent concentration against *A. solani*.

### 2. Integrated management of blight diseases of potato in field condition.

- ❖ Experimental design-RCBD.
  - ❖ Treatments-6
  - ❖ Replication-4
  - ❖ Variety-Kufri jyothi
- Treatments details  
 T1= Tebuconazole (0.1%)  
 T2= Culture filtrate of *T.viride* (5%)  
 T3=Culture filtrate of *P. flourosense* (5%)  
 T4= Seaweed extracts (10%)  
 T5= Tebuconazole+ *Tviride* +*P. flourosense* + seaweed extracts.  
 T6= Control.

## Results

- ❖ Eight fungicides were evaluated against *A.solani* and found that Tebuconazole (97.66%), Hexaconazole +Zineb (94.44%) and Tebuconazole +Trifloxystrobin (87.77%) were showed maximum inhibition of growth of *A.solani*.

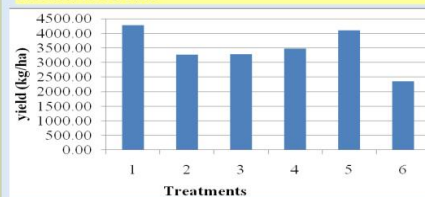
- ❖ The anti-chemical properties of various host extract were evaluated and found that Lantana, Simarouba, Tulsi and nagadhale extracts at 30 per cent concentration showed greater inhibition of *A. solani*.
- ❖ Tebuconazole, *Trichoderma viride*, *Pseudomonas flourosense* and seaweed extracts were evaluated for the management of the early disease and found that Tebuconazole was effective against *A.solani* followed by combination of Tebuconazole, *Trichoderma viride*, *Pseudomonas flourosense* and seaweed extracts.
- ❖ Maximum yield was obtained from the Tebuconazole (4250kg/ha.) treated plots followed by combination of Tebuconazole, *Trichoderma viride*, *Pseudomonas flourosense* (4100kg/ha) and seaweed extracts. The yield in untreated check was 2400kg/ha.



Fig.1 In vitro evaluation of fungicides and plant extracts against *A.solani*



Fig.2 Integrated management of early blight disease in Field at GKVK



Effect of different treatments on yield of Potato

## Discussion

- ❖ The fungicide Tebuconazole, Hexaconazole +Zineb and Tebuconazole +Trifloxystrobin were inhibited maximum fungal growth at all the concentration tested Ferial *et al.*(2010) also reported the triazole fungicides were effective against early blight disease of potato caused by *A.solani*.
- ❖ Nine plant extract were evaluated in *in vitro* against *A.solani* and found that, plant extracts lantana, simarouba, tulsi and nagadhale were showed greater inhibition was found. The plant extract from *Lantana* showed 50 per cent inhibition of *R. solani* was reported by Mangang *et al.*, (2014).
- ❖ Tebuconazole, *Trichoderma viride*, *Pseudomonas flourosense* and seaweed extracts were evaluated against early blight under field condition. The fungicide Tebuconazole was effective against early blight The same observation was reported by Shitienberg *et al.*, (2001).

- ❖ Maximum yield was obtained in Tebuconazole treated plots compared to other treatments. Daves *et al.*,(2002) reported that yield of potato more in tebuconazole treated plots.

Table 1: Effect of fungicides on mycelia growth of *A. solani*

Sl. No.	Fungicides	Concentration (ppm)			
		100	250	500	1000
		Per cent inhibition			
1	Metalaxyl +Mancozeb	29.22	50.33	57.00	63.00
2	Mancozeb	49.66	63.00	63.33	65.88
3	Captan	31.11	61.11	63.26	67.77
4	Fenamidone +Mancozeb	61.4	67.00	74.77	74.00
5	Hexaconazole +Zineb	82.2	87.77	91.11	94.44
6	Chlorothalonil	34.11	57.4	57.77	59.22
7	Tebuconazole	95.22	95.55	95.55	97.66
8	Trifloxystrobin + Tebuconazole	76.33	81.44	83.33	87.77
C.D.@5%		1.280	0.728	1.70	0.282
S.Em±		0.431	0.245	0.394	0.282

Table 2: Field evaluation of fungicides, bioagents and Plant extracts against blight diseases of potato.

Sl. N	Treatments	Disease severity (%)		
		Early blight		
		45days	60days	75days
1	Tebuconazole(0.1%)	20.8	32.03	35.6
2	<i>Trichoderma viride</i> (5%)	35.6	53.63	56.8
3	<i>P. flourosense</i> (5%)	32.8	51.03	55.6
4	Seaweed (10%)	31.8	46.65	50.8
5	Tebuconazole+ <i>Trichoderma viridae</i> + <i>Pseudomonas flourosense</i> + seaweed	25.6	36.50	38.9
6	Control	45.5	87.5	91.5
C.D. @ 5%		5.40	6.61	9.61
S.Em±		1.98	2.19	3.85

## Summary

- ❖ Eight fungicides were evaluated under *in vitro*. Tebuconazole, Tebuconazole + Trifloxystrobin and Hexaconazole +Zineb were found effective against *A.solani*
  - ❖ Nine host extract tested and found that, Simarouba, Tulasi, Naghadhale were effective.
  - ❖ In field condition Tebuconazole (0.1%) was found effective against blight disease .
  - Maximum yield was obtained from Tebuconazole(0.1%) treated plots.
- Reference**  
 ❖ Mangang, H.,C and Chetty, G.,K., 2012. *Int. J. Sci. Res. Pub.*, 2 :147-153.  
 ❖ Shitienberg, D., 2001. *Afr. Crop. Sci. J.*, 9 : 203-207.

## Advisory Committee:

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## I INTRODUCTION

The potato is one of the most important staple food crops, ranking fourth and fifth place in production and area in the world, respectively. Potato is one of the most important crop in the world, and it occupied an area about 18.2 million ha and total yield produced up to 314.1million ton. In India cultivable area under potato is about 1.96m ha with annual production of 44.72 mt. In Karnataka cultivable area under potato is 44,000 ha with annual production of 6, 28,000 mt. The per capita availability of potatoes in India is only 20 kg /year/ person which is almost one third of the world average. In India, potato is cultivated in almost all the states under diverse agro-climatic conditions. Nearly 82 per cent of the potatoes are grown in the plains during short winter days, about 10 per cent in hills under long day conditions during summer and rest of eight per cent in the plateaus region in the South Eastern and peninsular India as a rainfed crop during *Kharif* season. Karnataka is one of the most important potato growing states in peninsular India, which has only 2.54 per cent of the total area under potato in the country.

Potato is the only non-cereal food crop to commend such a high position in the world since being nutritious it can solve the problem of malnutrition and under nutrition if adopted as a major food crop. It has been recognized as a wholesome food and richest source of energy in most countries of the world where it forms important part of the human diet. Potato contains significant levels of phenolic compounds and vitamin C as potent antioxidants (Brown, 2005), which inactivate reactive oxygen species, reduce oxidative damage, lead to improved immune functions and reduce risk of cardiovascular diseases, cancer, cataract, diabetes and aging (Kour *et al.*, 2004). The mineral production in case of potato is 3.70 times more than wheat and 11 times more than rice. Potato produces more carbohydrates, fiber and vitamins per unit area Potato is a low energy food; 200 g of boiled potatoes provide about 138 Kcal of energy. It is rich in potassium and phosphorus. Tubers contain at least 12 essential vitamins and are a good source of vitamin 'C' containing about 14-25 mg/10 g of fresh weight of tuber .Potato is one of the main cash crops in six of its twenty districts. The state agro-ecology favours its cultivation during two seasons a year.

Commercially potato is propagated through tubers. Many disease causing agents *viz.*, viruses, fungus, bacteria, nematodes, viroid and phytoplasmas are reported on potato. Among the fungal diseases, early blight and late blight are most catastrophic diseases. The disease appears on leaves, stems, petiole, twig and tubers under favorable conditions resulting in defoliation, drying off of twigs and thus causing loss from 50 to 86 per cent in tuber yield (Mathur and Shekhawat, 1986).

Early blight of potato caused by *Alternaria solani* (Jones and Groul), is one of the most common and destructive diseases of potato in areas of heavy dew, rainfall and relative humidity. The disease becomes wide spread and serious, causing large economic loss to the growers when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavourable for the host and help in rapid disease development. The fungus can cause disease on foliage (leaf blight), stem (collar rot) and fruit, has result in severe damage during all stages of plant development. The

leaf blight phase, commonly referred to as early blight, is the most important phase of the disease and can result in complete loss of the crop when incidence is severe (Kallo and Banerjee, 1993). High humidity and the ineffectiveness of fungicides due to frequent heavy rainfall increase the disease intensity. Even under irrigated conditions, susceptible hybrids severely damage by early blight incurring a loss of 50 to 80 per cent (Mathur and Shekhawat, 1986). When *Alternaria* attacks the host leaf, morphologically it produces a series of concentric rings around the initial site of attack. This gives a "target spot" effect that is associated with early blight. Species of the genus are cosmopolitan and can survive as saprophytes as well as weak parasites. The genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal as well as oblique septa and having longer or short beaks. The spores of these polyphagous fungi occur commonly in the atmosphere and also in the soil. The telomorphs (sexual stage) are known in a very few species and placed in the genus *Pleospora* of Loculoascomycetes (under Sub-division: Ascomycotina), in which sleeper-shaped, muriform ascospores are produced in bitunicate asci (Verma and Verma, 2010).

The fungus overwinters in the form of conidia on crop debris but the main source of inoculum is probably seed. Fungus takes part on damping off and later infects older leaves. During vegetation period it spreads by conidia. They are spread by winds and splashing water. Wet weather with higher temperature support disease development. Spores and mycelium in infected plant refuse. Conidia are borne abundantly in most atmospheres and are disseminated readily by air currents.

These aspects prompted to understand the pathogen thoroughly for evolving effective management practices. The present investigations are taken up to keeping the following objectives:

1. Cultural and physiological studies of *Alternaria solani*.
2. *In vitro* evaluation of new molecules of fungicides, bio agents and plant extracts against *Alternaria solani*.
3. Effect of fungicides, bio agents and plant extracts on growth parameters and yield of potato affected by early blight disease under green house condition.
4. Integrated management of early blight disease of potato under field condition.

## II REVIEW OF LITERATURE

The potato crop affected by early blight disease is severe during cropping period and reduce potato yield. The management of early blight disease is very difficult by application of fungicides during rainy season. The major work on early blight of potato caused by *Alternaria solani* are reviewed and presented in this chapter:

### 2. 1 Cultural and physiological studies

#### 2.1.1 Growth of *A. solani* on different media

A suitable substrate is required for the growth of a pathogen. The morphological and cultural characteristics of the pathogen are essential for proper taxonomic status. The growth of *A. solani* on different media has been reviewed.

Pawar and Patel (1957) reported that growth of the pathogen was best on Oat meal agar, while it was also profuse in Richard's agar, Potato dextrose agar and Limabean agar. Sporulation was also profuse in Richard's agar.

Several reports indicated that PDA was a good medium for the growth and sporulation of *A. solani* (Bonde, 1929; Neergard, 1945 and Rotem, 1966). Arshour and El-Kadi (1959) reported that Potato dextrose agar and Richard's agar were the best for growth of *A. alternata*.

Barksdale (1968) reported that Potato agar and Lima bean agar were the best media for growth and sporulation of *A. solani*. Singh and Prasad (1973) obtained maximum growth of *A. cyamosis* on PDA followed by Richard's, Sabouraud's, Czapek's and Oat meal, while excellent sporulation was observed on Corn meal agar, followed by PDA and nutrient agar. Similarly, Cheema *et al.* (1976) reported that isolates of *A. citri* grow most rapidly on PDA, followed by Yeast extract agar and Czapek's-dox agar. Mohapatra *et al.* (1977) recorded the maximum growth of *A. sesame* on PDA followed by Richard's medium, Czapek's-Dox and Oat meal medium. Padmanabhan and Narayanaswamy (1977) observed that *A. macrospora* grow well on host leaf extract and Czapek's solution.

Kumar and Arya (1978) reported, Glucose Asparagine medium supported the best growth of *A. triticina* causing leaf blight of wheat. Roy (1969) found good growth of *Alternaria dauci* in Potato dextrose medium. Narasimha Rao and Rajagopalan (1979) observed that growth of *A. helianthicola* on Horne and Mitters medium was outstandingly superior and it was significantly inferior on Czapek's medium. The remaining media like Barne's, Potato dextrose agar, Oat meal, Host leaf extract dextrose, Czapek's malt extract and Richard media supported moderate growth of the fungus.

Desai (1979), Mahabaleswarappa (1981) and Joshi (1981) observed that more than 90 per cent spore of *A. macrospora*, *A. carthami* and *A. gomphrenae* respectively, germinated in Richard's solution, 2 per cent sucrose solution, Sabouraud's solution and sterile tap water. Among different media tried by Sitarama and Mehta (1982), Potato

dextrose supported best growth in both solid and broth, followed by Richard's and Czapek's solution for *A. porri*.

Pandey and Vishwakarma (1988) reported that vegetable based media like Capsicum dextrose agar significantly supporting maximum radial growth than other media; this was closely followed by Radish dextrose agar and Brinjal dextrose agar. The next best suitable media was V-8 juice agar. The synthesis media like CZDA, BA and RA along with most common media like PDA support least growth of *A. alternata*. Manika Sharma *et al.* (2013) reported that Potato dextrose agar, Cauliflower (Host) agar medium and Carrot potato agar were good for all the cultures of *A. brassicae*.

Swati Deep *et al.* (2014) reported that among seven types of media (PDA, Cauliflower leaf extract agar, Carrot potato agar, Oat meal agar, Czapek dox agar, V8 juice agar and Corn meal agar) were tested to determine their effect on growth and sporulation of the *A. brassicicola*. The results indicated that PDA (Potato dextrose agar) and Cauliflower leaf extract agar was found optimum for all isolates.

### 2.1.3 Effect of temperature on growth of *A. solani*.

Temperature affects almost every function of the fungi. For each fungus, there is a particular temperature below which, it will not grow. Likewise, there is a particular temperature above which growth ceases. These temperatures indicate the range and requirements of an organism. A few fungi are capable at growing below 0°C but for most of the species the minimum temperature is between 0 to 5°C.

Jackson(1959) reported that germ tube development, intracellular growth of hyphae and serious outbreak of *Alternaria* leaf blight occurred on muskmelon when temperature ranged from 20 to 32 °C. Khandelwal and Prasad (1970) studied the growth requirement of *Alternaria cucumerina* and reported that fungus required 25 to 30 °C and 92 to 100 per cent relative humidity for maximum growth and sporulation. However, typical symptoms on muskmelon were produced within 5 days alter inoculation at a temperature of 25 to 27 °C. Kumar and Arya (1978) reported the best growth of *A. triticina* at 25 °C.

Humperson-Jones *et al* (1983) stated that *Alternaria brassicae* and *A. brassicicola* required free water with optimum temperatures 15 and 25 °C respectively for infection on cabbage plants. Mridha and Wheeler (1993), who reported that most *A. brassicicola* infections in *Brassica napus* occur at 25 °C and fewer are seen at 15, 20 and 29 °C. Researchers have reported different optimal temperatures (19–30 °C) for infection due to differences in inoculation techniques, rating criteria and genotypes (Rotem, 1998).

Kucharek (2000) reported that in culture, optimum temperatures for spore production are between 75 to 82°F for all three species (*Alternaria brassicicola*, *A. brassicae* and *A. raphani*). Spore production was low below 61°F and above 82°F. However, if leaf wetness was prolonged for 20 hours or more, *A. brassicicola* is capable of producing many spores outside the optimum range of temperatures. *Alternaria raphani* produces fewer spores than the other two species (*A. brassicae* and *A. brassicicola*).

Spore germination of *A. brassicae* and *A. brassicicola* occurs between 34 to 104° F with the optimum being 59 to 95° F for *A. brassicae* and 91 to 95°F for *A. brassicicola*. Fontem *et al.* (1991) reported that sporulation in *A. brassicae* and *A. brassicicola* on naturally-infected leaf discs of oilseed rape and cabbage required humidities equal to or higher than 91.5 per cent and 87 per cent. The optimum temperatures for sporulation were 18 to 24 °C for *A. brassicae* and 20 to 30 °C for *A. brassicicola* at which temperatures both fungi produced spores in 12 to 14 h. Above 24 °C sporulation in *A. brassicae* was inhibited.

Kundu and Patra (1991) reported that, the spore germination of *Alternaria brassicicola* was high over a temperature range of 25-35 °C and high at neutral pH. Rotem (1994) reported that the conidia of *A. brassicicola* germinate over a wide range of temperatures, with the optimum at 28 to 31 °C. Infection was optimal at 25 °C, but infections occurred at temperatures as low as 10 °C. Sporulation occurred over a wide range of temperatures and optimal was at 20 to 30 °C. The disease appeared in the first fortnight of July and maximum disease intensity was noticed when the temperature ranged in between 25 to 28 °C and average relative humidity was more than 80 per cent. However, Singh and Suhag (1983) found that the disease developed at a temperature range of 15 to 25 °C and 100 per cent relative humidity for 10 to 12 hours on radish leaves.

Ronald and Diana (2011) reported that under warm weather (75-82° F), spores of *Alternaria brassicae* and *A. brassicicola* produced within a week. Sporulation occurred at a temperature range of 8 to 24 °C, where mature spores occur after 14 to 24 hours. Optimum temperatures are between 16 and 24 °C where sporulation time ranges from 12 to 14 hours. Moisture in the presence of rain, dew or high humidity were essential for infection and a minimum of 9 to 18 hours were required for majority of the species (Humperson-Jones and Phelps, 1989). Optimum germination of the conidia of the *Alternaria brassicicola* occurred at 25 to 30 °C, at pH 6 to 8 and at RH of 99 to 100 per cent (Samaddar, 1986)

### **2.1.2 Effect of pH on the growth of *Alternaria solani***

The nature of the activities of microorganism is such that the pH of the environment of a metabolizing culture will not remain constant for long (Munro, 1970). Lilly and Barnett (1951) reported that, the pH of the medium affected the rate and amount of growth and many other life processes. Kumar and Arya (1978) reported that, pH 6.0 was optimum for growth of *A. triticina*. Nishikado *et al.* (1941) reported that, pH 2, 5 and 10 are minimum, optimum and maximum respectively for the growth of *A. macrospora*, while Pabmanabhan and Narayaswamy (1977) found pH 5 to 7 to be optimum for the growth of *A. macrospora*. Diener (1955) reported a wide range of pH 4.1 to 9.1 for good growth and conidial production in *Stemphylium solani* Weber. a causal agent of grey leaf spot of tomato.

Rane and Patel (1956) reported that *A. macrospora* grew well between pH 4.8 and 5.2. Good growth and sporulation of *A. ricini* (Yoshii) Hansford, occurred between pH 4.8 and 5.5 are reported by Pawar and Patel (1957) while, Tandon (1961) observed that

the optimum pH for the growth of *A. alternata* was 5.0. Similar type of results was also reported by Ponnappa (1970). According to Mahabaleswarappa (1981) the optimum pH range for the germination of conidia of *A. carthami* was 5.0-6.0. He also noticed maximum conidial germination at 85 per cent relative humidity.

Taber *et al.* (1986) reported that *A. raphani* grow well at pH ranging between 4.8 and 7.2. Hasija (1970) studied the optimum pH requirement for *A. citri* and *A. alternata* and found that growth of *A. citri* was best between pH 4.4 and 6.4, moderate at pH 7.4 and poor at pH 2.7, 3.4 and 8.0, however *A. alternata* gave good growth in the pH range of 5.4 to 7.4, moderate growth at pH 4.4 and poor growth at pH 2.7, 3.4 and 8.0. Verma (1970) reported that optimum pH for growth of *A. alternata* was 6.6; the growth was moderate at a pH of 4.4; poor at a pH of 2.7, 3.4 and 8.0. Mohapatra *et al.* (1977) reported that *A. sesame* grows well in pH range of 3 to 10 and the best at 4.5 pH. Mathur and Sarboj (1977) found maximum growth and sporulation of *A. alternata* at pH of 5.5.

Narasimha Rao and Rajagopalan (1978) found that, *A. helianthi* could grow and sporulation over a fairly wide range of pH 4.5 to 10.0 with maximum at neutral pH (7.0). The growth gradually increases up to neutral pH with a steep fall afterwards. The fungus grows poorly at pH 3.5 and 10.0 but fails to grow at pH 3.0, 10.5 and 11.

Reddy and Gupta (1981) reported that the maximum growth of *A. helianthi* was at pH 6.0.

### **2.2.1 *In vitro* evaluation of fungicide**

The various fungicides were evaluated under laboratory conditions for the management of early blight pathogen. *In vitro* studies of the fungus toxicity provide information on toxicity against specific pathogen and therefore serve as a reliable guide for field testing. These studies deal with the pathogen and chemicals under controlled condition.

Ponnappa (1970) studied *Alternaria solani* efficiency of fungicides against *in vitro* and found that, the fungicides RH1261 was the most effective fungicide in checking the mycelial growth at all concentration tested. Aureofungin, Dithane M-45 and Duter showed complete inhibition of *Alternaria solani* at 0.2 per cent concentration.

Mukewar and Gera (1980b) tested nice fungicides *viz.*, Bavistin, Benlate, Brassicol, Captan, Dithane M-45, Dithan Z-78, Fytalon, Thiride and Vitavax in the laboratory for inhibition of the conidial germination of *A. helianthi*. They reported that Dithane M-45 and Vitavax were more effective followed by Bavistin and Benlate.

Gupta *et al.* (1981) reported Dithane M-45 as the most effective fungicide in inhibiting the growth of *Alternaria porri* under *in vitro* condition. Wadiphasm *et al.* (1994) tested six non-systemic and there systemic fungicides *in vitro* against *A. helianthi* by poison food technique. They found that Dithane M-45 was the most effective followed by Fytalon and Dithane Z-78.

Sashtrahidayat (1994) reported that Difenconazole 0.8ml/liter inhibiting the growth of *Alternaria porri* under laboratory condition. Haq *et al.* (1994) evaluated five fungicides *viz.*, Rovral (0.2 %), Mancozeb (0.2 %), Cuprovit (0.2 %), Copper oxy chloride and Antracol (0.2 %) inhibited *Alternaria solani* cause early blight of potato. The Rovral was the best for inhibition of growth of *Alternaria solani* followed by Dithane M-45. Datar (1994) reported that, the fungicides *viz.*, Carbendazim, Copper oxy chloride, Zineb, Mancozeb, Iprodione and Ziram tested at 100, 250 and 500 ppm significantly reduced conidial germination of *A. porri in vitro* and *in vivo* and increased yield over control.

Mallikarjun (1996) reported to out of the eight fungicides tested *in vitro* against *Alternaria alternata*. Propiconazole (Tilt) was found to be superior in inhibiting the growth of the fungus, followed by Mancozeb and Ziram.

Kamble *et al.* (2000) tested six fungicides against *A. alternata* under *in vitro* conditions. They reported that Mancozeb was highly effective in inhibiting the mycelia growth followed by copper oxy chloride and Iprodione at 1000, 2000 and 3000 ppm. Urbanszki *et al.* (2003) tested *in vitro* the efficacy of 16 fungicides against *A. alternata*. They reported that Tridemorph fungicide proved to be very efficient in controlling the pathogen.

Prasad and Naik (2003) reported Copper oxy chloride was one among the non systemic fungicide was effective in inhibiting the growth of *A. solani* and *A. alternata*.

Suralirajan and Janki (2003) reported the efficacy of Propiconazole and Hexaconazole were effective against *Alternaria solani* under *in vitro* condition.

Amaresh and Nargund (2004) reported Hexaconazole was effective at 200 ppm among systemic fungicides against *Alternaria* leaf blight of sunflower.

Singh and Singh (2006) tested, the efficacy of seven fungicides *viz.*, Chlorothalonil, Copper oxychloride, Azoxystrobin, Propineb, Copper hydroxide, Mancozeb at 250, 500, 1000, 2000 and 2500 ppm and Hexaconazole at 50, 100, 200, 500, 1000 ppm against *A. alternata* causing early blight of tomato. Their observations revealed that all the fungicides significantly reduced the radial growth of the fungus. However, Hexaconazole was very effective as it inhibited 100 per cent growth.

Arunakumara (2006) observed that among the systemic fungicides evaluated against *A. solani*, Propiconazole (84.57 %) gave maximum inhibition of the mycelial growth of pathogen. Arunkumar (2008) reported the efficacy of Carbendazim 25 % + Iprodione 25 % (Quintal) and Carbendazim 12 % + Mancozeb 63 % in inhibiting mycelia growth of *Alternaria alternate*.

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

Patel and Nandi *et al.* (2012) tested Captan and Mancozeb different formulated product at different concentrations (100 and 250 mg kg<sup>-1</sup>) and found that significant inhibition of the mycelial growth of *A. solani*, which was 61.95 and 65.33 per cent respectively as compared to control. Chetana *et al.* (2012) reported that among the contact fungicides tested, Mancozeb followed by Propineb were highly effective by recording 89.65 and 89.40 per cent inhibition respectively at 0.2 per cent concentration. There was significant difference between Mancozeb (80.71 %), Propineb (78.80 %), Copper oxy chloride (62.60 %) and Chlorothalonil (14.63 %) in inhibiting the growth of the pathogen at 0.25 per cent concentration. Significant increase in inhibition of the pathogen in Mancozeb, Propineb and Copper oxychloride recorded 88.25, 89.4 and 67.45 per cent respectively. The complete inhibition of the pathogen was observed at 0.3 per cent in Mancozeb and Propineb whereas, Copper oxychloride recorded maximum inhibition of 72.27 per cent.

Vikas *et al.* (2013) reported that the inhibition of the growth over the control of the *A. alternata* ranged from 70.20 to 100.00 per cent irrespective of the concentrations, Tebuconazole, Myclobutanil and Hexaconazole which proved to be the more effective fungicides and recorded the highest reduction of mycelial growth (100 %) which were superior than other fungicides followed by Tricyclazole (89.73 %) and Mancozeb (74.67 %), whereas in Carbendazim was found least inhibition of the fungal growth (70.20 %).

Sujoy *et al.* (2014) reported that, the best inhibitor of the fungal growth was exhibited by the combi product Nativo (Trifloxystrobin 25 % + Tebuconazole 50 %) at 350 mg kg<sup>-1</sup>, The that is 75.1 per cent and this was at on par with the same at 300 mg kg<sup>-1</sup> (74.87 %).

Deepti Sadana, and Nidhi Didwania (2015) reported that the maximum per cent inhibition of *Alternaria solani* in tomato was observed in Mancozeb at different concentrations and this was followed by Thiram and Captan. The least inhibition was observed in Carbendazim which range from 24.1 to 33.7 per cent.

### **2.2.2. *In vitro* evaluation of bio agents:**

The various bio agents were attempted for the management of various pathogens Vannacci and Harman (1987) have reported *Chetobium globosum* was one of the most effective antagonist for the management of *Alternaria brassicola*.

Quiser-Ahmed (1987) reported that *Pseudomonas chrysogenum*, *Pseudomonas thomii* and *Stachybotry satra* were antagonistic towards *Botrytis cineria*, *Fusarium moniliforme* and *Alternaria alternata*.

Casida and Lukezie (1992) reported that *Pseudomonas* strain 679-2 able to reduce the severity of the leaf spot disease caused by *A. solani*. Various volatile metabolites *viz.*, derivatives of lactones, alcohols and terpenes etc., produced by *T. viride* and chemicals like gliotoxin and gliovirin etc. produced by *Gliocladium* sp. are reported to be responsible for the formation of inhibition zone. Martinez and Solano (1995) studied the antagonism of ten *Trichoderma* strains against *A. solani* on tomato and strains L12 and

L17 showed 45.7 and 38.77 per cent of inhibition respectively. Liu-chienhui *et al.* (1997) reported that *Bacillus megaterium* MBS4 *B. brevis* OBS1 and *B. subtilis* var. *globigii* CBS10 were antagonistic to *A. solani*.

Three *Streptomyces* sp. (*S. pulcher*, *S. canescens* and *S. citreofluorescens*) were used to evaluate the potential of microbial antagonism for the control of some potato diseases including bacterial (*Pseudomonas solanacearum*) *Fusarium* (*F. oxysporum* sp. *lycopersici*) and *Verticillium* (*V. alboatrum*) with early blight (*Alternaria solani*) and bacterial canker (*Clavibacter michiganensis* sub sp. *michiganensis*). *In vivo* studies involved different treatments such as soaking potato seeds in filtrate of the antagonist prior to sowing, inoculation of the soil with the antagonists 7 days before sowing and coating potato seeds with spores of the antagonist before sowing. The seed-coating treatment will be the most effective in controlling all the pathogens up to 42 and 63 days after sowing. Soil inoculation with the antagonist 7 days prior to sowing will be less effective in controlling the potato pathogens than seed coating. The seed soaking treatment will be the least effective in controlling the diseases. The results also revealed that seed coating with antagonistic *Streptomyces* spp. significantly improved potato growth (El Abyad *et al.*, 1993).

Prasad and Kulashrestha (1999) observed all *Pseudomonas fluorescence* I to IV and *Pseudomonas putida* and *Pseudomonas cepacia* showed high antagonistic activity against *Alternaria helianthi* as compared to *Bacillus* spp.

Babu *et al.* (2000a) evaluated the efficacy of six *Trichoderma* species on early blight of tomato. Among the six species of *Trichoderma harzianum* exerted the highest inhibition of the mycelial growth (50.22 %) of the pathogen over control followed by *T. viride*. Babu *et al.* (2000b) reported that all the six *P. fluorescens* used were significantly inhibited the growth of *A. solani* compared to control. Among the antagonists, *Bacillus subtilis*, *Trichoderma viride* and *Gliocladium virens* also inhibited mycelial growth of *A. solani* causing leaf blight of tomato.

Amaresh (2000) reported that among fungi *Trichoderma viride* and *T. harzianum* overgrew and inhibited the growth of *A. helianthi* while the bacterium *Pseudomonas fluorescens* produced maximum inhibition zone.

Munshi and Dar (2004) reported that the formation of inhibition zone by *Gliocladium* sp. Against *Fusarium pallidoroseum*. *T. viride* and *T. harzianum* have also been reported to be effective fungal antagonists against *Sclerotium rolfsii* and *Rhizoctonia solani*.

Parveen and Vijay Kumar (2004) studied the mode of antagonism of *Trichoderma viride*, *in vitro* against *Alternaria triticina* causing leaf blight of wheat by using dual culture technique as a bio-control agent *Trichoderma viride* inhibited the growth of the pathogen and its mycelia coiled around hyphae of the test pathogen, forming a rope like structure and finally distinguishing the test pathogen *Alternaria tritician*.

Sharma and Sharma (2006) reported that the *Streptomyces* species and *Bacillus subtilis*; were also being used to inhibit mycelial growth and spore germination of *A. solani*

Antagonistic bacteria strains B-916, H-91, G-329, P-6854 and JND were used to control early blight of tomato caused by *A. solani* in pot and field experiments. It was observed that the antagonistic bacterial solution containing  $2 \times 10^6$  spores/ml when applied at 1ml/pot and 100 ml/m<sup>2</sup> in field, reduced the growth of hyphae of *A. solani* by 29-85 per cent and 61-80 per cent respectively. In another experiment, *Brevibacillus brevis* strain KH-7 and *Bacillus firmus* strain M-10 exhibited antagonistic activity against *A. solani* and also enhanced the growth and yield of potato (Panwar *et al.*, 2006).

Imtiaz and Lee (2008) studied on the efficacy of three different sp. of *Trichoderma* against *A. porri* revealed *T. harzianum* and *T. virens* are most effective in inhibiting the pathogen. Fungicidal activities of the secondary metabolic products of an actinomycetes strain, A 19 were determined *in vitro*. Its fermentation broth had inhibitory effect against *A. alternata* and some other fungi.

The culturable leaf-associated bacteria inhabiting a plant were considered as a promising biological control agent (BCA) candidate because they could survive on the plant. It was also found that *Bacillus* and *Pantoea* had strong antifungal activity both in *in vitro* as well as *in vivo* conditions, but *Curtobacterium* and *Sphingomonas* showed antifungal activities only *in vitro* against *A. solani* isolated from tomato. A washed cell suspension of the antagonistic yeast *Pichia guilliermondii* was effective in inhibiting the pathogen; also, tomato fruits treated with the antagonist inoculum concentration 107-108 CFU ml<sup>-1</sup> had significantly lower incidences of disease and smaller lesion diameters (Zhao *et al.*, 2008).

Dalpati *et al.* (2010) evaluated four different bio-agents (*Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*) and ten botanicals *viz.* Neem, Custard apple, Lantana, Eucalyptus, Marigold, Tamarind, Kanher, Garlic, Datura and Congress grass against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*.

Sabriye *et al* (2011) reported that all the twenty three bacterial isolates strongly inhibited the growth of *A. solani* by forming inhibition zones larger than 5 mm on NA. *Serratia plymuthica* (İK-139) isolate had the highest inhibitory effect with inhibition zone of 31.3 mm on *A. solani*. Second most effective bacterium was *S. plymuthica* (İK-150) isolate with inhibition zone of 27.55 mm, followed by *B. subtilis* (İK-92), *Serratia marcescens-GC subgroup A* (İK-174), *Pantoea agglomerans* (İK-147) and *Bukholderia pyrrocinia* (İK-145) with inhibition zone of 26.6, 23.75, 21.05 and 20.25 mm, respectively., As well. *B. subtilis* (İK-159) and *Brevibacillus brevis* (İK-146) produced zone of inhibitions lower than 10 mm.

Ganie *et al.* (2013) reported that *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. Ex Gray inhibited the mycelial growth of *A. solani* causing early blight of

potato foliar spray with *Trichoderma harzianum* Rifai ( $1 \times 10^7$  spore/ml) were highly effective in controlling the disease severity as compared to control.

### 2.2.3 *In vitro* evaluation of plant extracts

Skinner (1955) suggested that the presence of some antibiotic constituents or some unknown substances contribute to the inhibitory activity of the plant extracts. The extracts of *Canna indica* L., *Convolvulus arvensis* L., *Ipomoea palmate* Forsk., *Cenchrus catharticus* Delite., L., *Prosopsis spicigera* L. (Mant), *Allium cepa* L., *A. sativum* L., *Lawsoni ainermis* L., *Argemone mexicana* L., *Datura stramonium* and *Clerodendron inerme* completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower.

Dharma and Sharma (1985) reported that the preparation from neem inhibited the growth of *Alternaria alternate* by 61.1 per cent at a concentration of 1 per cent and by 100 at 10 per cent concentration.

Vijayan (1989) reported that the bulb extract of *A. sativum*, leaf extract of *Aegleamar melos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*.

Babu *et al.* (2000a) reported the effect of plant extracts, oils and Neem products (Neem leaf, neem seed kernel and neem cake) on early blight. Among the plant products, *Acacia concinna* pod extract resulted in the lowest per cent disease index (23.1 %) followed by neem oil (30.9 %).

Plant extracts can be directly used or substances responsible for the antimicrobial properties can be isolated. A new extract with potent antifungal properties the extract obtained from *Aloevera*, antifungal activity against four common post-harvest pathogens: *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *Alternaria alternate* (Barkai-Golan, 2001).

Lemon grass leaves extract inhibited the spore germination and reduce the mycelia growth of two pathogenic fungi. *In vivo* tests, detached leaf technique showed that the TAMPE decreased the disease infection with both *P. infestans* and *A. solani*. The efficacy of plant extracts in controlling the late and early blight (Stephan and Koch, 2002). Muto *et al.* (2005) showed that the extracts derived from fresh and dry tissues of 14 plant species were evaluated for activities against *P. infestans* and *A. solani*.

Pramodkumar (2007) reported *Clerodendron* leaf extract as one of the best plant extract in inhibiting the mycelial growth of *Alternaria porri*.

Dalpati *et al.* (2010) evaluated ten botanicals *viz.* Neem, Custard apple, Lantana, Eucalyptus, Marigold, Tamarind, Kanher, Garlic, Datura and Congress grass against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*. The per cent inhibition of botanicals ranged from 44.59 to 8.25 per cent. Lantana and Datura were found effective

as it restricted 44.59 and 30.88 per cent respectively. Botanicals like Tamarinds found least effective as it prohibited the growth by 8.25 per cent only.

Vanitha (2010) reported that EC formulation of winter green oil exhibited 100 per cent inhibition of mycelial growth of *Alternaria chlamydospora*.

Suleiman (2010) reported that methanol extracts of leaves of pawpaw showed highest mycelial growth inhibition against *A. solani*. Nashwa and Abo-Elyousr (2012) reported that the leaf extracts of *Datur astramonium*, *Azadirachta indica* (Neem) and *Allium sativum* (Garlic) at 5 per cent concentration caused the highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2 % respectively), while *Ocimum basilicum* (Sweet Basil) at 1 and 5 per cent concentration and *Nerium oleander* (Oleander) at 5 per cent concentration caused the lowest inhibition of mycelial growth of the pathogen.

Ravikumar and Rajkumar (2013) reported that out of the 39 plants selected, 13 plant extracts significantly reduced the mycelial growth of *A. solani*

Bioefficacy of fifteen plant extracts (*Polyalthi alongifolia*, *Azadirachta indica*, *Datura stramonium*, *Ocimum sanctum*, *Calotropi procera*, *Crotalaria juncea*, *Eucalyptus obliqua*, *Cassia fistula*, *Agelemar melos*, *Croton bonplonadium*, *Pergularia daemia*, *Cleome viscosa*, *Phyllanthus amarus*, *Bauhinia purpurea*, *Euphorbia hirta*) were evaluated under *in vitro* conditions. Among plant extracts evaluated, fresh aqueous extract of *Eucalyptus oblique* (15 %) was effective in causing 88 per cent inhibition of mycelial growth in A1 strain of *Alternaria solani*. Followed by *Datura stamonium*, *Azadirachta indica*, *Calotropis procera* and *Polyalthia longifolia* (Deepti Sadana, and Nidhi Didwania, 2015).

### **2.3.1 Effect of new molecules of fungicides, bio agents and plant extracts on growth parameters and yield of potato affected by early blight disease under green house condition.**

Rochecoste (1984) reported that purple blotch of garlic caused by *Alternaria porri* was effectively controlled by Metalaxyl +Mancozeb. Choulwar and Datar (1992) reported the least disease intensity both at pre and postharvest stages with increased yield where six early sprays of Mancozeb at 0.2 per cent followed by six late and five early sprays were given. Krilaxyl and Dithane M-45 found more effective than the control in reducing early blight development and they increased yield over the control by 17 to 41 per cent.

Sattar and Kaseem(1991) studied the effectiveness of Iprodione, Dithane M- 45, Zineb, Topsin M-7 and Ridomil 5G against *Alternaria* disease in tomato at 5 per cent and found that, among these Iprodione gave the best control with maximum yield.

Dahren and Staub (1992) reported that physiological effects may contribute to greater yield, even in the absence of disease. The application of Difenconazole is likely to be more effective than a protective fungicide, as it has both protective and post-

infection activity and can provide greater efficacy and potential yield/economic response. In an experiment disease was effectively by 8 per cent Metalaxyl + 64 per cent Mancozeb (Dhanbir *et al.*, 1994).

In addition to disease control, Strobilurin fungicides are known to have beneficial physiological/growth-promoting effects on plants, including delaying of leaf senescence (Bertelsen *et al.* 2001), increased chlorophyll content (Butkute *et al.* 2008) and greater stress tolerance (Jabs *et al.*, 2002). Fugro *et al.* (1994) evaluated some fungicides for disease control and found that Dithane M-45 was significantly superior to others against *A. cucumerina* causing leaf blight of watermelon.

Shtienberg and Blachinsky (1996) conducted to the lesion expansion rate (LER) of *A. solani* on potato plants was not affected significantly by Chlorothalonil, but Tebuconazole decreases it significantly.

Jovancev (1998) reported the efficacy of Acrobat plus (Dimethomorph + Mancozeb) and Mancozeb 80 WP for controlling late blight (*Phytophthora infestans*) and early blight (*A. solani*) diseases of tomato.

Sarkar and Chaudhary (2004) observed that when polyram was applied thrice at 2.5kg/ha at 15 days intervals it was significantly superior to the Captan 50 per cent and Mancozeb, in reducing early blight of tomato caused by *A. solani* and also increasing the yield.

Singh (2002) observed that Dithane M-45 + Kavach were found to be on par, in controlling *A. helianthi*. However, Bavistin and Dithane M-45 were the most superior formulations.

Pyraclostrobin significantly reduced the early blight and increased the yield in tomato and potato has reported by many workers (Ganeshan and Chethana, 2009 ) and MacDonald *et al.*, (2007) .Kumar *et al.* (2007) reported that Hexaconazole (0.05 %) and Azoxystrobin (0.2 %) was very effective in managing early blight of tomato.

Ping *et al.* (2007) isolated 29 strains of endophytic actinomycetes from surface sterilized plant tissues of wild plants. Out of these the fermentation filtrate of SG 2 metabolites exhibited greater antagonism against *A. solani*. Under greenhouse conditions the control efficacy of SG 2 and SG 4 reached 89.7 per cent on *A. solani*.

Rahman *et al.* (2008) reported reduced leaf infection (43 %) and increased yields (20.56 %) comparative to control by applying foliar sprays of Filthane M-45.

The spray program trials confirmed the importance of fungicide use for the control of *A. solani* in the production of potato. Yield increases were primarily a result of controlling early blight and prolonging green leaf areas, which increases the period of tuber bulking. (Stevenson and James 2005; Stevenson and James 2007; Franc and Stump 2008).

Verma *et al.* (2008) reported that disease severity of *A. solani*, the causal agent of early blight of tomato could be significantly reduced with foliar spray of *Clerodendron aculeatum* leaf extract (15 %) immediately after appearance of symptoms or foliar spray of *T. viride* ( $10^7$  cfu/ml) followed by two sprays of Mancozeb.

Jambhulkar *et al.* (2012) reported spray of Azoxystrobin showed promising results by reducing disease index by 38.9 per cent as compare with control.

Majeed *et al.* (2014) reported that foliar blight and disease progress was significantly reduced by foliar application of systemic and contact fungicides. Increments in tuber yields were recorded for systemic fungicides Curzate and Ridomil gold; contact fungicides did not affect yield of potato.

## **2.4 Integrated management of early blight disease of potato**

Singh and Milne (1974) evaluated the efficacy of 15 fungicides against five fungi causing chrysanthemum flower blight, *viz.* *A. alternata*, *Botrytis cinerea*, *Itersonilia perplexans*, *Mycosphaerella ligulicola* and *Stemphylium vesicarium*. No one fungicide was outstanding at low concentrations against all five fungi. Captafol, Chloroneb, Mancozeb and Thiram appeared the most promising.

Abraham *et al.* (1976) stated that Miltox was found to be effective in controlling *Alternaria* leaf spot, followed by Benomyl, Mancozeb and Captan. They also noticed differences in the reaction of different varieties and fungicides.

Ramakrishan and Kandaswamy (1978) reported that spraying of broad spectrum fungicides like Mancozeb and Captan has been recommended for the control of early blight and from their field trials in 1972 and 1974 reported that *A. solani* on tomato was controlled and yield also increased with the application of Dithane M-45 at 0.2 per cent followed by Difolaton and Benlate.

Savanur (1984) reported that different systemic and non systemic fungicides were tried for the control of leaf spot disease of cotton. The least per cent disease incidence was observed in Dithane M-45, which gave maximum kapas yield. While, Benomyl did not effectively reduce the disease incidence and also recorded the least kapas yield.

Choulwar and Datar (1992) reported the least disease intensity both at pre and postharvest stages with increased yield where six early sprays of Mancozeb at 0.2 per cent followed by six late and five early sprays were given. In general early sprays were most effective that equal number of late sprays.

Four fungicides sprayed 4 times after the first appearance of tomato early blight and thereafter at 10 days intervals were evaluated for control of *A. solani*. Polyram-combi (Metiram) at 1.5 g/ litre or Dithane M-45 (Mancozeb) at 2.5 g/litre gave effective control, whereas bavistin (Carbendazim) at 0.5 g/ litre and Captan at 2.5 g/litre were less effective (Mohammad, 1988).

Bhardwaja (1991) reported that sequential application of Captofol, Mancozeb and Copper oxychloride 40, 55, 70 days after transplanting, increased yield by 50.5 per cent by reducing the incidence of *A. solani*.

Roval (Iprodione), Dithane M-45 (Mancozeb), Zineb, Topsin M-7 (Thiophanate-methyl) and Ridomil (Metalaxyl) were tested for their ability to control early blight of tomato caused by *A. solani* in Yemen. Iprodione gave the best control. Significant differences were observed between Iprodione, the other fungicides and the control. Maximum yield was recorded after 5 per cent Iprodione treatment during 1986-1987. During 1988-1989, similar results were observed and significant differences were recorded between Iprodione, Metalaxyl and control treatments. *In vitro* tests confirmed the field results (Sattar and Kassem, 1991).

Maheswari *et al.* (1991) conducted field trials using six fungitoxicants, the most effective control (64.7 %) of *A. solani* was given by Copper oxychloride followed by Mancozeb (61.7 %) the increase in field was recorded on plots spray with Mancozeb.

Choulwar and Datar (1992) reported that out of nine fungicides (Copper oxychloride, Zineb, Ziram, Mancozeb, Carbendazim, Dithionon, Thiophenate methyl, Iprodione and Captafol) tested against early blight in tomato, Mancozeb was found most effective in reducing disease intensity and increasing yield in cultivar Pusa ruby, followed by Captofol and Zineb.

Brammatta (1993) reported that application of Chlorothalonil (0.2 %) decreased early blight severity. Combined application of talc-based antagonistic formulation, as bulb, soil and foliage treatment was the most effective method for reducing the disease. Possibly both rhizosphere and phyllosphere population of antagonists helped in control of the disease.

A field experiment was carried out to manage the leaf spot and bight disease using seven fungicides *viz.*, Carbendazim (0.1 %), Copper oxychloride (0.2 %), Iprodione (0.2 %), Chlorothalonil (0.2 %), Mancozeb (0.25 %), Captan (0.25 %) and Thiophanate methyl (0.1 %) used in this trial considerable reduced the disease incidence as compared to control. Maximum disease reduction was recorded in the experimental plots spray with Thiophanate methyl (0.1 %) followed by Mancozeb (0.25 %) and Chlorothanil (0.2 %) and were statistically on par with each other (Bhaskar, 1996).

Babu *et al.* (2000a) reported the effect of plant extracts, oils and neem products (neem leaf, neem seed kernel and neem cake) on tomato leaf blight in the field. Among the plant products, *Acacia concinna* pod extract resulted in the lowest per cent disease index in the field (23.1 %), followed by *B. latifolia* oil cake and neem oil (27.2 and 30.9 %) respectively.

Pandey *et al.* (2000) conducted a field experiment to control brinjal leaf spot caused by *Alternaria alternata*, and reported that Mancozeb 0.2 per cent was superior to

other tested fungicides. It reduced disease severity by 65.26 per cent over control. The next best fungicides were Dithianon, Thiram, Folpet and Zineb.

The best control of *Alternaria* leaf spot disease of bottle gourd was obtained by spraying recommended at 0.2 per cent Indofil M-45 followed by Chlorothalonil, Cuman L., Ridomil, Indofil Z-78, Copper oxychloride, Jkstein and Topsin-M (Katiyar *et al.*, 2001b).

Monaco *et al.* (2001) conducted *in vitro* studies to investigate a possible integrated use of chemical approach to control *A. solani*. Six fungal antagonists *viz.*, *Fusarium semitectum* (*F.pallidoroseum*), *Trichoderma polysporum*, *Tolypocladium niveum*, *Chaetomium globosum*, *Rhodotorula* sp., *Cladosporium cladosporioides* and *Nigrospora* sp and two fungicides (Daconil (chlorothalonil) and Dithane M-45) were used. *Rhodotorula* sp and *Cladosporium cladosporioides* were tolerant to Daconil with high ED 50 values (142.89 and 112.14 ppm, respectively), while *Chaetomium globosum* was tolerant to Dithane M-45 with ED 50 value of 38.72 ppm. The other isolates were sensitive to both fungicides with ED 50 values similar or lower than those presented by *A. solani*. These results suggest that successful integrated control programme can be implemented when *Chaetomium globosum* was used in combination with Dithane M-45 and when *Cladosporium cladosporioides* and *Rhodotorula* sp. were used in combination with daconil.

Bhatti *et al.* (2002) evaluated four fungicides: Dithane M-45, Topsin-M, Liromanzeb, and Vitigran blue were applied. Vitigran blue significantly inhibited the colony growth of *Alternaria brassicae* followed by Dithane M-45. The number of leaf spots on inoculated leaves were reduced by spraying Vitigran blue followed by Dithane M-45. It is recommended that leaf spots can be controlled by pre-application of both Vitigran blue and Dithane M-45 fungicides.

Dillard *et al.* (2002) evaluated fifteen fungicides against *Alternaria* leaf spot caused by *A.brassicicola* under field condition. They reported that none of the treatment resulted in acceptable disease control in this severe test, although many treatments were significantly better than the control. Final disease severity in all of treatments was less than the control except for Plant yield, Bravo Weather Stick, Messenger, Spent mushroom mulch and QRD 137. Bass 16, Folicur plus Induce and Quadris plus Induce were the most effective treatments in the trial as measured by final per cent disease severity.

Prasad and Naik (2003) tested the efficacy of non-systemic fungicides (Iprodione Mancozeb, Copper oxychloride and SAAF), systemic fungicides (Thiophenatemethyl, Triadimefon Benomyl and Carbendazim) in controlling the early blight of potato. Mancozeb treatment gave the highest cost-benefit ratio of 1:11.4 in addition to reducing the disease incidence Pyraclostrobin significantly reduced the early blight and increased the yield in tomato and potato has reported by many workers (Ganeshan and Chethana, 2009 and McDonald *et al.*, 2007).

The efficacy of botanicals *i.e.*, Neem seed and leaf extract (each at 5 %) and Tobacco decoction (2 %) was evaluated in a field experiment for the management of early blight of tomato. The plant products, namely Neem seed extract (19.75 PDI), Neem leaf extract (20.36 PDI) and Tobacco decoction (23.87 PDI) were also effective in reducing disease incidence and increasing fruit yield by 168.56, 156.43 and 147.66q/ha, respectively (Patil *et al.*, 2003).

Sobolewski and Robak (2004) tested the fungicides Unikat 75 WG zoxamide (mancozeb), ethaboksam (ethaboxam). MC 72.5 WP (mancozeb) cymoxanil and Acrobat MZ69 WP, dimethomorphyl (mancozeb) to control early blight (*A. solani*) in potato.

Pyraclostrobin alternated with maneb and pyraclostrobin + boscalid alternated with maneb significantly reduced the anthracnose incidence in bell pepper as compared to control. Best disease control with highest yields and fruit quality was reported in combination product of pyraclostrobin+metiram effective against both early blight and late blight has reported by Capriotti *et al.* (2005).

Kamal *et al* (2007) found that *Alternaria* blight and *Alternaria* fruit rot of tomato were lower when foliar spray was done with Indofil M-45, with disease incidence of 1.7 per cent and 4.0 per cent respectively. Indofil M-45 was found as the most effective fungicide for reducing the early blight disease of bottle gourd followed by Indofil Z-78, Kavach, Blue copper-50, Bavistin and Ziram.

Kumar *et al.* (2007) reported that Hexaconazole (0.05 %) and Azoxystrobin (0.2 %) were very effective in managing early blight of tomato. In fields, more recent report (McDonald *et al.*, 2007) showed efficacy against *A. solani* of other active ingredients belonging to the same family of Strobilurin (Azoxystrobin, Pyraclostrobin).

Singh (2008) found three sprays of 0.25 per cent Ridomil MZ (*i.e.*, @ 2 kg/ha) at 10 days intervals were most effective in controlling early blight of potato caused by *A. solani* followed by Melody Duo 66.75 at 2.5 kg/ha and 2 kg/ha) and Antracol. Lower dose of Acrobat MZ (2.25 kg/ha) and Dithane M-45 both were equally effective followed by Delan (0.5 kg/ha) and Acrobat MZ (1.79kg/ha). Copper oxychloride (1.5 kg/ha) being the least effective.

Ashour (2009) reported that fungicides were the most efficient in managing the natural infection of the early blight and resulted in producing the highest fruit yield compared with antioxidants as well as the alternation between them.

Earlier workers reported application of fungicides is the most effective method of *Alternaria* blight control and found that Tetra methyl Thiram Disulphide (TMTD), Dithane M-45, Bavistin, Dithane Z-78, Difoltan, Blitox, Captafol and Bordeaux mixture effectively manage the disease fungicides (Verma and Verma, 2010).

Mancozeb as effective fungicide for the management of early blight and maximum fruit yield was reported by several workers (Maheshwari *et al.*, 1991; Choulwar and Datar, 1992 and Singh *et al.*, 2001).

Archana and Jamadar (2014) reported that Propiconazole at 0.1 per cent recorded significantly the least disease incidence in early blight of tomato (PDI-4.37 %) followed by Thiophanate methyl and Hexaconazole recording 11.70 and 14.47 per cent disease index respectively. Carbendazim showed the least efficacy with 52.43 and 23.33 per cent disease index at concentrations *viz.*, 0.05 and 0.1 per cent respectively. Propiconazole recorded significantly the highest yield (36.15 ton/ha) followed by Thiophanate methyl (31.70 ton/ha), Azoxystrobin (31.31 ton/ha), Hexaconazole (29.65 ton/ha), Difenconazole (23.80 ton/ha) and Carbendazime (22.51 ton/ha), while the least fruit yield of (20.70 ton/ha) was recorded in untreated control.

The field experiments were conducted to control *Alternaria* leaf spot disease in Bt cotton by Propiconazole (0.1 %) at 35, 55, 75, 95 and 115 days after sowing and recorded significantly lowest *Alternaria* leaf spot per cent disease index (3.78 PDI). Similarly the significantly maximum yield of 2894.5 kg/ha was recorded in Propiconazole (0.1 %) at 55, 75, 95 and 115 DAS which was on par with Propiconazole (0.1 %) at 55, 75, 95 and 115 DAS (2802.72 kg/ha.) and Propiconazole (0.1 %) at 35, 55, 75, 95 and 115 DAS (2691.23 kg/ha.), respectively. The maximum percent avoidable yield loss was recorded in Propiconazole (0.1 %) at 35, 55, 75, 95 and 115 DAS (32.38 %) (Hosagoudar *et al.*, 2014).

### III MATERIAL AND METHODS

The experiments pertaining to studies on early blight of potato caused by *Alternaria solani* were carried out during the period of 2014-2015. Laboratory and experiments were carried out in the Department of Plant Pathology, University of Agricultural Science, GKVK Bengaluru-65. Field experiment was conducted at ZARS, GKVK, Bengaluru-65, in the sandy loam soil with neutral pH. The details of experiment and methodology adopted for the present investigation are briefly described below.

#### 3.1 General laboratory procedure

##### 3.1.1 Glassware cleaning

For all the laboratory experimental studies, Corning and Borosil glass wares were used. The glass wares were kept in cleaning solution containing 60.0 g of potassium dichromate ( $K_2Cr_2O_7$ ), 60.0 ml of concentrated sulphuric acid ( $H_2SO_4$ ) in 1000 ml of water for 24 h. They were washed with vim powder followed by thorough washing in running tap water and then rinsed in distilled water before use.

##### 3.1.2 Sterilization

All glass wares, solid and liquid media were subjected to sterilization by autoclaving at  $1.1 \text{ kg/cm}^2$  ( $121.6^\circ\text{C}$ ) for 15 min. The seeds and plant tissues were surface sterilized in 1 per cent sodium hypochlorite solution for one minute followed by three changes in sterile water. All cultural studies were conducted under aseptic conditions in laminar flow. The tip of inoculation needle and forceps were sterilized over flame.

#### 3.2 Preparation of media

##### 3.2.1. Potato dextrose agar medium

For all the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for culturing the *Alternaria solani*

The composition of PDA used is given below.

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

Two hundred grams of peeled potatoes were cut into pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. Each of 20 g of dextrose and agar-agar were dissolved in potato extract and the final volume was made up to 1000 ml by adding distilled water. A known quantity of such medium was dispensed into number of conical flasks and plugged with non-absorbent cotton and finally wrapped with paper. The flasks containing dispensed medium were sterilized at  $1.1 \text{ kg cm}^{-2}$  pressure for 20 min.

### **3.3 Isolation and identification of the pathogen**

*Alternaria solani* infected leaves of Potato were collected from infected field and isolated by following standard tissue isolation method.

#### **3.3.1 Isolation of the fungi**

The part of leaf with concentric rings region showing typical symptoms of the early blight disease infected parts were cut into small pieces. These pieces were surface sterilized with 0.1 per cent Mercuric chloride solution for one minute. Such pieces were washed thoroughly in sterile distilled water thrice to remove traces of sodium hypochlorite solution, if any and then aseptically transferred to sterilized potato dextrose agar (PDA) plates for *Alternaria solani*. They were incubated at  $27\pm 1^\circ\text{C}$  for three days to facilitate growth of the fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by following hyphal tip culture technique under aseptic conditions.

#### **3.3.2 Maintenance of pure cultures**

The isolated fungus was sub-cultured on PDA slants and allowed to grow at  $27\pm 1^\circ\text{C}$  temperature for ten days. The cultures so obtained were stored in a refrigerator at  $4^\circ\text{C}$  and they were cultured periodically once in a month.

#### **3.3.3 Soil sterilization**

For testing the effect of new molecules, bio agents and leaf extract on growth parameters and yield of early blight of potato under glass house condition. The soil was sterilized by using formaldehyde to avoid contamination through soil.

A raised soil bed was made and watered the soil up to saturation level and left undisturbed for two days. After two days the soil was moistened by 4 per cent formaldehyde solution (40 ml formaldehyde/lit. of water) up to saturation level then covered by polythene sheet and kept undisturbed for five days. Polythene sheet was removed after five days and soil was exposed to open environment for seven days to remove the traces of formaldehyde present in the soil. This soil was filled to the disinfected pots to carry out further studies.

### **3.4 Cultural and physiological study of early blight potato caused by *A.solani***

#### **3.4.1 Cultural studies**

##### **3.4.1.1 Growth on solid and liquid media.**

*A. solani* was grown on twelve different media (solid and liquid) to study its growth and colony characters. Liquid medium was made in flask without agar.

### **Solid media**

1. Potato dextrose Agar
2. Malt extract agar
3. Czapek's agar
4. Oat meal agar
5. Sabouraud's agar
6. Potato carrot extract agar
7. Rose Bengal agar.

### **Liquid media**

1. Potato Dextrose Broth
2. Malt extract Broth
3. Czapek's Broth
4. Oat meal Broth
5. Sabouraud's Broth
6. Potato carrot extracts Broth
7. Rose Bengal Broth

The composition and preparation of the above mentioned synthetic and non-synthetic/semi- synthetic media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawksworth *et al.* (1983). The composition and preparation of the media are given below.

#### **1. Potato Dextrose Agar (PDA)**

In most of the experimental studies PDA medium was used .The composition of PDA is as follows

Peeled potato	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000 ml (volume to make up)

Two hundred g of peeled potato was cut into small bits and boiled in distilled water and extract was collected by filtering through muslin cloth. Dextrose 20 g and Agar- Agar powder 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Later, it was sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min and preserved for future use.

### 1. Malt extract agar

Malt Extract	20.00 g
Agar - agar	20.00 g
Distilled water	1000 ml (volume to make up)

All the ingredients were dissolved in 400 ml distilled water and agar was dissolved separately in 500 ml of distilled water and mixed with the above solution and the volume was made up to one liter. The medium was sterilized at 1.1-kg/cm<sup>2</sup> pressure for 15 min.

### 2. Czapek's agar

Sucrose	30.00 g
Sodium nitrate (NaNO <sub>3</sub> )	2.00 g
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.00 g
Magnesium sulphate (MgSO <sub>4</sub> 7H <sub>2</sub> O)	0.50 g
Ferric chloride (FeCl <sub>3</sub> 6H <sub>2</sub> O)	0.01 g
Potassium chloride (Kcl)	0.50 g
Agar - agar	20.00 g
Distilled water	1000 ml (volume to make up)

Agar - agar was melted in 500 ml distilled water. The other ingredients were dissolved in remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter. The medium was sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min.

### 3. Oat meal agar

Oat meal powder	40.00 g
Agar - agar	20.00 g
Distilled water	1000 ml (volume to make up)

The oat meal powder was dissolved in 500 ml of distil water and Agar Agar was melted in 500 ml of distilled water separately. Both the solutions were mixed thoroughly. Then the volume was made up to one liter and sterilized.

### 4. Sabouraud's agar (SA)

Dextrose	40.0 g
Neo peptone	10.0 g
Agar agar	20.0 g
Distilled water	1000 ml.

All the ingredients one by one were dissolved in 400 ml distilled water. Agar was dissolved separately in 500 ml distilled water and mixed with the above solution to make up the volume was made to one liter before sterilization.

### **5. Potato carrot agar**

Carrot	200 g
Potato	250.00 g
Agar-agar	15.00 g
Distilled water	1000 ml

Carrots were boiled for 10-15 min in 400 ml distilled water; the extract was squeezed and filtered through muslin cloth. The extract was collected in a beaker, remaining ingredients were added to same and volume was made to one liter before sterilization.

### **6. Rose Bengal agar**

Papaic digest of soyabean meal	5.000 g
Dextrose	10.000 g
Monopotassium phosphate	1.000 g
Magnesium sulphate	0.500 g
Rose Bengal	0.050 g
Agar	15.000 g
Distilled water	1000 ml

All the ingredients one by one were dissolved in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution to make up the volume to one liter before sterilization.

Twenty ml of each solid media was poured into 90 mm diameter Petri-plates of 3replications were maintained. Inoculation was made by transferring half a cm disc of mycelium taken from periphery of seven days old culture on different media. The plates were incubated at  $27\pm 1^{\circ}\text{C}$  for five days. For each different solid media, differences in colony colour, type of growth, type of margin, radial growth were recorded on different media.

### **3.3 Effect of pH levels on growth of *A. solani***

The growth of *A. solani* was tested at thirteen pH levels viz., 4; 4.5; 5; 5.5; 6; 6.5; 7; 7.5; 8; 8.5; 9; 9.5 and 10. Fifty ml of sterilized Potato dextrose broth (PDB) was poured into the sterile 100 ml flask. Then the flasks were inoculated with the 0.5 cm mycelial discs of *A. solani*. Each treatment with three replications was maintained. The flasks were incubated for seven days at room temperature. After the incubation period,

fungus was filtered through whatmanNo. 42 filter paper of 9 cm diameter and kept in hot air oven at 60°C. The mycelial weight (g) was recorded.

### 3.4 Physiological studies

#### 3.4.1 Effect of temperature levels on growth of *A. solani*

Twenty ml of sterilized PDA was poured into the sterile petriplates aseptically and allowed to solidify. Then the plates were inoculated with the 0.5cm mycelial discs of *A.solani*. The plates were incubated for seven days in incubators at different temperature of 15- 40 °C. Each treatment with four replications was maintained. After the incubation period, the mycelial growth (mm) was recorded.

### 3.4 *In vitro* evaluation of new molecules of fungicides, bio agents and plant extracts against of potato caused by *A.solani*.

#### 3.4.1 *In vitro* evaluation of fungicides.

Both non-systemic and systemic fungicides viz., Chlorothalonil, Copper oxy chloride, Mancozeb, Tebuconazole, Tebuconazole + Trifloxystrobin, Hexaconazole+ Zineb,, Metalaxyl, Fenamidone +Mancozeb were tested in laboratory against *A.solani* at different concentration viz., 100,250,500,1000 ppm. Each treatment was replicated thrice in Completely Randomized Design (CRD). Potato dextrose agar was used as nutrient medium for evaluation of fungicide against *A.solani* in *invitro*. The fungicide was thoroughly mixed to medium by proper stirring and then poured to petriplates and allowed for solidification. The actively growing periphery of the nine day old culture of *A. solani* was carefully removed using a disc cutter and transferred aseptically to the center of each Petridish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without adding any fungicides. The plates were incubated at 27±1°C for nine days and the colony diameter was recorded at regular interval. Per cent inhibition of mycelial growth was calculated using the formula of Vincent (1947):

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

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

#### 3.4.2 Evaluation of bio agent's against *A. solani*.

Antagonistic organisms viz., *T.viride*-GKVK1, *T.viride*-GKVK2, *T.viride*-GKVK3, *T.viride*-NBAIR, *T.harzianum*-NBAIR, *T.harzianum*-2-IIHR, *T.viride*-13-IIHR, *T.viride*-14-IIHR, *T.viride*-16-IIHR, *T.harzianum*-19-IIHR, *T.harzianum*-20-IIHR, *T.viride*-21-IIHR, *T.viride*-22-IIHR, *T.harzianum*-41-IIHR, *T.viride*-52-IIHR, *T.viride*-55-IIHR, *T.harzianum*-58-IIHR, *B. subtilis*-NBAIR and *P. fluorescens*-NBAIR were evaluated in laboratory condition by dual culture technique. Interactions between *A. solani* with antagonistic organisms *in-vitro* were used to study their interaction with

**Table 1: List of fungicides used for *in vitro* and field evaluation against *A. solani***

Sl. No.	Common name	Chemical name	Trade name	Active Ingredient	Source
1.	Hexaconazole+ Zineb	Hexaconazole (4 %) + Zineb (68 %)	Avatar	4 + 68 WP	Indofil, Industries Limited, Mumbai
2.	Captan	N-trichloromethyl-thio-4-cyclohexene-1,2 dicarboxamide	Captan	75 WP	Agro Life Science Corporation, New Delhi.
3.	Chlorothalonil	Tetrachloroisophthalo nitrite	Kavach	75 WP	Syngenta India Ltd. 14, J. Tata Road, Mumbai.
4.	Mancozeb	Zinc-manganese ethylene bis-dithiocarbamate	Indofil	75 WP	Indofil, Industries Limited, Mumbai.
5.	Tebuconazole	-1- <i>p</i> -chlorophenyl)-4,4-dimethyl-3-(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)pentan-3-ol	Folicur	25 EC	Bayer crop science limited, Mumbai.
6.	Tebuconazole+ Trifloxystrobin	Tebuconazole +methyl (aE)-(a- ethoxyimino)-2-[[[( <i>E</i> )-[1-[3-(trifluoromethyl) phenyl]ethylidene]amino]oxy]methyl] benzeneacetate	Nativo	50 +25 WG	Bayer crop science limited, Mumbai.
7.	Metalaxyl+ Mancozeb	Metalaxyl (4 %)+Mancozeb (64 %)	Ridomil gold	4+64 WP	Syngenta India Ltd. 14, J. Tata Road, Mumbai.
8.	Fenamidone+ Mancozeb	Fenamidone (10 %) +Mancozeb (50 %)	Sectin	60WG	Bayer crop science limited, Mumbai-400 076

*A. solani*. Twenty ml of PDA will be poured into 90 mm diameter petridishes and allowed to solidify. 5 mm discs of *A. solani* taken from 9 days old culture was placed at one end of petridish and respective antagonistic organisms were inoculated at the opposite side. A control will be maintained by inoculating only *A. solani* at one end in case of fungal antagonist. In case of bacterial antagonist *A. solani* will be placed at one of the corner of the medium and the then antagonist streaked in opposite direction. Control will be maintained by inoculating *A. solani* at both the ends of Petri plate. Each treatment will be replicated thrice and incubated for 6 days at  $27\pm 1^{\circ}\text{C}$ . The activity of antagonistic organisms will be recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control. List of Bio agents presented in Table 2.

**Table 2: List of bio agents used for *in vitro* evaluation against *Alternaria solani***

Sl. No.	Bioagents	Isolates	Source of Bioagents
1.	<i>Trichoderma harzianum</i>	Th-2	IIHR, Hesaragatta, Bengaluru.
2.	<i>Trichoderma viride</i>	Tv-13	IIHR, Hesaragatta, Bengaluru.
3.	<i>Trichoderma viride</i>	Tv-14	IIHR, Hesaragatta, Bengaluru.
4.	<i>Trichoderma viride</i>	Tv-16	IIHR, Hesaragatta, Bengaluru.
5.	<i>Trichoderma harzianum</i>	Th-19	IIHR, Hesaragatta, Bengaluru.
6.	<i>Trichoderma harzianum</i>	Th-20	IIHR, Hesaragatta, Bengaluru.
7.	<i>Trichoderma viride</i>	Tv-21	IIHR, Hesaragatta, Bengaluru.
8.	<i>Trichoderma viride</i>	Tv-22	IIHR, Hesaragatta, Bengaluru.
9.	<i>Trichoderma harzianum</i>	Th-41	IIHR, Hesaragatta, Bengaluru.
10.	<i>Trichoderma viride</i>	Tv-52	IIHR, Hesaragatta, Bengaluru.
11.	<i>Trichoderma harzianum</i>	Th-55	IIHR, Hesaragatta, Bengaluru.
12.	<i>Trichoderma harzianum</i>	Th-58	IIHR, Hesaragatta, Bengaluru.
13.	<i>Trichoderma viride</i>	Tv-AI	GKVK, Hebbal, Bengaluru.
14.	<i>Trichoderma viride</i>	Tv-GKVKB-1	GKVK, Bengaluru.
15.	<i>Trichoderma viride</i>	Tv-GKVKB-2	GKVK, Bengaluru.
16.	<i>Trichoderma viride</i>	Tv-NBAIR	NBAIR, Hebbal, Bengaluru.
17.	<i>Trichoderma harzianum</i>	Th-NBAIR	NBAIR, Hebbal, Bengaluru.
18.	<i>Bacillus subtilis</i>	B.s	IIHR, Hesaragatta, Bengaluru.
19.	<i>Pseudomonas fluorescens</i>	P.f	IIHR, Hesaragatta, Bengaluru.

### 3.4.3 Evaluation of plant extracts against *A. solani* in vitro

#### 3.4.3.1 Preparation of plant extract

Nine plants viz., Calotropis, Lantana, Lemon grass, Neem, Simarouba, Tulsi, Nagadhale, Pongamia, Subabul and Sea weed were selected for the study.

Mature fresh leaves were collected from various locations of UAS, GKVK Bengaluru and confirmed their taxonomical identification. The leaves were washed with running tap water and finally rinsed with distilled water. The washed leaves were blotted with filter paper. Twenty grams of the leaf material was ground well with mortar and pestle. Then water is added in the ratio of 1:2 (weight by volume) and strained through muslin. List of plant extracts presented in Table 3.

**Table 3: List of plant extracts evaluated against *Alternaria solani* using poison food technique**

Sl. No.	Botanical name	Common name	Family
1	<i>Azadirachta indica</i>	Neem	Meliaceae
2	<i>Pongamia pinnata</i>	Hongae tree	Leguminaceae
3	<i>Leucaena leucocephala</i>	Subabul	Fabaceae
4	<i>Calotropis gigantean</i>	Calotropis	Apocynaceae
5	<i>Lantana camera</i>	Lantana	Verbenaceae
6	<i>Cymbopogon citratus</i>	Lemon grass	Poaceae
7	<i>Simarouba glauca</i>	Simarouba	Simaroubaceae
8	<i>Ruta graveolens</i>	Nagadhale	Rutaceae
9.	<i>Ascophyllum sps</i>	Sea weed	Fucaceae

#### 3.4.3.2 In vitro evaluation

The poisoned food technique was followed to evaluate the efficacy of plant extracts. Different plant extracts were tested in laboratory against *A. solani* at 1:1, 1:2, 1:3 dilution concentrations with three replications. Potato dextrose agar was used as a medium for evaluation of leaf extract against *A. solani* in vitro. The plant extracts were thoroughly mixed to the medium by proper stirring and then poured to petriplates and allowed for solidification. From nine day old culture of *A. solani*, mycelium from the periphery was carefully removed by using a disc cutter and transferred aseptically to the center of each Petridish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without adding any plant extracts. The plates were incubated at 27±1 °C for nine days and the colony diameter was recorded at regular interval Per cent inhibition of mycelial growth was calculated using the formula of Vincent (1947):

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

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

### **3.5 Evaluation of fungicides, bio agents and sea weed extract on growth and yield of potato affected by early blight disease under glass house condition**

Evaluation the effective fungicides, bio agents and sea weed extract on growth and yield parameters of potato affected by early blight disease in glass house conditions the following procedures was carried out in Plant Pathology glass house, UAS, GKVK, Bengaluru-65. The tubers were planted in sterilized soil as three transplants per pot and six treatments with four replications. Foliar spraying with tested bio-agents, fungicide and sea weed extracts applied once in a week.

#### **3.5.1 Soil sterilization**

The soil was sterilized by using formaldehyde by following procedure. Raised soil bed was made and watered the soil up to saturation level and left undisturbed for two days. After two days the soil was moistened by 4 per cent formaldehyde solution up to saturation level then covered by polythene sheet and kept undisturbed for five days. Polythene sheet was removed after five days and soil was exposed to open environment for seven days to remove the traces of formaldehyde present in the soil. These soils were filled to the disinfected pots to carry out further studies. The sterilized soil, sand and FYM were mixed in 1:1:0.5 proportion (w/w basis) and filled in disinfected earthen pots

#### **3.5.2 Plant material:**

Tubers were planted in plastic pots 30 cm diameter and containing a soil mixture of sand 3 kg/pot and 10 g slow-release fertilizer per kg (N:P:K 12:4:6). All pots were placed in a controlled greenhouse and watered regularly.

#### **3.5.3 Artificial inoculation of *A.solani***

Potato early blight pathogen *A.solani* was sprayed with pathogen suspension ( $2 \times 10^4$  cfu/ml) on potato leaves and covered using polythene bags. Control plants were sprayed with water and incubated under green house condition. The observations on plant height, tuber weight, fresh and dry weight per plant were recorded.

#### **3.5.4 Treatments Details**

T<sub>1</sub>- Sea weed extract @ 10 %

T<sub>2</sub>- Culture filtrate of *T.viride* @ 5 %

T<sub>3</sub>- Culture filtrate of *P. fluorescens* @ 5 %

T<sub>4</sub>- Tebuconazole @ 0.1 %

T<sub>5</sub>- Tebuconazole @ 0.1 % > *T. viride* @ 5 % > *P. fluorescens* @ 5 % > Sea weed extract @ 10 %,

T<sub>6</sub>- Untreated check.

### 3.5 Integrated management of early blight of potato in field at GKVK.

#### 3.5.1 Imposition of treatments

The experiment was conducted field for the management of early blight of potato at GKVK. The experimental layout was laid out with plot size of 2 m×1.5 m with three replication. The planting material of potato tubers cv. Kufri Jyothi was used .The tubers were treated with Metalaxyl +Mancozeb fungicide at the rate of 6g/ kg of tubers for the period of 30 min and then shade dried. The treated tubers were planted with a spacing of 20×30 cm.

The pressure of early blight disease was created by artificial spraying of *A. solani* culture during heavy rain fall period with good relative humidity. The treatment imposed after getting sufficient disease pressure in the field.

**Location** : ZARS, UAS, GKVK, Bengaluru-65.

**Variety** : Kufri Joyth

**Treatments** : 13

**Replication** : 3

**Plot size** : 2m x 1.5 m

**Spacing** : 20cm x 30 cm.

#### 3.5.2 Details of treatments

T<sub>1</sub>- Mancozeb > Avtar> Mancozeb > Avtar> Mancozeb >Avtar@0.2 %

T<sub>2</sub>- Folicur > Metalaxyl> Folicur> Metalaxyl> Folicur@0.1 %>Metalaxyl@0.2 %

T<sub>3</sub>- Nativo > Sectin > Nativo >Sectin >Nativo > Sectin @0.1 %

T<sub>4</sub>- Mancozeb > Mancozeb > Mancozeb > Mancozeb >Mancozeb > Mancozeb@0.2 %

T<sub>5</sub>- Folicur > Folicur > Folicur > Folicur > Folicur > Folicur@0.1 %

T<sub>6</sub>- Nativo > Nativo > Nativo > Nativo > Nativo > Nativo@0.1 %

T<sub>7</sub>- Metalaxyl > Kavach > Metalaxyl > Kavach >Metalaxyl@0.2 %

T<sub>8</sub>- Mancozeb@0.2 %>Avtar@0.2 % > Folicur@0.1 %> Metalaxyl@0.2 % > Nativo@0.1 % > Sectin@0.2 %

T<sub>9</sub>- Folicur@0.1 % >T.viride@ 5 % >P. fluorescens @ 5 % > Sea weed extract@ 10 %

T<sub>10</sub>- Sea weed extract@10 %> Sea weed extract@ 10 % > Sea weed extract@ 10 %> Sea weed extract@ 10 %> Sea weed extract@ 10 %

T<sub>11</sub>- T.viride@ 5 % >T.viride@ 5 % >T.viride@ 5 % >T.viride@ 5 % >T.viride@ 5 % >T.viride@ 5 %

T<sub>12</sub>- P. fluorescens @ 5 % >P. fluorescens @ 5 % > P. fluorescens @ 5 % > P. fluorescens @ 5 % > P. fluorescens @ 5 % > P. fluorescens @ 5 %

T<sub>13</sub>- Untreated check.

The new molecules of fungicides, along with bio agents, sea weed extract sprayed in the field at weekly regular intervals. The fungicides combinations were made totally six sprays were taken with weekly intervals with different combination of fungicides. The observation on severity of early blight disease, growth and yield parameters were recorded during the experimental period. At end of the experiment, the potato plants were uprooted and recorded fresh weight , dry weight and tuber weight per plant in each plot.

### **3.6 STATISTICAL ANALYSIS**

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage was converted to angular transformed values.

## IV EXPERIMENTAL RESULTS

The experiments were carried out on various aspects of early blight of Potato. With respect to cultural and physiological study of *Alternaria solani*. *In vitro* evaluation of new molecules of fungicides, bio agents and plant extracts against *Alternaria solani*, effect of new molecules of fungicide, bio agents and sea weed extract on growth parameters and yield of potato infected by early blight disease under green house condition and integrated management of early blight disease of potato under field condition. The results obtained on these aspects are presented here under.

### 4.1 Cultural and physiological studies of early blight of potato caused by *A. solani*

#### 4.1.1 Growth of *A. solani* on different solid media

The fungus showed slight difference in its growth on different solid media. Maximum radial growth of *A. solani* was measured on Potato Dextrose Agar with mean colony diameter of 78.11 mm followed by Oat meal agar (73.65 mm) and least mean colony diameter of 36.00 mm was observed in Malt extract agar. The optimum growth of the fungus was observed in other media, Potato carrot extract agar (59.64 mm) Malt extract agar (36.00mm) Czapek dox agar (59.26 mm) Rose Bengal agar (52.22 mm) Sabouraud's agar (62.36 mm). The results are presented in Table 4.

**Table 4: Growth of *A. solani* on different solid media**

Sl. No.	Media	Colony diameter (mm)
1.	Potato dextrose agar	78.11
2.	Malt extract agar	36.00
3.	Czapek dox agar	59.26
4.	Oat meal agar	73.65
5.	Sabouraud agar	62.36
6.	Potato carrot extract agar	59.64
7.	Rose Bengal agar	52.22
<b>Mean</b>		<b>61.80</b>
<b>CD @ 1 %</b>		<b>2.38</b>
<b>S.Em±</b>		<b>0.91</b>
<b>CV %</b>		<b>3.02</b>

#### 4.1.2 Growth of *A. solani* on different liquid media

Among the seven different liquid media tested, mean dry mycelial weight of *A. solani* was maximum in Potato dextrose broth (188 mg), followed by Oat meal broth (180 mg), whereas minimum in case of Malt extract broth (136mg). The results are presented in Table 5.

**Table 5: Growth of *A. solani* on different liquid media**

Sl. No.	Culture media	Mean dry mycelial weight (mg)
1.	Potato dextrose broth	188
2.	Malt extract broth	136
3.	Czapek's broth	141
4.	Oat meal broth	180
5.	Sabouraud's dextrose broth	145
6.	Carrot extract broth	173
7.	Rose Bengal broth	151
<b>Mean</b>		<b>159</b>
<b>SEm±</b>		<b>0.02</b>
<b>CD at 1 %</b>		<b>0.06</b>
<b>CV (%)</b>		<b>2.96</b>

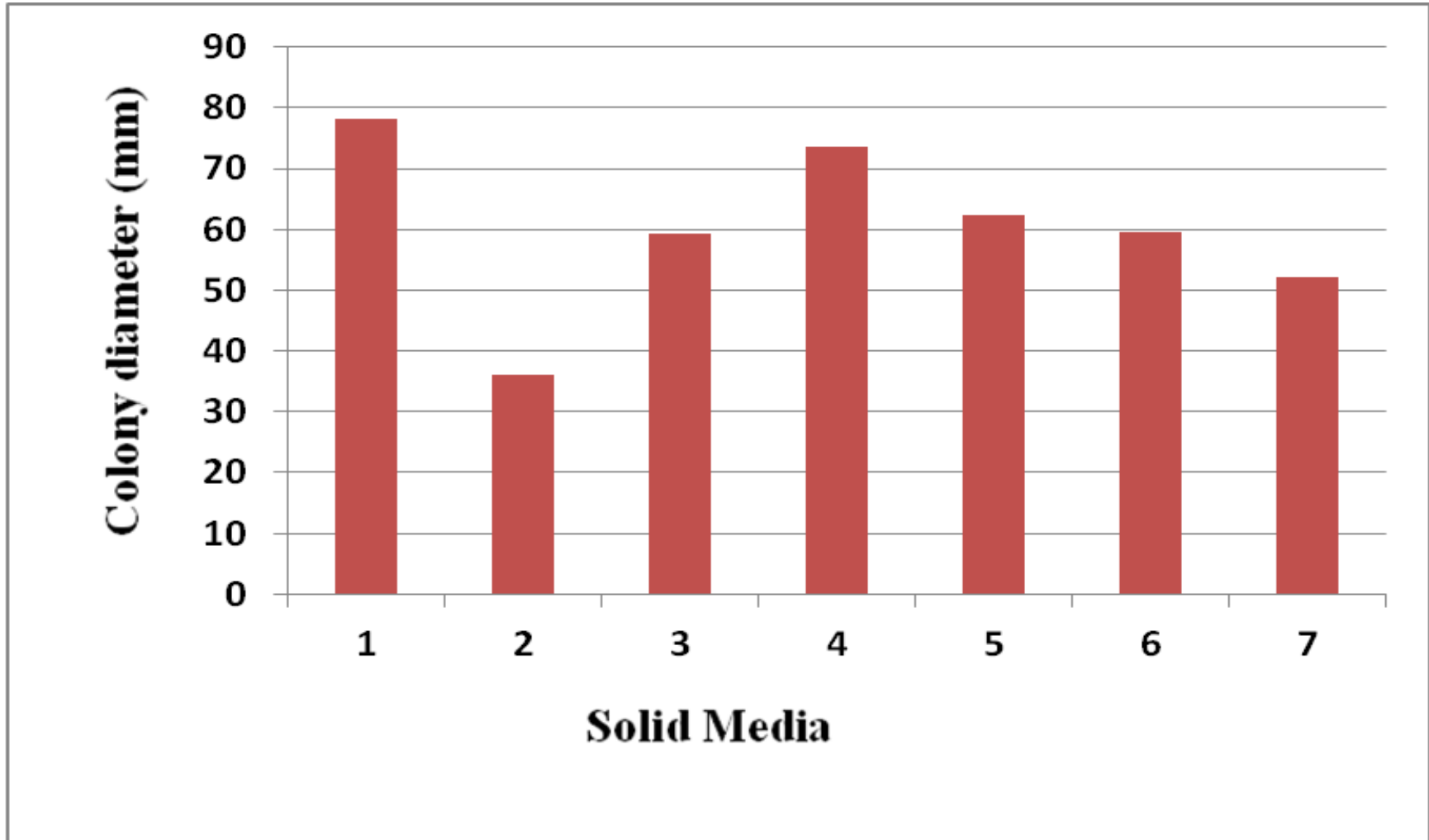
## 4.2 Physiological studies

### 4.2.1 Influence of temperature on growth of *A. solani*

This experiment was conducted to study the effect of temperature on growth at different temperature and results are presented in the Table 5. The maximum mycelial growth of *A. solani* was observed at 30 °C, with a growth of 73.83 mm, followed by 35 °C and the growth of the fungus in this temperature was 65.67 mm. The least fungal growth of the 25 mm was observed at 15 °C. The fungus grew well at 30 °C. The results are presented in Table 6.

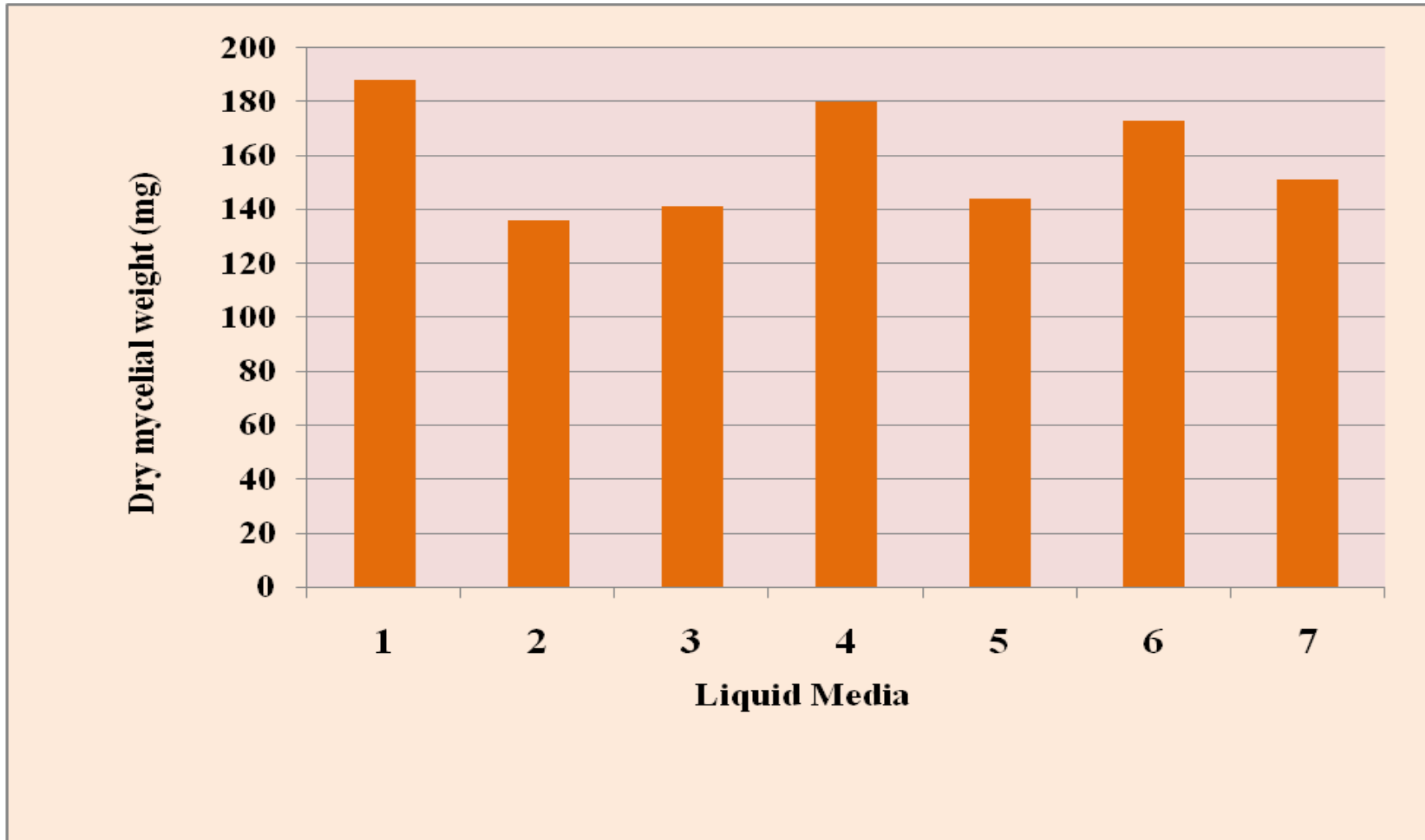
**Table 6: Influence of different temperature on growth of *A. solani***

Sl. No.	Temperature	Mycelium growth (mm)
1.	15 °C	25.00
2.	20 °C	45.50
3.	25 °C	64.00
4.	30 °C	73.83
5.	35 °C	65.67
6.	40 °C	43.67
<b>Mean</b>		<b>48.26</b>
<b>CD @ 1 %</b>		<b>3.56</b>
<b>S.Em±</b>		<b>0.54</b>
<b>CV %</b>		<b>3.60</b>



1. Potato dextrose agar 2. Malt extract agar 3. Czapek's agar 4. Oat meal agar 5. Sabouraud's agar 6. Potato Carrot extract Agar 7. Rose Bengal agar

**Fig. 1: Growth of *A. solani* on different culture media**



1. Potato dextrose agar 2. Malt extract agar 3. Czapek's agar 4 Oat meal agar 5. Sabouraud's agar 6. Potato Carrot extract Agar 7. Rose Bengal agar

**Fig. 2 : Growth of *A. solani* on different liquid media**

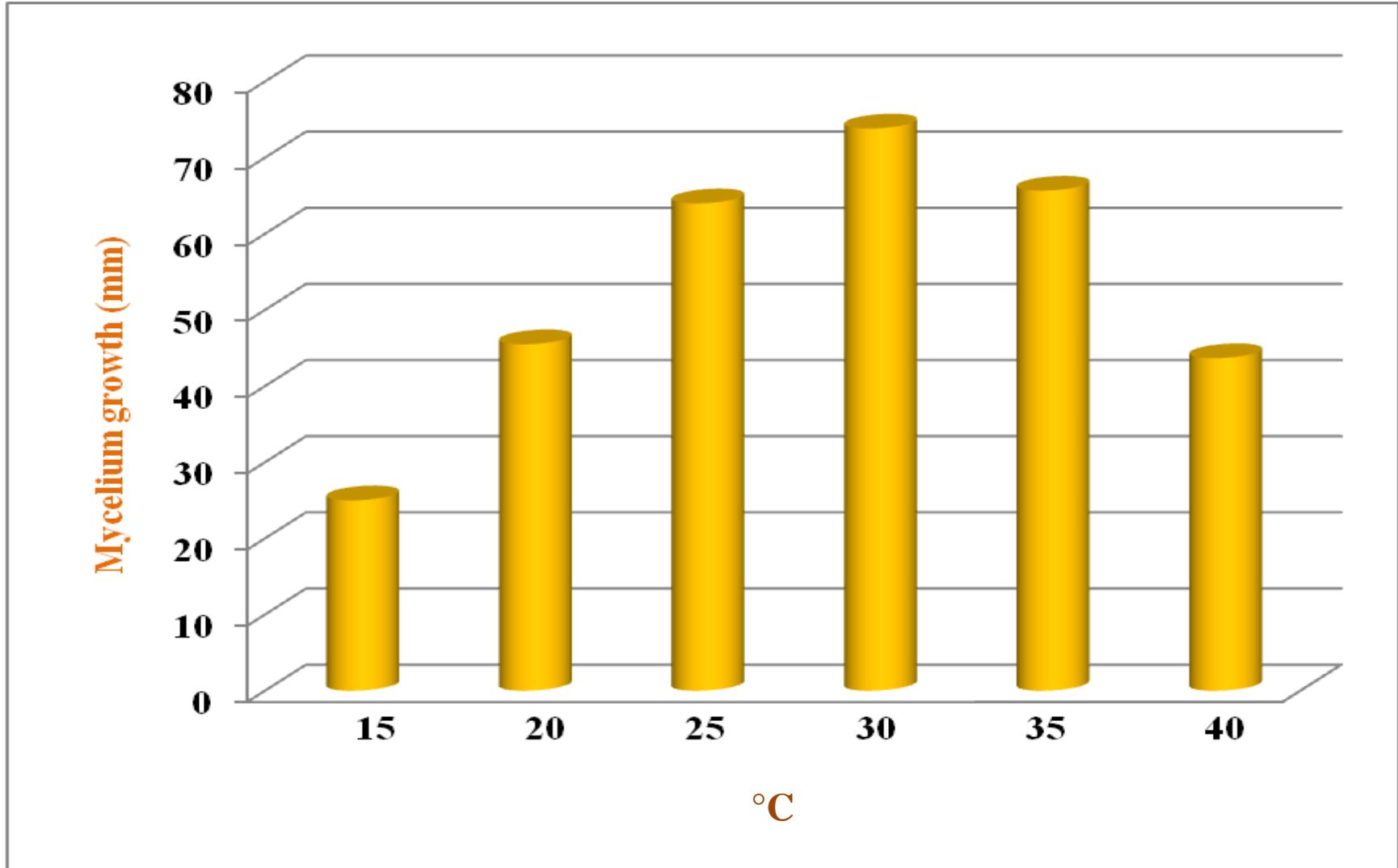


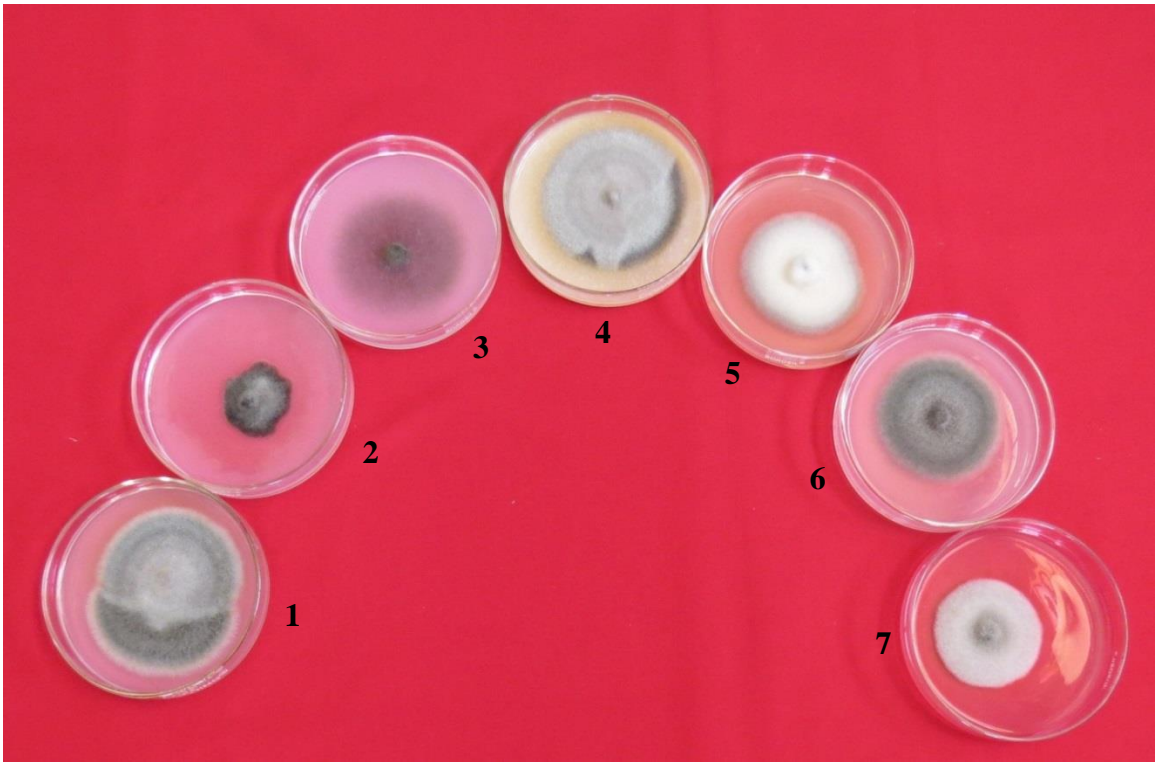
Fig. 3: Effect of different temperature on growth of *A. solani*



**Plate 1: Colony of *Alternaria solani***

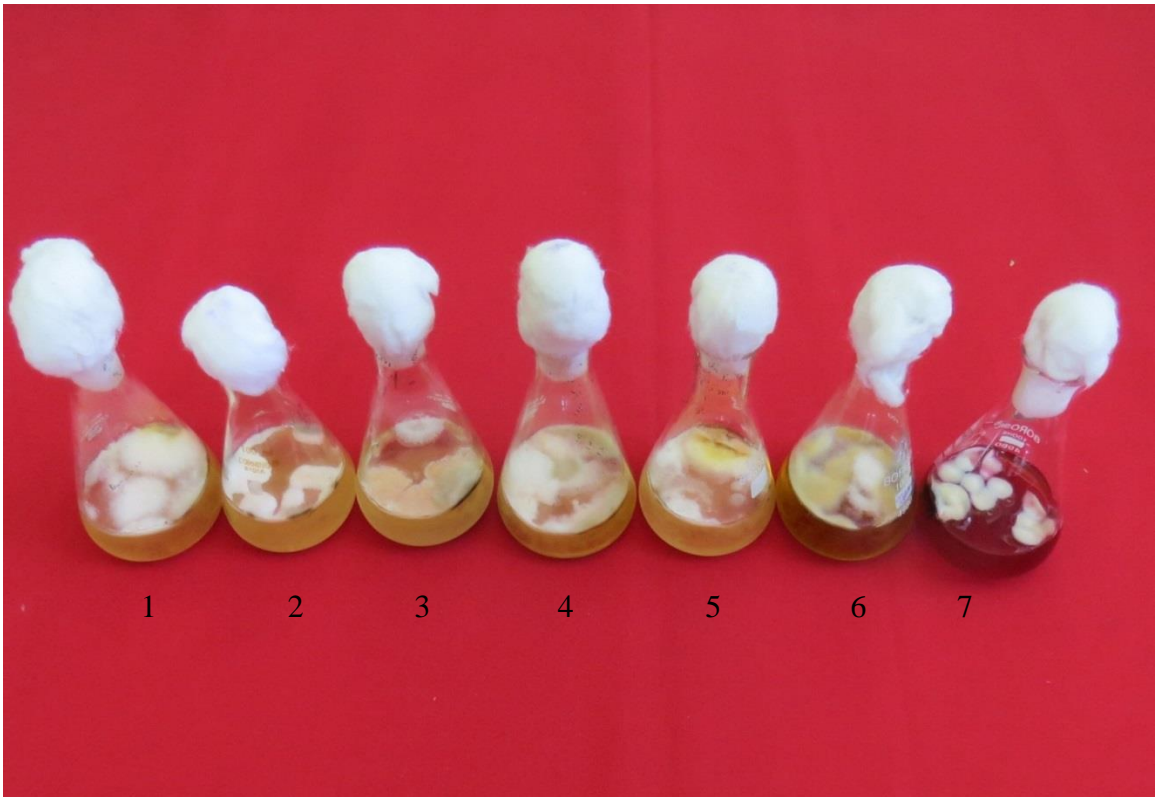


**Plate 2: Microphotographs of conidia of *Alternaria solani***



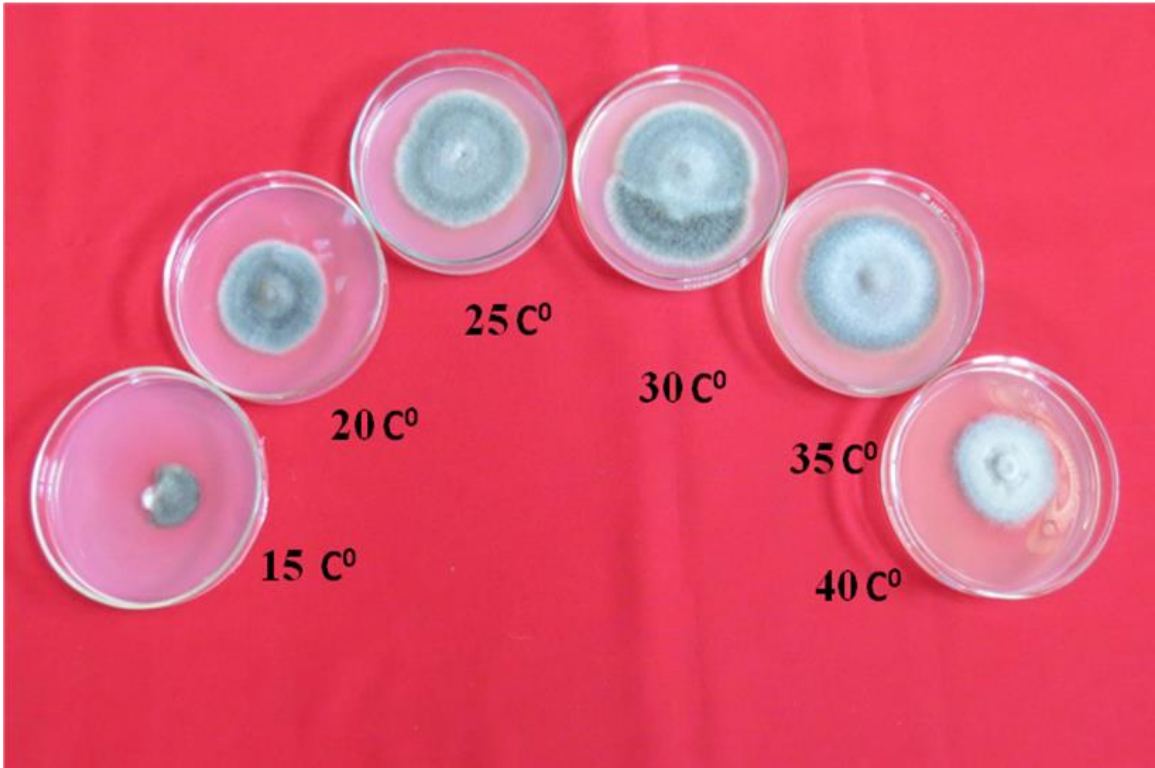
1. Potato Dextrose Agar 2. Malt extract agar 3. Czapek's agar 4. Oat meal agar  
5. Sabouraud's agar 6. Potato carrot extract agar 7. Rose Bengal agar

**Plate 3: Growth of *Alternaria solani* on different solid media**



1.Potato Dextrose broth 2. Malt extract broth 3.Czapek's broth 4.Oat meal broth  
5. Sabouraud's broth 6. Potato carrot extract broth 7. Rose Bengal broth

**Plate 4: Growth of *Alternaria solani* on different liquid media**



**Plate 5: Effect of different temperature on growth of *A. solani***

#### 4.2.2 Effect of different level of pH on growth of *A. solani*.

This experiment was conducted to study the effect of pH on growth at different temperature.

The maximum mycelial growth of *A. solani* was observed at pH of 6 with a dry matter weight of 157 mg this was followed by the pH 6.5, the dry mater weight of the fungus in this pH was 152 mg and in pH 7 it was 142 mg. The least dry weight of the fungus (120 mg) was observed in pH 4. The fungus grows well at the pH 6. The results are presented in Table 7.

**Table 7: Effect of different levels pH on growth of *A. solani* in Potato dextrose broth**

Sl. No.	pH level	Mean dry mycelial weight (mg)
1	4.0	120
2	4.5	133
3	5.0	138
4	5.5	142
5	6.0	157
6	6.5	152
7	7.0	142
8	7.5	139
9	8.0	133
10	8.5	132
11	9.0	131
12	9.5	129
13	10.0	128
<b>Mean</b>		<b>135</b>
<b>SEm±</b>		<b>0.07</b>
<b>CD at 1 %</b>		<b>0.16</b>
<b>CV (%)</b>		<b>1.82</b>

#### 4.3 *In vitro* studies

##### 4.3.1 *In vitro* evaluation of fungicides

The fungicides were evaluated against *A. solani* in laboratory by adopting Poison food techniques at different concentration. The systemic and non systemic fungicides used in this study were Hexaconazole + Zineb, Tebuconazole, Chlorothalonil, Mancozeb, Trifloxystrobin + Tebuconazole, Metalaxyl + Mancozeb, Fenamidone + Mancozeb at

100, 250, 500 and 1000 ppm concentrations. The data are presented in the Table-4. The radial growth of the mycelium was recorded on 7 days after inoculation.

The maximum radial growth observed in Chlorothalonil was recorded 65.7, 58.67, 46.78 and 39.07 mm at 100, 250, 500 and 1000 ppm concentration respectively. The fungicide Metalaxyl + Mancozeb also found maximum radial growth of 67.85, 58.9, 44.67 and 38.44 mm at 100, 250, 500 and 1000 ppm concentration respectively. The fungicides Tebuconazole, Hexaconazole + Zineb and Trifloxystrobin + Tebuconazole were found to be effective against *A.solani* at all concentration.

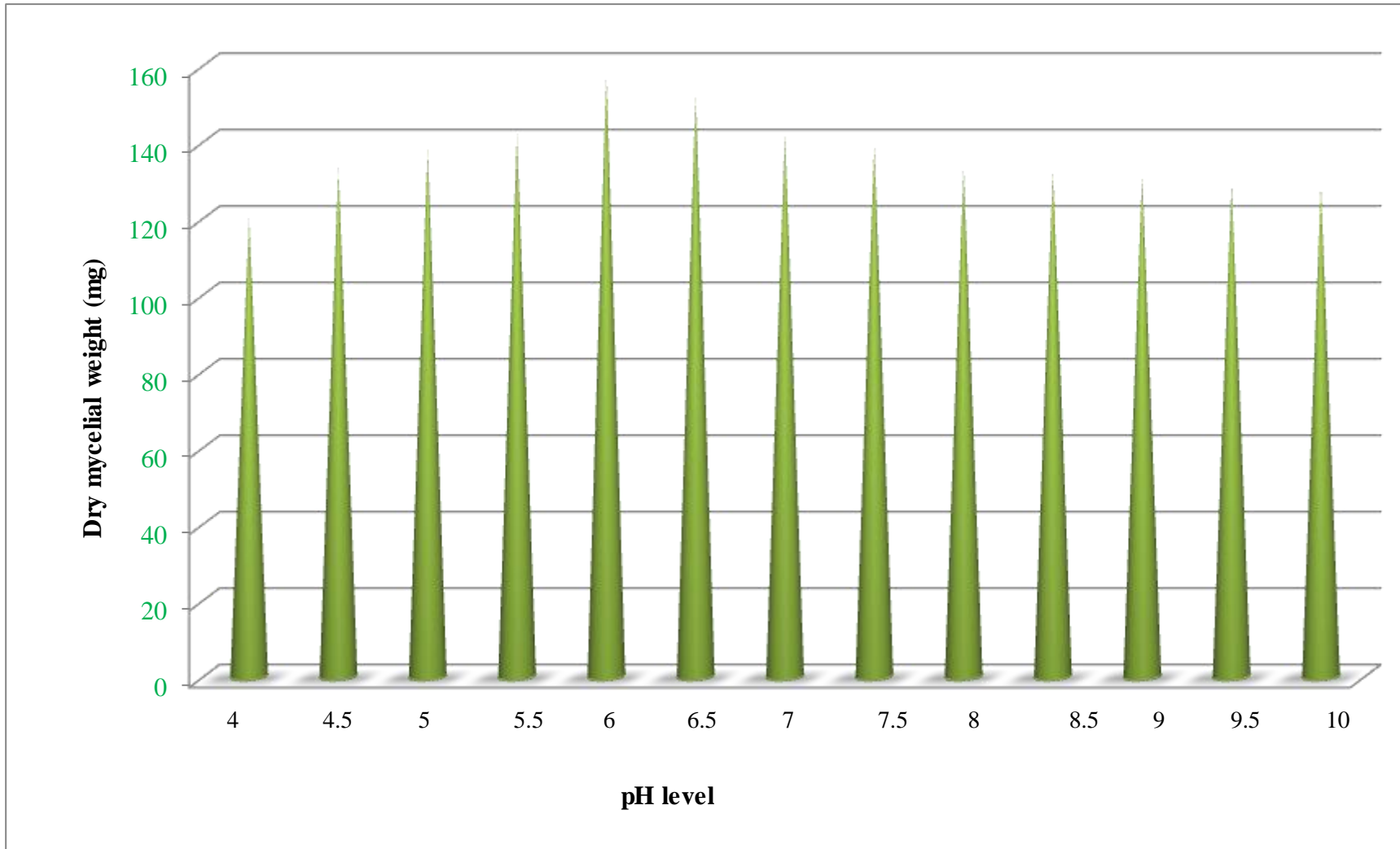
Per cent inhibition of the fungus over control was calculated and found that maximum inhibition of 100 per cent was found in the fungicide Tebuconazole at 100, 250, 500 and 1000 ppm followed by Hexaconazole + Zineb, whereas the maximum inhibition (95.18 %) was obtained at 1000 ppm concentration. The fungicide Trifloxystrobin + Tebuconazole inhibited 85.81 and 88.15 per cent inhibition at 500 and 1000 ppm concentration.

Among the fungicides evaluated *in vitro* at different concentrations, the fungicide Tebuconazole, Hexaconazole + Zineb and Trifloxystrobin + Tebuconazole were effective against *A. solani* . The results are presented in Table 8.

**Table 8: *In vitro* evaluation fungicides against *A.solani* .**

Sl. No.	Fungicides	Per cent inhibition of mycelial growth over control				
		100 ppm	250 ppm	500ppm	1000 ppm	Mean
1.	Metalaxyl + Mancozeb	23.33 (28.85)	35.55 (36.59)	43.33 (41.16)	52.59 (46.48)	38.70 (38.27)
2.	Mancozeb	41.48 (40.08)	42.69 (40.73)	40.74 (41.16)	52.49 (46.48)	44.35 (42.09)
3.	Captan	30.00 (33.19)	37.03 (37.47)	41.85 (40.30)	47.03 (43.29)	38.97 (38.56)
4.	Fenamidone + Mancozeb	50.37 (45.21)	56.67 (48.83)	60.74 (51.25)	67.03 (54.98)	58.70 (50.07)
5.	Hexaconazole + Zineb	84.06 (66.74)	88.88 (70.52)*	92.22 (73.91)	95.18 (77.34)	90.08 (72.13)
6.	Chlorothalonil	24.07 (29.36)	34.44 (35.92)	41.48 (40.05)	46.26 (42.86)	36.36 (37.05)
7.	Tebuconazole	100 (89.69)	100 (89.69)	100 (89.69)	100 (89.69)	100 (89.69)
8	Trifloxystrobin + Tebuconazole	72.29 (60.87)	81.85 (64.81)	85.81 (67.38)	88.15 (69.89)	82.02 (65.74)
<b>Mean</b>		53.20 (49.25)	59.76 (53.07)	63.27 (55.61)	68.57 (58.87)	61.14 (54.20)
		<b>Fungicides (F)</b>		<b>Concentrations (C)</b>		<b>F x C</b>
<b>S.Em ±</b>		0.38		0.27		0.69
<b>C.D at 1 %</b>		2.193		1.551		4.387

\*The value in the parenthesis is arc sine transformed



**Fig. 4: Effect of different pH level on growth of *A. solani***

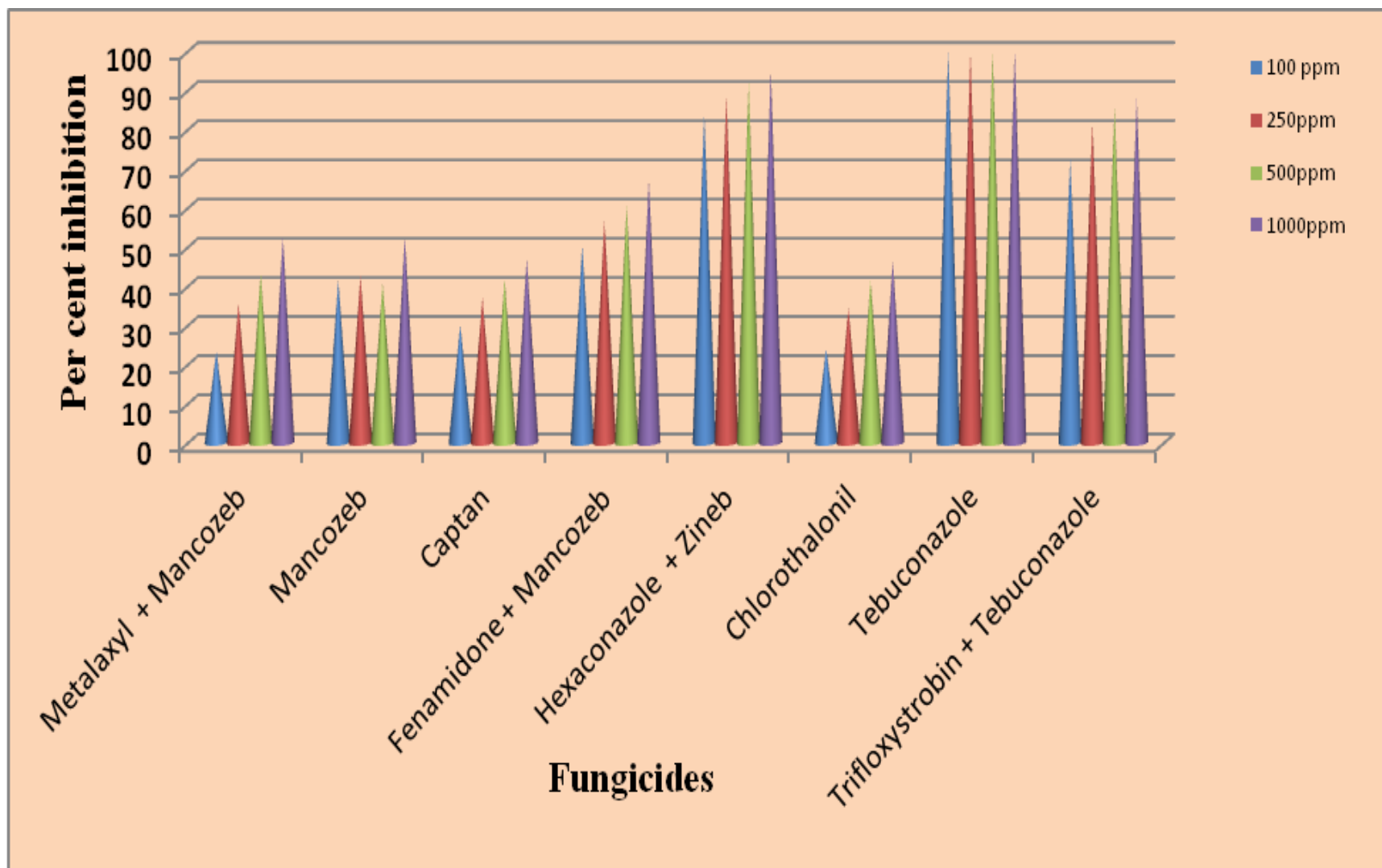
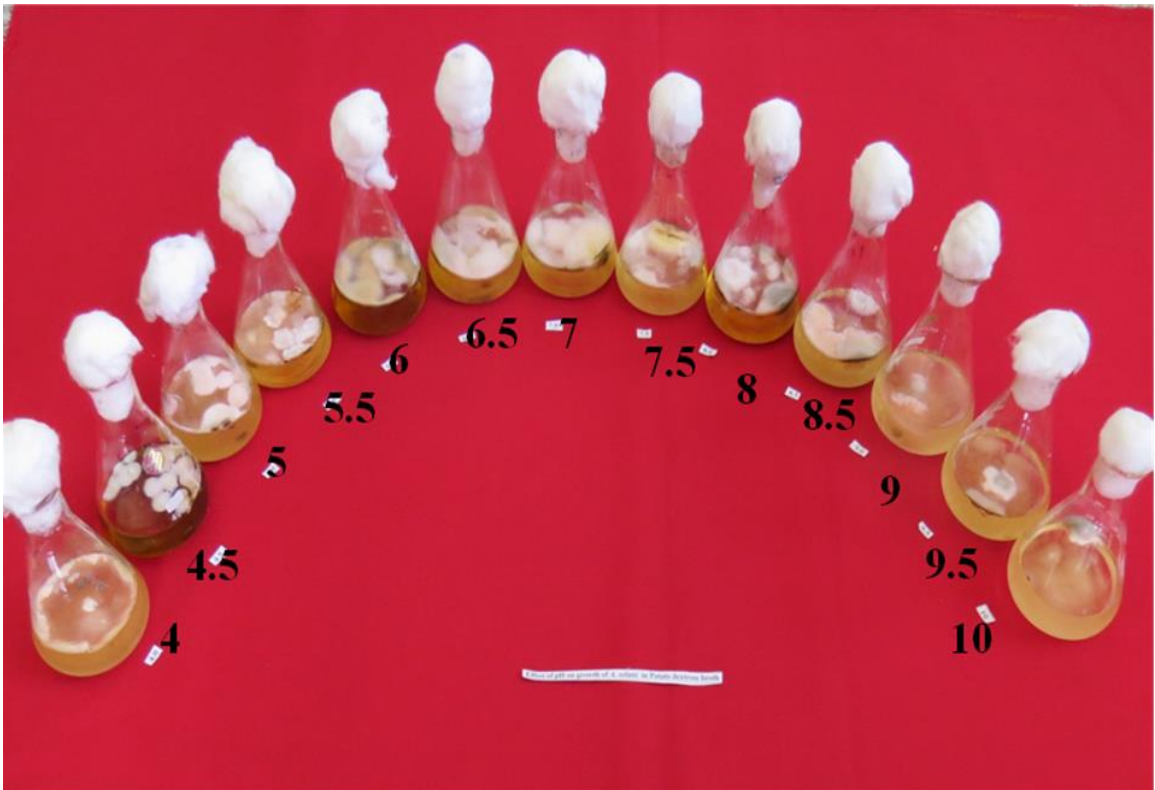
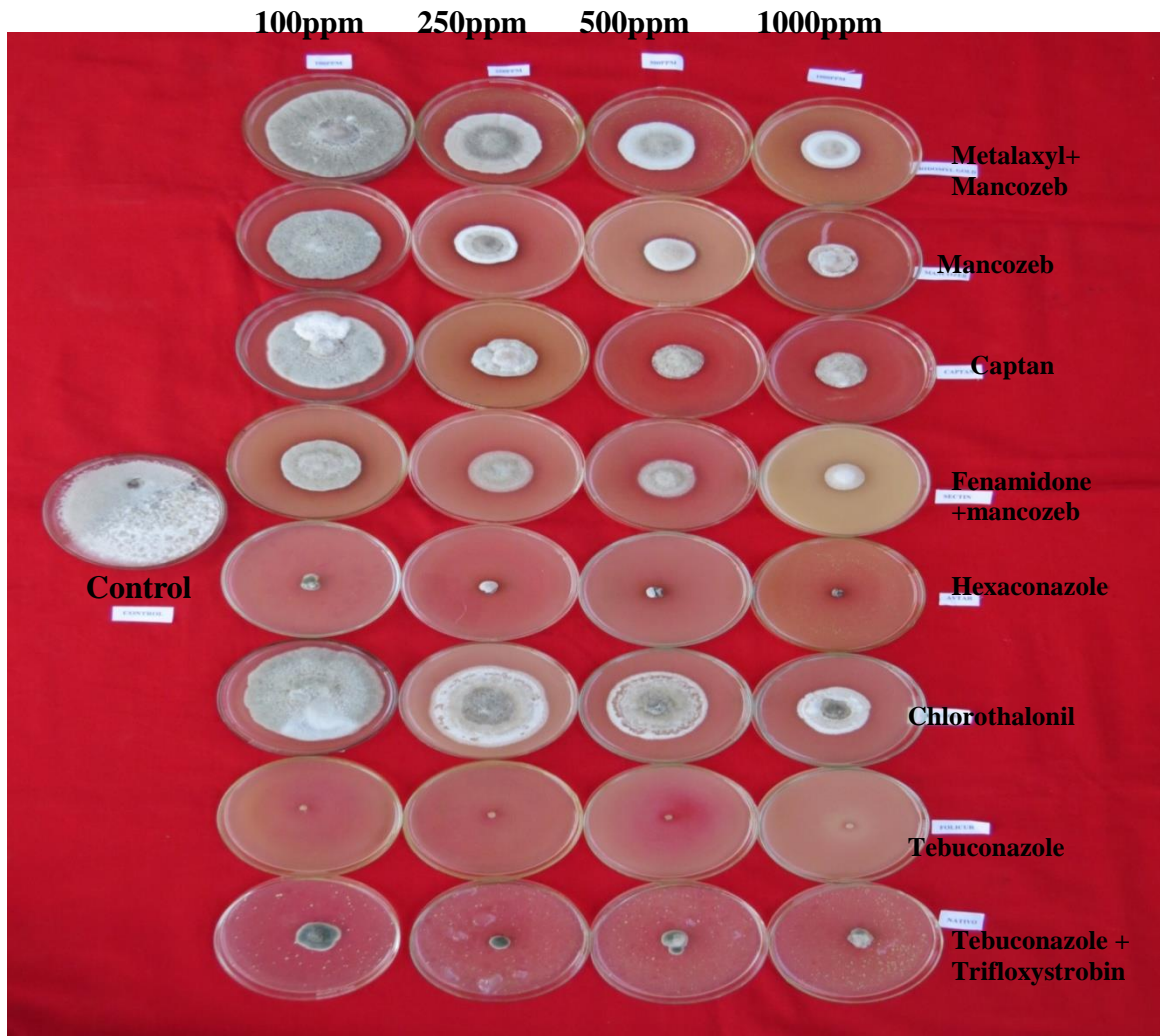


Fig. 5: Per cent inhibition of mycelial growth of *A.solani* in different fungicides (*in vitro*)



**Plate 6: Effect of different pH levels on growth of *A. solani***



**Plate 7: Per cent inhibition of mycelial growth of *A. solani* in different fungicides (*in vitro*)**

#### 4.3.2 *In vitro* studies of bio-agents against *A. solani*

The bio control agents viz., *T. viride* - GKVK1 *T. viride* - GKVK2, *T. viride* - GKVK3, *T. viride* - NBAIR, *T. harzianum* - NBAIR, *T. harzianum* -2- IIHR, *T. viride* - 13 - IIHR, *T. viride* -14- IIHR, *T. viride* -16- IIHR, *T. harzianum* -19- IIHR, *T. harzianum* -20- IIHR, *T. viride* -21-IIHR, *T. viride* -22- IIHR, *T. harzianum* -41- IIHR, *T. viride* -52 –IIHR, *T.viride* -55- IIHR *T. harzianum* -58- IIHR, *B. subtilis* – NBAIR, *P. fluorescens* - NBAIR were evaluated by dual culture technique against *A. solani* under *in-vitro* conditions as explained in ‘Material and Methods’. The per cent inhibition was recorded and the per cent inhibition over control was calculated.

**Table 9: *In vitro* evaluation bio agents against *A. solani***

Sl. No.	Bio agents	Per cent inhibition of <i>A. solani</i> by antagonist
1.	<i>T. viride</i> -GKVK1	56.30(48.61)
2.	<i>T. viride</i> -GKVK2	56.30(48.61)
3.	<i>T. viride</i> GKVK3	63.33(52.75)*
4.	<i>T. viride</i> -NBAIR	63.33(52.75)
5.	<i>T. harzianum</i> -NBAIR	62.96 (52.54)
6.	<i>T.harzianum</i> -2-IIHR	51.11 (45.63)
7.	<i>T.viride</i> -13-IIHR	50.30(45.17)
8.	<i>T.viride</i> -14-IIHR	59.83(50.55)
9.	<i>T.viride</i> -16-IIHR	56.16(58.31)
10.	<i>T.harzianum</i> -19-IIHR	52.96(46.72)
11.	<i>T.harzianum</i> -20-IIHR	55.19(48.83)
12.	<i>T.viride</i> -21-IIHR	69.63(56.56)
13.	<i>T.viride</i> -22-IIHR	66.30(54.51)
14.	<i>T.harzianum</i> -41-IIHR	59.63(50.57)
15.	<i>T.viride</i> -52-IIHR	46.67(43.08)
16.	<i>T.viride</i> -58-IIHR	48.56(43.93)
17.	<i>T.harzianum</i> -55-IIHR	52.22(46.27)
18.	<i>B. subtilis</i> -NBAIR	24.07(29.36)
19.	<i>P. fluorescens</i> -NBAIR	27.41(31.54)
20.	Control	---
<b>Mean</b>		<b>47.12</b>
<b>CD @ 1 %</b>		<b>3.778</b>
<b>S. Em ±</b>		<b>0.90</b>
<b>CV %</b>		<b>4.833</b>

**\*The value in the parenthesis is arc sine transformed**

At 12 days after incubation, the maximum inhibition of mycelial growth (69.63 %) was observed in *T. viride* -21- IIHR which was on par with *T. viride* -22- IIHR (66.30 %) whereas *T. viride* -52- IIHR recorded least (46.67 %) in *Trichoderma viride*- 52- IIHR

followed by *T. viride*-58- IIHR (48.56 %). *Pseudomonas fluorescens* (27.41 %) and *Bacillus subtilis* (20.45 %) showed least inhibition of mycelial growth of *A. solani*.

Impact of dual culture on population of bio agents is presented in Table 6. The data revealed that, mycelial growth of *A. solani* was significantly reduced in the presence of *T. viride*-21- IIHR, *T. viride* -22- IIHR and *T. viride* - GKVK-2. The results are presented in Table 9.

#### 4.3.3 *In vitro* evaluation of Plant extracts against *A. solani*

The efficacy of nine Plant extracts were tested at 1:1, 1:2, and 1:3 per cent concentrations by 'Poisoned food technique' as described in 'Material and Methods'. The radial growth of the mycelium was recorded in 12 days after incubation and the data converted into per cent inhibition over the control.

**Table 10: *In vitro* evaluation plant extracts against *A. solani*.**

Sl. No.	Host plants	Common name	Per cent inhibition over control			
			Concentrations			
			1:1	1:2	1:3	Mean
1.	<i>Simarouba glauca</i>	Simarouba	54.44 (45.90)	49.63 (42.96)	44.81 (40.01)	49.62 (42.96)
2.	<i>Cymbopogon flexuosus</i>	Lemon grass	47.41 (41.60)	40.37 (37.22)	32.59 (32.15)	40.12 (36.99)
3.	<i>Azadirachta indica</i>	Neem	40.74 (37.46)	37.41 (35.33)	30.37 (30.64)	36.17 (34.48)
4.	<i>Calotropis gigantea</i>	Calotropis	40.00 (36.98)	32.22 (31.91)	27.41 (28.51)	33.21 (32.47)
5.	<i>Ocimum tenuiflorum</i>	Tulasi	40.00 (36.99)	37.78 (35.57)	27.78 (28.77)	35.18 (33.78)
6.	<i>Ruta graveolens</i>	Nagadhale	54.07 (45.67)	49.26 (42.74)	42.96 (38.86)	48.76 (42.42)
7.	<i>Lantana camera</i>	Lantana	47.41 (41.60)	42.22* (38.39)	38.89 (36.28)	42.84 (38.76)
8.	<i>Pongamia pinnata</i>	Pongamia	47.04 (41.38)	38.89 (36.29)	27.78 (32.41)	37.90 (36.69)
9.	<i>Leucaena leucocephala</i>	Subabul	35.19 (33.89)	40.00 (34.37)	30.37 (30.62)	35.18 (32.96)
10	<i>Ascophyllum nodosum</i>	Sea weed extract	33.36 (32.06)	29.35 (29.65)	26.35 (27.36)	29.36 (26.35)
<b>Mean</b>			45.14 (40.16)	40.80 (37.20)	33.66 (33.14)	35.97 (36.83)
		<b>Plant extracts(P)</b>	<b>Concentrations (C)</b>		<b>P x C</b>	
<b>S.Em±</b>		0.38	0.27		0.69	
<b>C.D at 1 %</b>		1.812	1.046		3.139	

\*The value in the parenthesis is arc sine transformed

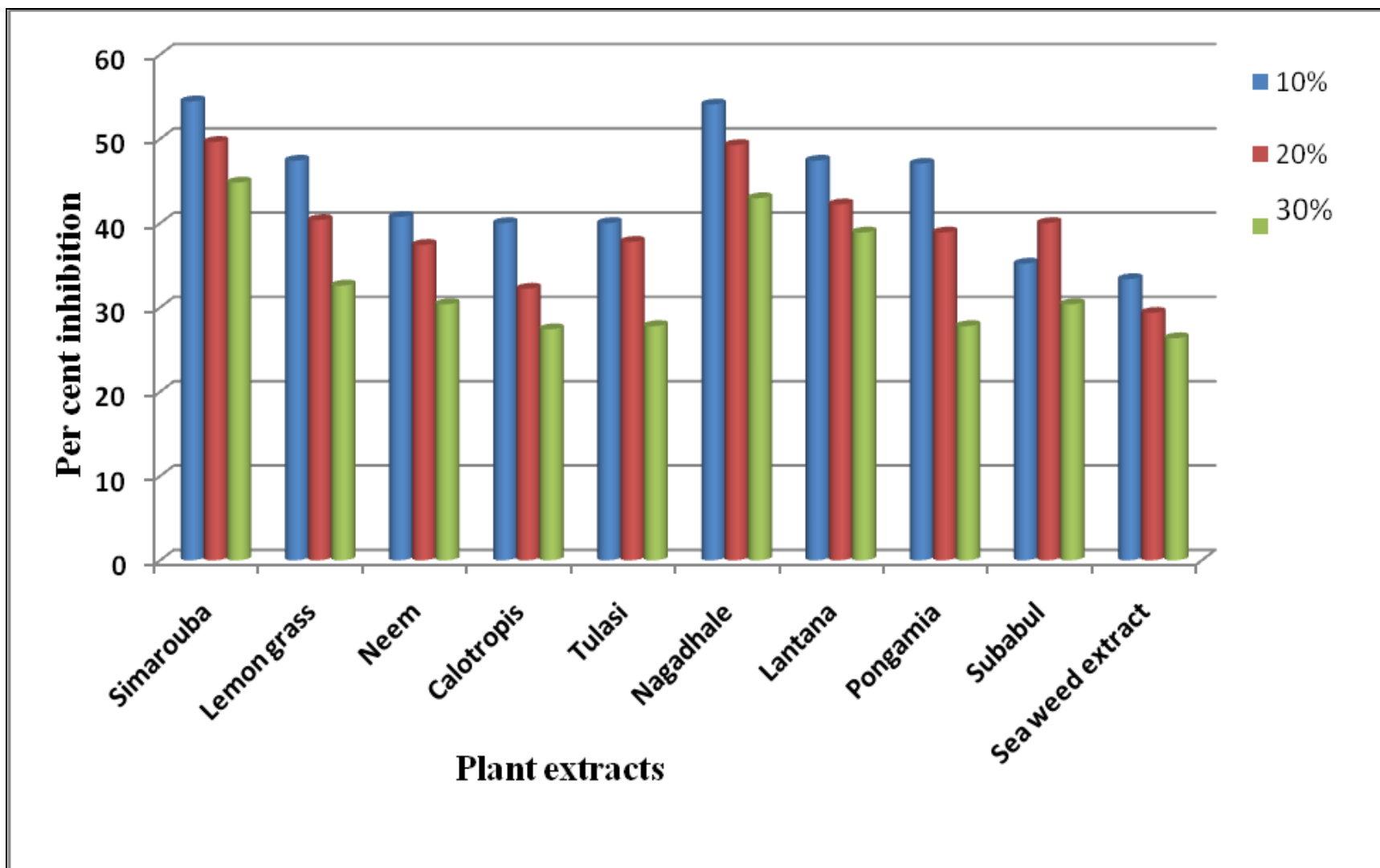
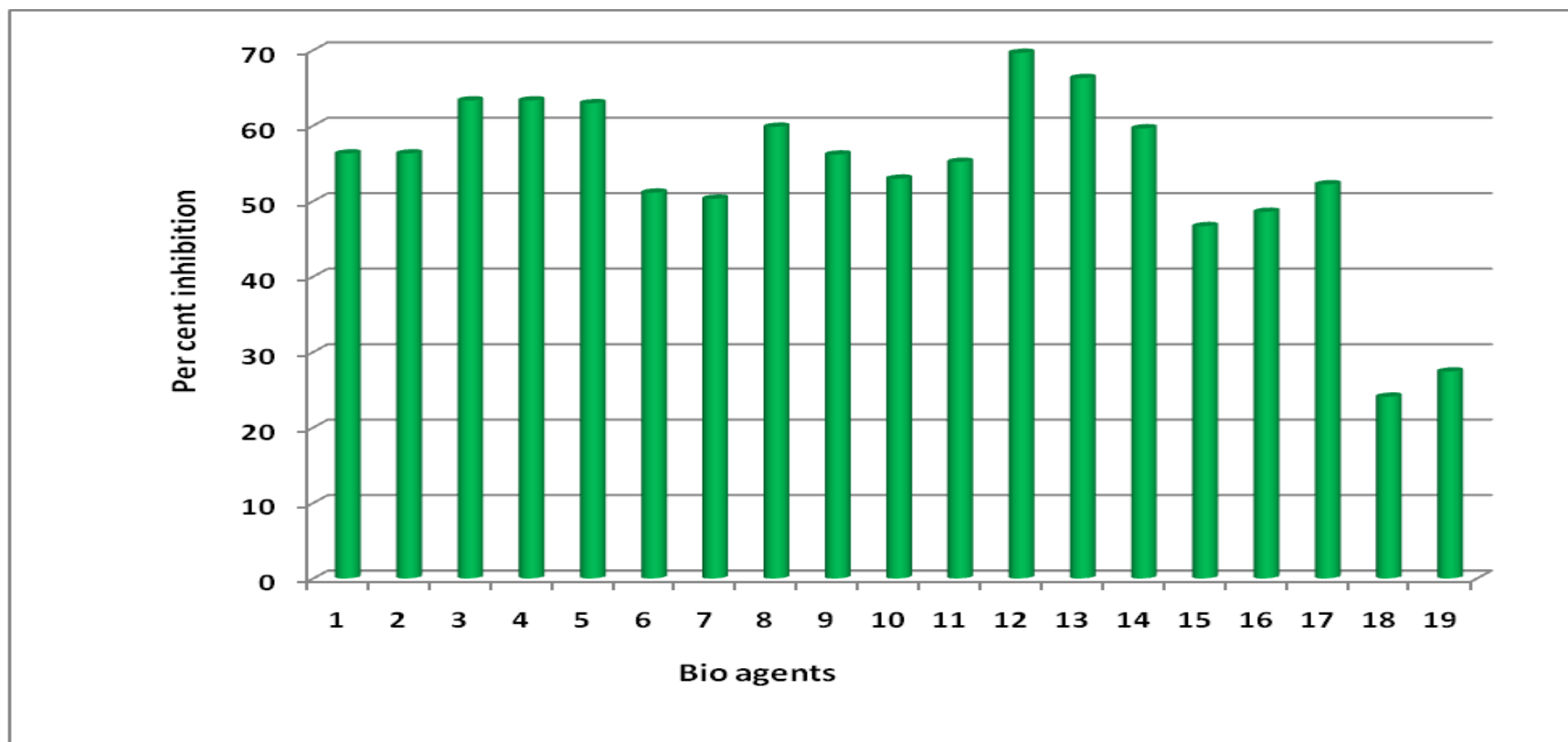


Fig. 6: Per cent inhibition of mycelial growth of *A. solani* in different plant extracts (*in vitro*)

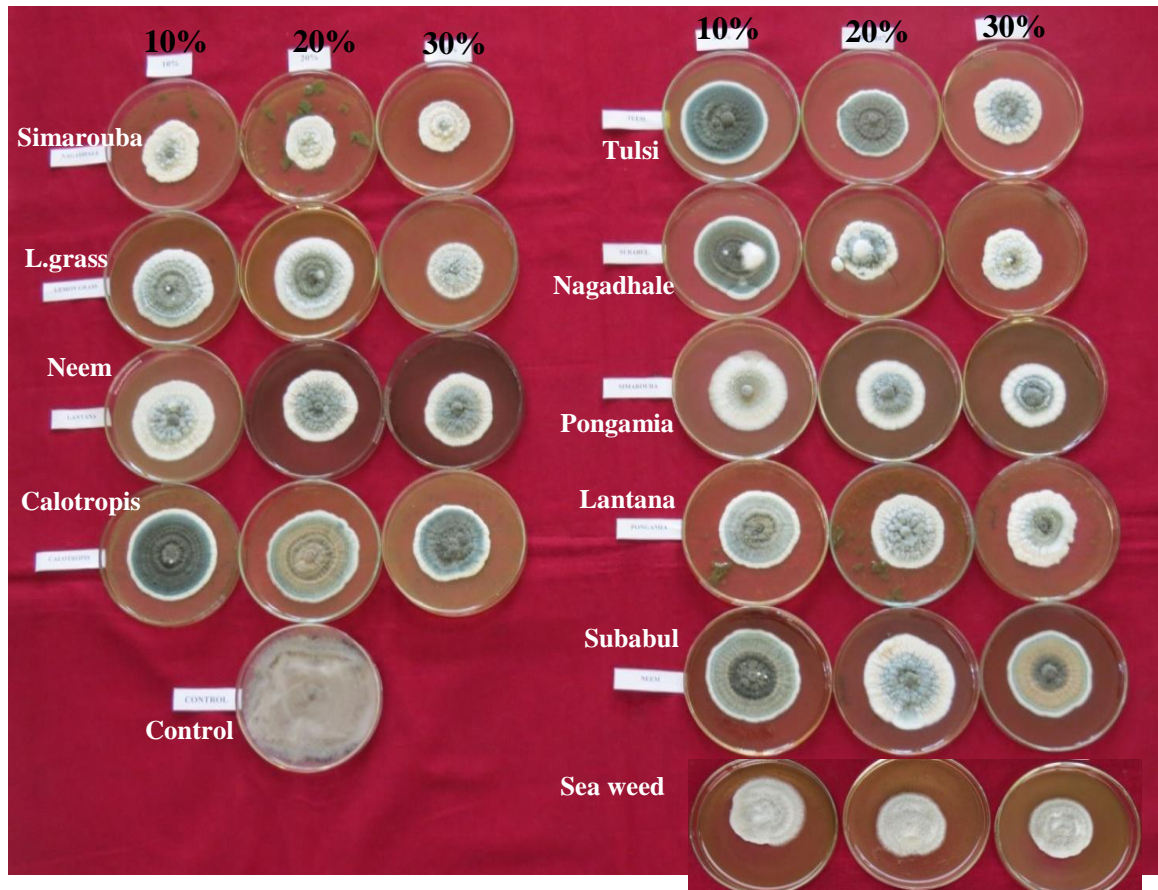


1. *T. viride*-GKVK1
2. *T. viride*-GKVK-2
3. *T. viride* GKVK3
4. *T. viride*-NBAIR
5. *T. harzianum*-NBAIR
6. *T. harzianum*-2-IIHR
7. *T. viride*-13-IIHR

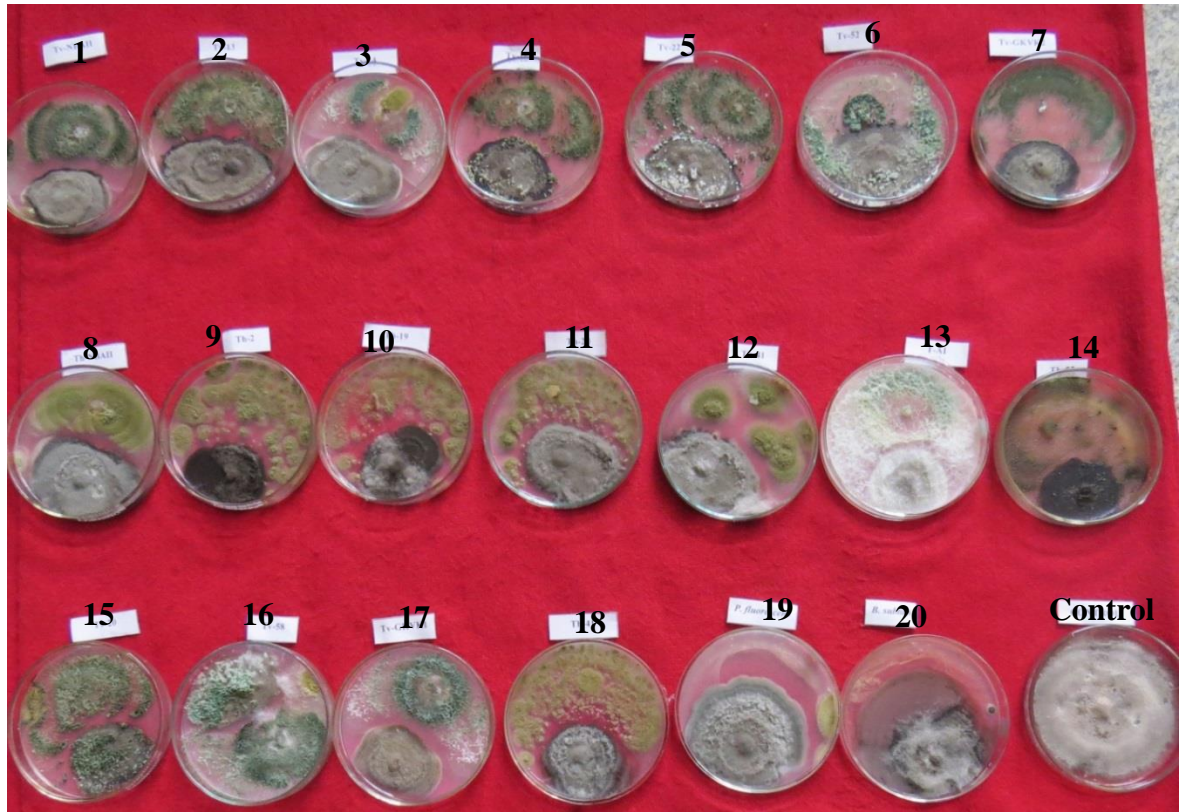
8. *T. viride*-14-IIHR
9. *T. viride*-16-IIHR
10. *T. harzianum*-19-IIHR
11. *T. harzianum*-20-IIHR
12. *T. viride* -21-IIHR
13. *T. viride* -22-IIHR
14. *T. harzianum*-41-IIHR

15. *T. viride*-52-IIHR
16. *T. viride*-58-IIHR
17. *T. harzianum*-55-IIHR
18. *B. subtilis*-NBAIR
19. *P. fluorescens*-NBAIR

**Fig. 7: Per cent inhibition of mycelial growth of *A. solani* in different bio agents (*in vitro*)**



**Plate 8: Per cent inhibition of mycelial growth of *A. solani* in different leaf extracts (*in vitro*)**



1. *T.viride*-NBAII  
 2. *T.viride*-13 IIHR  
 3. *T.viride* 14 IIHR  
 4. *T.viride*-21IIHR  
 5. *T.viride*-16-IIHR  
 6. *T.viride*-52-IIHR  
 7. *T.viride*-22-IIHR

8. *T.harzianum*-NBAIR  
 9. *T.harzianum*-2-IIHR  
 10. *T.harzianum*-19-IIHR  
 11. *T.harzianum*-20-IIHR  
 12. *T.harzianum*-41-IIHR  
 13. *T.harzianum*-55-IIHR  
 14. *T.viride*-GKVK-1

15. *T.viride*-20-IIHR  
 16. *T.viride*-58-IIHR  
 17. *T.viride*-GKVK-2  
 18. *T.viride*-GKVK-3  
 19. *P. flourecense*-NBAIR  
 20. *B. subtilis*-NBAIR  
 21. Control

**Plate 9: Per cent inhibition of mycelial growth of *A.solani* in different bio agents (*in vitro*)**

At 12 days after incubation Simarouba leaf extract recorded maximum per cent inhibition of the fungus, was 44.81, 49.63 and 54.44 per cent at 1:1, 1:2 and 1:3 dilutions respectively. Nagadhale leaf extract showed the maximum of 42.96, 49.26 and 54.07 per cent at 1:1, 1:2 and 1:3 dilutions respectively. Leaf extracts of lantana showed 38.89, 42.22 and 47.41 per cent inhibition at 1:1, 1:2 and 1:3 dilutions respectively. Sea weed showed least per cent fungus inhibition was 27.41, 32.22 per cent at 1:1 and 1:2 dilutions concentration respectively. But least per cent inhibition of fungus mycelium was recorded in Subabul plant extract at 1:3 per cent concentration. However, least per cent inhibition were recorded in Calotropis and Subabul leaf extract at all concentration. Among nine plant extract Simarouba, Nagadhale and Lantana were effective against of *A. solani*. The results are presented in Table 10.

#### **4.4 Effect of fungicide, bio agents and sea weed extract on growth parameters and yield of potato affected by early blight disease under glass house condition.**

The experiment was carried out in glass house to know the effect of fungicides, bio agents and Sea weed extract on growth parameters and yield of potato with 6 treatments and 4 replication. The observation on plant height, number of tubers /plant, weight of tubers/plant, fresh weight/plant, dry weight /plant, and also disease severity were recorded at different growth stages of the crop.

##### **4.4.1 Effect of fungicides, bio agents and sea weed extract on early blight severity under glass house condition.**

The minimum of disease severity was recorded from Tebuconazole (7.56 %) treated plants at 45 days followed by Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts (8.12 %) treated plants. At 60 days the minimum of disease severity was recorded from Tebuconazole (14.65 %) treated plants followed by Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts (16.54 %) treated plants. The minimum of disease severity was recorded from Tebuconazole (29.25 %) treated plants at 75 days followed by Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts (34.24 %) treated plants. At 90 days the minimum of disease severity was recorded from Tebuconazole (48.33 %) treated plants followed by Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts (51.10 %) treated plants. The maximum of disease severity was observed in control plants were 10, 24, 27, 35, 48 and 74.03 per cent at 45, 60, 75 and 90 days after planting respectively. The results pertained disease severity presented in the Table 11.

##### **4.4.2. Effect of fungicide, bio agents and sea weed extract on growth and yield parameters of Potato infected by early blight disease under green house condition.**

The maximum plant height was recorded in the treatment Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts treated plants (97.55 cm) followed by Tebuconazole (86 cm) treated plants and the minimum plant height was recorded in control untreated check, in this treatment the plant height was 60.33 cm.

**Table 11: Effect of fungicides, bio agents and plant extracts on disease severity of early blight of Potato under glass house condition**

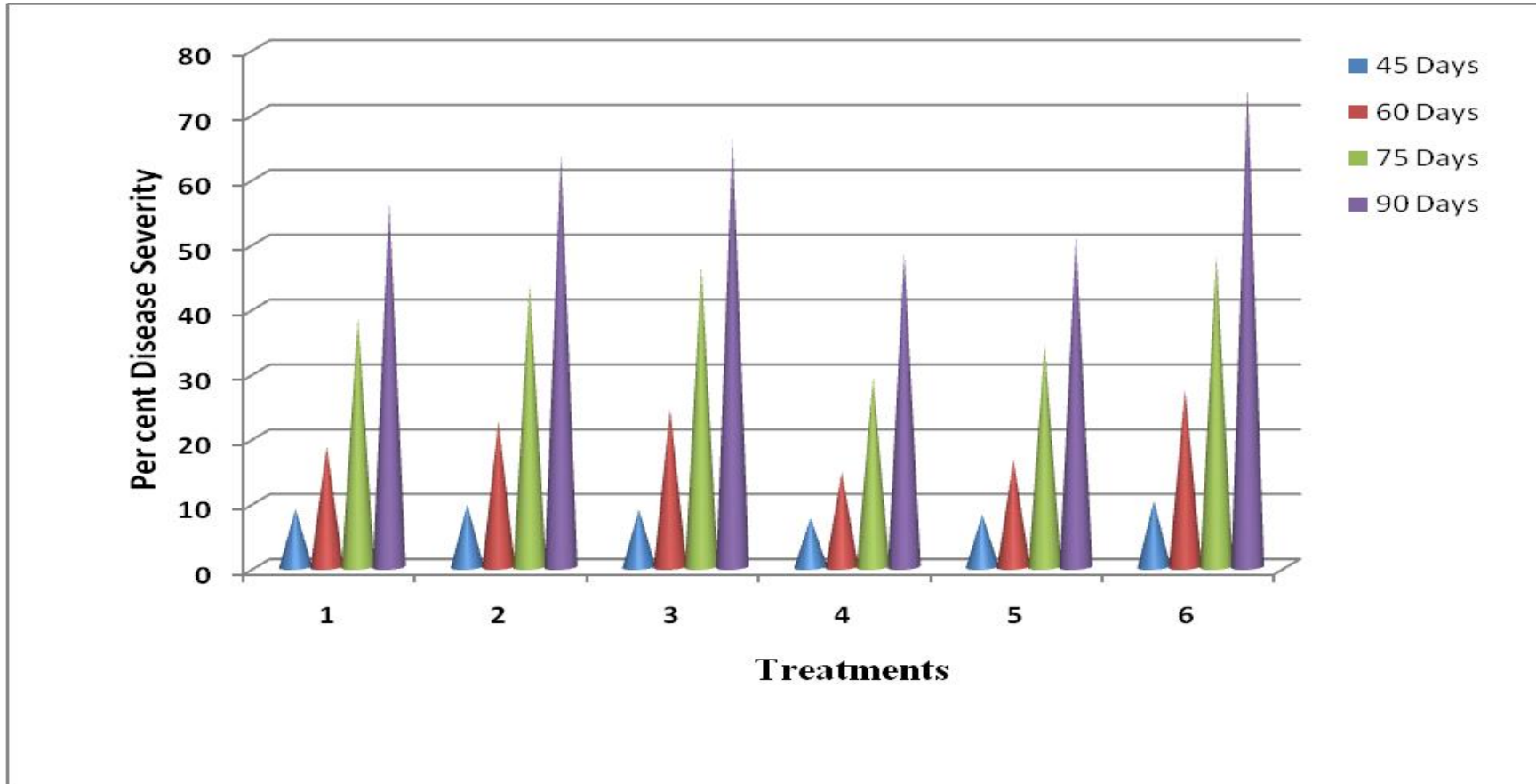
Sl. No.	Treatments	Per cent Disease Severity			
		45 days	60 Days	75 Days	90 Days
1	Seaweed extract @10 %	8.98(17.24)*	18.63(24.46)	38.50(37.22)	56.33(48.65)
2	<i>T. viride</i> @ 5 %	9.64(17.98)	22.50(26.64)	43.62(43.88)	63.64(51.39)
3	<i>P. fluorescens</i> @ 5 %	8.89(17.26)	24.36(31.25)	46.37(48.34)	66.23(53.11)
4	Tebuconazole @ 0.1 %	7.56(16.36)	14.65(21.68)	29.25(36.27)	48.33(39.85)
5	Seaweed @ 10 % > <i>T. viride</i> @ 5 % > <i>P. fluorescens</i> @ 5 % > Tebuconazole @ 0.1 %	8.12(17.02)	16.54(17.18)	34.24(35.68)	51.10(51.55)
6	Control	10.24(18.52)	27.35(32.26)	48.00(39.62)	74.03(60.67)
<b>Mean</b>		<b>8.94(17.56)</b>	<b>20.67(26.47)</b>	<b>39.98(40.12)</b>	<b>59.95(50.84)</b>
<b>SEm±</b>		<b>0.10</b>	<b>0.45</b>	<b>1.33</b>	<b>1.64</b>
<b>CD@5 %</b>		<b>0.31</b>	<b>1.31</b>	<b>3.88</b>	<b>4.80</b>
<b>CV %</b>		<b>10.01</b>	<b>5.36</b>	<b>7.02</b>	<b>5.20</b>

\*The value in the parenthesis is arc sine transformed.

The Maximum numbers of tuber per plants were noticed in Tebuconazole fungicide sprayed plants (3.75/plant) followed by Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts treated plants (2.25/plant) and least numbers of tuber was recorded in untreated plants. The Fresh weight was more in Tebuconazole treated plants was 140.75 g per pot followed by 107 g in Tebuconazole > *T. viride* > *P. fluorescens* > sea weed extracts and least fresh weight of 93.33 g was observed in control plants. After harvest of potato plants allowed drying in hot air oven and dry weight of the plants were recorded. Among six treatments the maximum dry weight was observed in Tebuconazole treated plants (22.83 g) and least was recorded in control plants. The highest tuber yield (138 g/plant) was recorded from Tebuconazole treated plants. The results pertained to growth and yield presented in the Table 12.

#### 4.5 Integrated management of early blight of potato in field condition

The field experiment was carried by Randomized Block Design (RBD) with various combination of fungicides, bio-agents, sea weed extract were tested against early blight and late blight with 3replications and 13 treatments during 2014-15 and disease severity observations were taken at 45, 60, 75 and 90 days after planting.



T<sub>1</sub>- Sea weed extract@10%

T<sub>2</sub>-Culture filtrate of *T.viride*, @ 5%

T<sub>3</sub>-Culture filtrate of *P. fluorescens*, @ 5%

T<sub>4</sub>-Tebuconazole@ 0.1%

T<sub>5</sub>-Metalaxy1+Mancozeb@0.1% >*T.viride*@5%>*P.flourescens*@5%>Sea weed extract@10%,

T<sub>6</sub>-Control.

**Fig. 8: Disease Severity of early blight disease of Potato under glass house condition**

**Table 12: Effect of different treatments on growth and yield parameters of potato infected by early blight disease under glass house conditions**

Sl. No.	Treatments	Plant height (cm)	Fresh weight/Plant (gm)	Dry weight /plant (gm)	Tuber weight/ plant (gm)
1	Seaweedextract@10 %	67.25	94.25	18.90	110.50
2	<i>T.viride</i> @5 %	70.85	80.25	19.70	92.25
3	<i>P.flourosense</i> @5 %	68.08	87.75	20.53	101.75
4	Tebuconazole@0.1 %	86.38	140.75	22.88	148.25
5	Seaweed@10 %> <i>T.viride</i> @5 %> <i>P.floursense</i> @5 %>Tebuconazole@0.1 %	97.55	107.75	22.83	119.00
6	Control	60.33	49.25	17.80	82.00
<b>Mean</b>		<b>74.12</b>	<b>93.33</b>	<b>20.44</b>	<b>109.40</b>
<b>SEm±</b>		<b>7.66</b>	<b>17.33</b>	<b>0.95</b>	<b>13.92</b>
<b>CD@5 %</b>		<b>22.77</b>	<b>51.49</b>	<b>2.84</b>	<b>41.38</b>
<b>CV %</b>		<b>12.63</b>	<b>7.55</b>	<b>11.34</b>	<b>15.05</b>

#### 4.5.1 Management of early blight disease of potato.

Among the fungicides, bio agents and sea weed extract tested against early blight disease under field condition. The minimum disease severity was recorded in Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin (0.91 %) treated plants followed by Mancozeb > Avtar > Mancozeb > Avtar > Mancozeb > Avtar (1.20 %) treated plants at 45 DAP. At 60 DAP the disease severity was minimum in Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin (8.63 %) treated plants which was on par with Folicur (8.73 %) treated plants. The minimum disease severity was noticed from Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin (21.46 %) followed by Folicur (23.86 %) treated plants at 75DAP. At 90 DAP 41.10 per cent of disease severity recorded from Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin followed by Folicur treated plants. The maximum disease severity was noticed from control plants. The results are presented in Table 13.

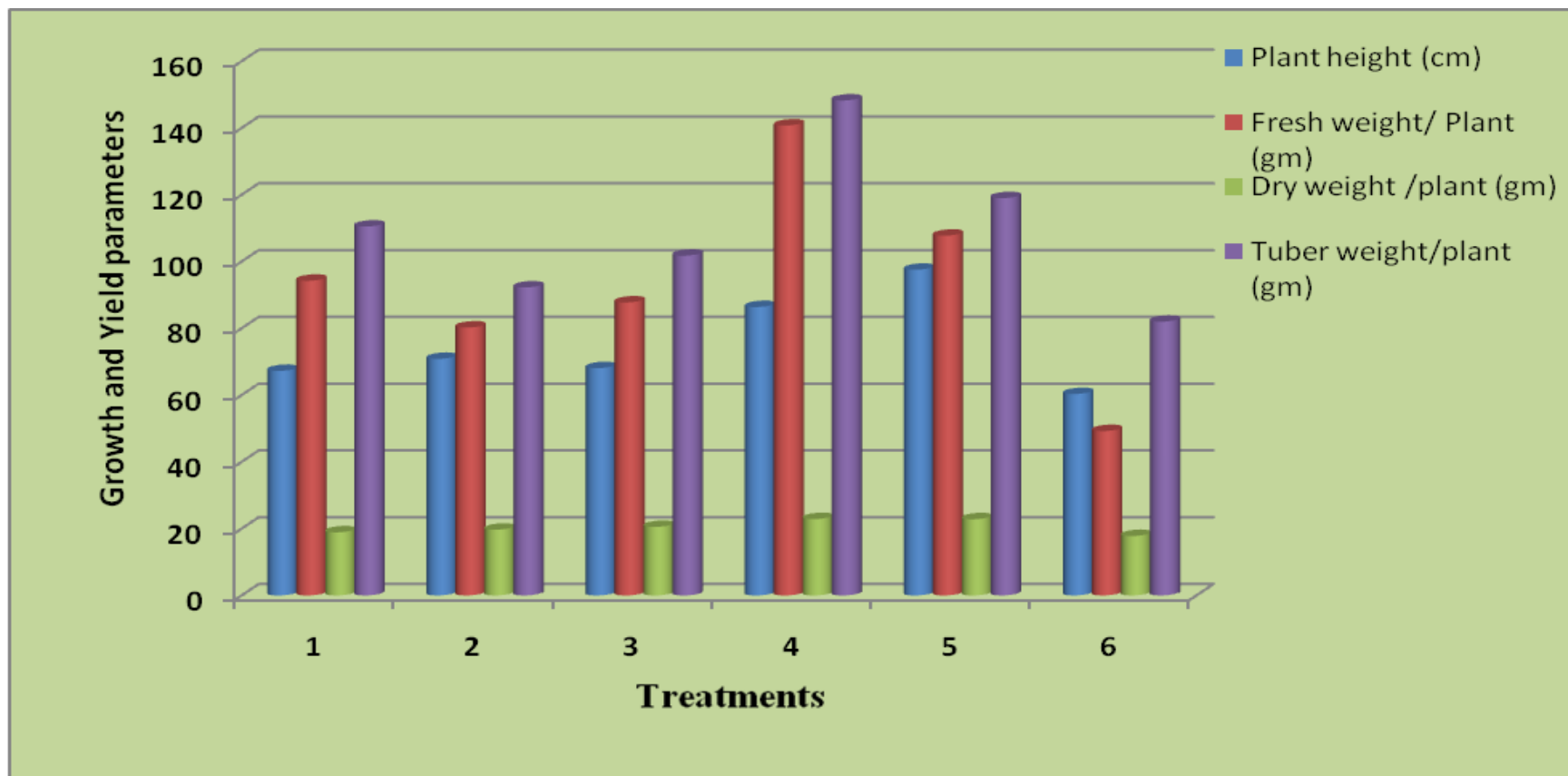
#### 4.5.2 Effect of different treatments on growth and yield parameters of Potato affected by early blight disease under field condition.

Among different treatments Folicur treated plants showed the maximum plant height (88.66 cm), number of tubers (7/plant), dry weight (13.30 g/plant) and tuber weight(560 g/plant). The maximum fresh weight (133.33 g/plant ) and tuber yield (61.33 kg/plot) per plot obtained from in Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin treated plants. The results are presented in Table 14.

**Table 13: Evaluation of sequence application of fungicides, bioagents and plant extract against early blight of Potato under field condition**

Sl. No.	Treatments	Percent Disease severity			
		45 days	60 days	75 days	90 days
1.	Mancozeb > Avtar>Mancozeb >Avtar > Mancozeb > Avtar	1.20 (6.28)*	9.50 (17.94)	28.50 (32.25)	46.73 (55.43)
2	Folicur > Metalaxyl >Folicur > Metalaxyl> Folicur > Metalaxyl	1.30 (6.51)	9.50 (17.94)	24.70 (29.76)	43.93 (41.49)
3	Nativo > Sectin > Nativo > Sectin > Nativo >Sectin	1.30 (6.54)	12.00 (20.25)	26.67 (31.14)	50.23 (45.11)
4	Mancozeb > Mancozeb > Mancozeb > Mancozeb >Mancozeb >Mancozeb	1.56 (7.16)	13.66 (21.68)	35.05 (36.27)	50.60 (39.85)
5	Folicur> Folicur >Folicur >Folicur >Folicur >Folicur	1.76 (7.63)	8.73 (17.18)	23.86 (29.21)	41.10 (41.55)
6	Nativo >Nativo >Nativo >Nativo> Nativo >Nativo	1.33 (6.62)	10.46 (18.86)	26.00 (30.62)	44.03 (45.67)
7	Metalaxyl> Kavach> Metalaxyl> Kavach>Metalaxyl	1.98 (7.98)	13.64 (21.69)	29.36 (33.56)	46.56 (55.54)
8	Mancozeb >Avtar>Folicur > Metalaxyl> Nativo> Sectin	0.90 (5.43)	8.63 (17.06)	21.46 (27.58)	36.63 (37.15)
9	Folicur> <i>T.viride</i> > <i>P. flourescens</i> >Sea weed extract	1.90 (7.91)	14.23 (22.13)	32.33 (34.61)	51.56 (43.10)
10	Sea weed extract	2.36 (8.84)	19.46 (26.16)	38.66 (38.43)	67.83 (57.69)
11	<i>T.viride</i>	2.30 (8.71)	19.46 (26.16)	41.40 (40.03)	71.46 (59.06)
12	<i>P. flourescens</i>	2.50 (9.09)	19.70 (26.35)	42.53 (40.68)	73.56 (60.52)
13	Control	3.96 (11.48)	30.83 (33.71)	53.89 (47.22)	83.03 (65.68)
<b>Mean</b>		<b>1.91 (7.98)</b>	<b>14.69 (25.63)</b>	<b>32.69 (37.32)</b>	<b>54.53 (48.36)</b>
<b>SEm±</b>		<b>0.10</b>	<b>0.45</b>	<b>1.33</b>	<b>1.64</b>
<b>CD@5 %</b>		<b>0.31</b>	<b>1.31</b>	<b>3.88</b>	<b>4.80</b>
<b>CV %</b>		<b>10.01</b>	<b>5.36</b>	<b>7.02</b>	<b>5.20</b>

\*The value in the parenthesis is arc sine transformed



T<sub>1</sub>- Sea weed extract@10%

T<sub>2</sub>-Culture filtrate of *T. viride*, @ 5%

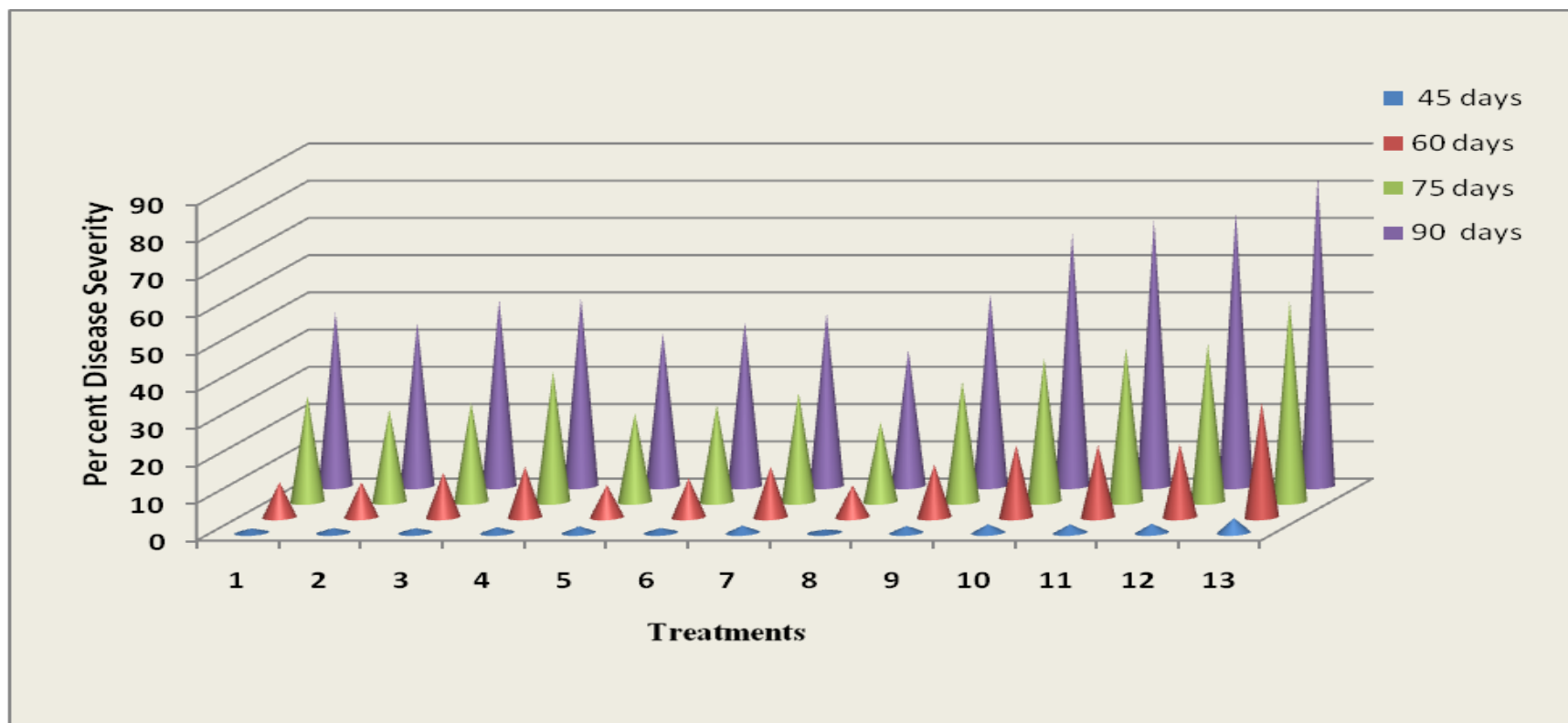
T<sub>3</sub>-Culture filtrate of *P. fluorescens*, @ 5%

T<sub>4</sub>-Tebuconazole@ 0.1%

T<sub>5</sub>-Metalaxyl+Mancozeb@0.1% >*T. viride*@5% >*P. flourescens*@5%>Sea weed extract@10%,

T<sub>6</sub>-Control.

**Fig. 9: Growth and yield parameters of potato in treated plants against early blight disease under glass house condition**



**T1**-Mancozeb >Avtar>Mancozeb >Avtar>Mancozeb >Avtar  
**T2**-Folicur >Metalaxyl >Folicur>Metalaxyl > Folicur > Metalaxyl  
**T3**-Nativo >Sectin > Nativo > Sectin >Nativo >Sectin  
**T4**-Mancozeb >Mancozeb >Mancozeb >Mancozeb >Mancozeb >Mancozeb  
**T5**-Folicur >Folicur >Folicur >Folicur >Folicur >Folicur  
**T6**-Nativo >Nativo >Nativo >Nativo >Nativo >Nativo  
**T7**-Metalaxyl>Kavach>Metalaxyl>Kavach>Metalaxyl

**T8**-Mancozeb > Avtar>Folicur >Metalaxyl >Nativo >Sectin  
**T9**-Folicur> *T.viride* >*P. flourescens* >Sea weed extract  
**T10**-Sea weed extract,  
**T11**-*T.viride*  
**T12**-*P. flourescens*  
**T13**-Control

**Fig. 10: Evaluation of sequence application of fungicides, bioagents and plant extract against early blight of Potato under field condition**



**Early blight symptoms**

**Plate 10: Development of Early blight disease under glass house**



**Tebuconazole treated Plants**



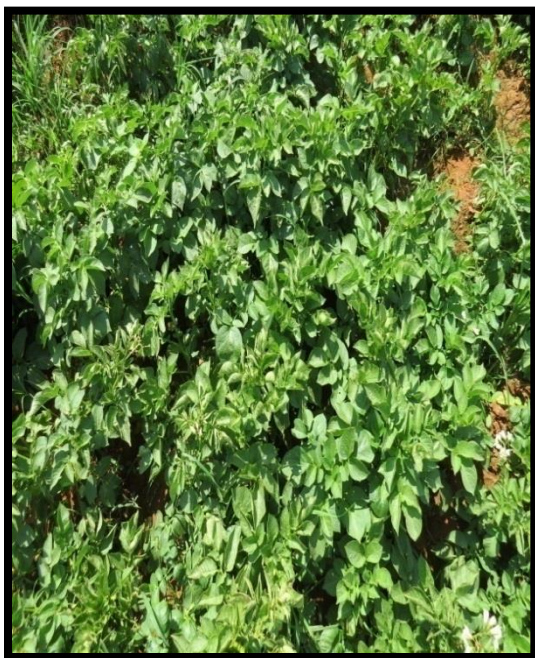
**Untreated Control**

**Plate 11: Evaluation of fungicide, bio agents and plant extract spray against early blight of potato under glass house**



**Early blight symptoms**

**Plate 12. Development of early blight disease in field**



**Sequence application of Mancozeb>  
Avtar>Folicur>Metalaxyl>Nativo  
>Sectin treated plot**

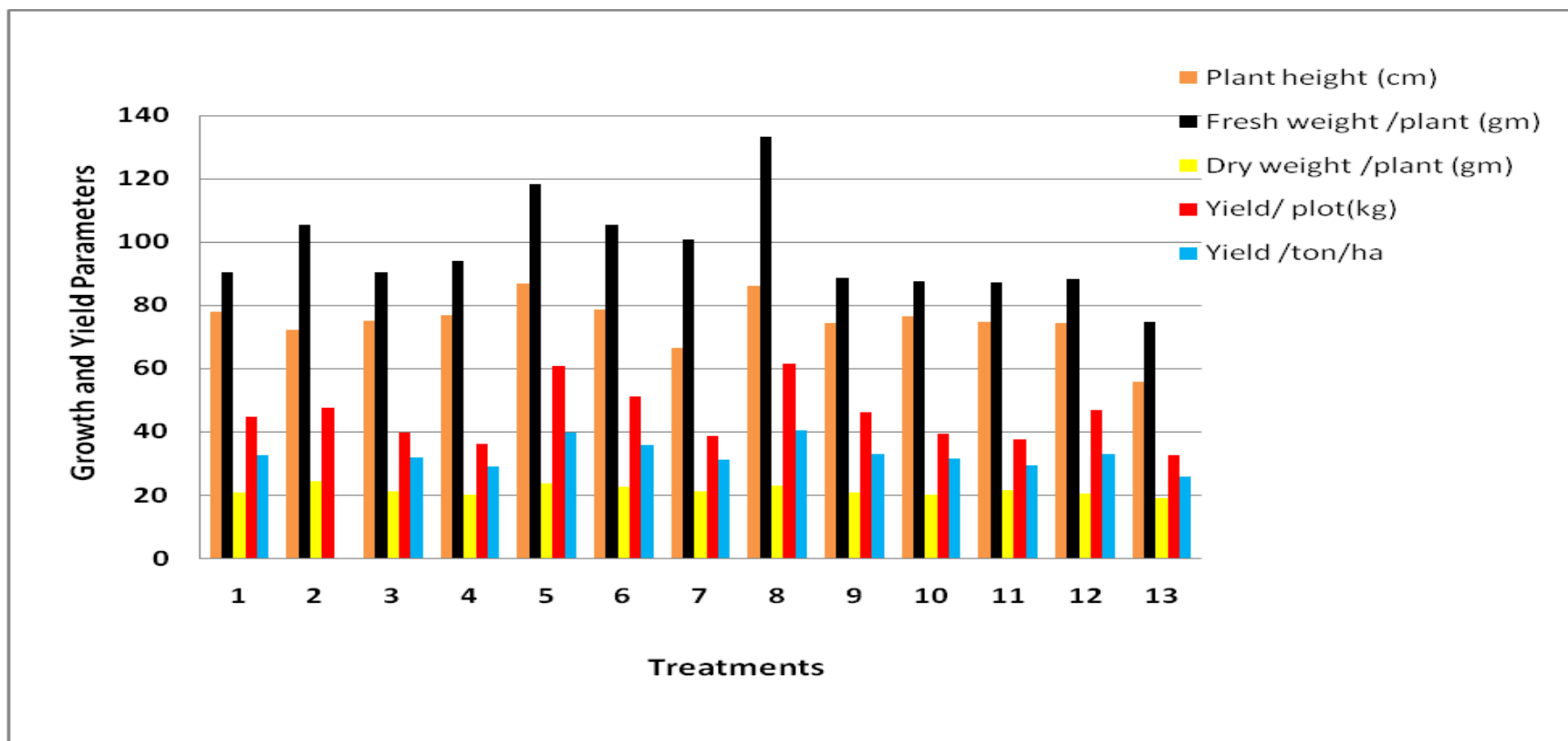


**Untreated control**

**Plate 13: Evaluation of sequence application of fungicides, bioagents and plant extract against early blight of Potato under field condition**

**Table 14: Effect of different treatments on growth and yield of Potato affected by early blight disease of Potato under field condition**

Sl. No.	Treatments	Plant height (cm)	Fresh weight /plant (gm)	Dry weight /plant (gm)	Yield/plot (kg)	Yield /ton /ha
1.	Mancozeb > Avtar>Mancozeb >Avtar> Mancozeb > Avtar	78.00	90.33	20.60	44.66	32.65
2	Folicur > Metalaxyl >Folicur > Metalaxyl> Folicur > Metalaxyl	72.30	105.33	24.20	47.33	33.02.
3	Nativo > Sectin > Nativo > Sectin > Nativo >Sectin	75.00	90.33	20.90	39.66	31.65
4	Mancozeb > Mancozeb > Mancozeb > Mancozeb >Mancozeb >Mancozeb	76.66	94.00	19.96	36.10	28.96
5	Folicur> Folicur >Folicur >Folicur >Folicur >Folicur	86.66	118.33	23.70	60.66	39.68
6	Nativo >Nativo >Nativo >Nativo>Nativo >Nativo	78.66	105.33	22.36	51.00	35.84
7	Metalaxyl> Kavach> Metalaxyl>Kavach>Metalaxyl	66.33	100.66	20.93	38.66	31.03
8	Mancozeb >Avtar>Folicur > Metalaxyl> Nativo> Sectin	86.00	133.33	22.70	61.33	40.36
9	Folicur> <i>T.viride</i> > <i>P. flourescens</i> >Sea weed extract	74.33	88.66	20.63	46.00	32.96
10	Sea weed extract	76.33	87.33	20.13	39.33	31.58
11	<i>T.viride</i>	74.66	87.00	21.40	37.66	29.34
12	<i>P. flourescens</i>	74.33	88.33	20.20	46.66	32.98
13	Control	55.66	74.66	18.96	32.66	25.64
	<b>Mean</b>	<b>74.36</b>	<b>103.63</b>	<b>21.23</b>	<b>43.56</b>	<b>33.67</b>
	<b>SEm±</b>	<b>7.54</b>	<b>7.59</b>	<b>0.91</b>	<b>4.36</b>	<b>1.62</b>
	<b>CD@5 %</b>	<b>22.55</b>	<b>22.15</b>	<b>2.66</b>	<b>13.53</b>	<b>2.38</b>
	<b>CV %</b>	<b>17.21</b>	<b>13.52</b>	<b>13.98</b>	<b>18.13</b>	<b>11.63</b>



**T1**-Mancozeb >Avtar>Mancozeb >Avtar>Mancozeb >Avtar  
**T2**-Folicur >Metalaxyl >Folicur>Metalaxyl > Folicur > Metalaxyl  
**T3**-Nativo >Sectin > Nativo > Sectin >Nativo >Sectin  
**T4**-Mancozeb >Mancozeb >Mancozeb >Mancozeb >Mancozeb >Mancozeb  
**T5**-Folicur >Folicur >Folicur >Folicur >Folicur >Folicur  
**T6**-Nativo >Nativo >Nativo >Nativo >Nativo >Nativo  
**T7**-Metalaxyl>Kavach>Metalaxyl>Kavach>Metalaxyl

**T8**-Mancozeb > Avtar>Folicur >Metalaxyl >Nativo >Sectin  
**T9**-Folicur> *T.viride* >*P. flourescens* >Sea weed extract  
**T10**-Sea weed extract,  
**T11**-*T.viride*  
**T12**-*P. flourescens*  
**T13**-Control

**Fig. 11: Effect of different treatments on growth and yield parameters of potato affected by blight disease under field condition**



**Plate 14: General view of experimental plot for management early blight disease of potato at GKVK**

## V DISCUSSION

Potato is one of the most important staple food crop in the world. The crop is vulnerable to a number of diseases, of which early blight disease is a major limiting factor in successful production of potato. The yield loss of Potato in India due to this disease under favorable conditions varies from 60-80 per cent. The experiment conducted on disease and the results obtained are described briefly in this chapter.

### 5.2 Cultural and morphological studies on pathogen

#### 5.2.1 Growth of *A. solani* on different media

The growth of *A. solani* was observed on different solid media. Among them PDA (78.11 mm) and Oat meal agar (73.65mm) medium showed better growth of the fungus. The least (67.98 mm) mycelia growth was observed in Malt extract agar (36.00 mm). PDA is the best media for the growth of *A. solani*. The growth of the pathogen on the different liquid media found the maximum dry mycelia weight (188 mg) in PDB medium, whereas mean dry mycelial weight was minimum in case of Malt extract broth (136mg). Present findings are supported by the observations made by earlier workers Swati Deep *et al.* (2014) reported that among seven types of media (PDA, Cauliflower leaf extract agar, Carrot potato agar, Oat meal agar, Czapekdox agar, V8 juice agar and Corn meal agar) were tested to determine their effect on growth and sporulation of the *A. brassicicola*. The results indicated that PDA (Potato dextrose agar) and Cauliflower leaf extract agar was found optimum for all isolates.

#### 5.2.2 Effect of temperature on growth of *A. solani*.

The maximum mycelial growth of *A. solani* was observed at 30<sup>0</sup> C, the growth of the fungus in this treatment was 73.83 mm, this was followed by 25<sup>0</sup> C, and the growth of the fungus in this temperature was 65.67 mm. The least fungal growth of the fungus (25 mm) was observed at 15<sup>0</sup> C. The fungal growth in 40<sup>0</sup> C was 43.67 mm respectively. The fungus grew well and at 30<sup>0</sup> C. The findings of the present study were in close conformity with these observations and also those of Rotem (1994) who reported that the conidia of *A. brassicicola* germinate over a wide range of temperatures, with the optimum at 28 to 31 °C. Sporulation can occur over a wide range of temperatures and is optimal at 20 to 30 °C. Further, Pawar and Patel (1957) showed that *Alternaria ricini* grows in temperature range of 7 to 38° C with optimum at 27 to 29° C. Hasija (1970) reported that several species of *Alternaria* had good growth between the temperature range of 27°C and 28°C. In addition, Kumar and Singh (1996) observed that, in sunflower, *Alternaria helianthi* was favoured by the temperature range of 27 to 29° C. Kundu and Patra (1991) reported that, the spore germination of *Alternaria brassicicola* was high at a temperature range of 25 to 35° C and neutral pH. Hence, in the present study was also found that the optimum temperature for the growth of *A. brassicicola* ranged from 25 to 30° C.

#### 5.2.3 Effect of pH levels on growth of *Alternaria solani*

The results indicated that pH range of 5.5 to 7.5 was optimum for the growth of *A. brassica*. Maximum dry mycelial weight was observed at pH 6.0 with 157 mg, followed

by pH 6.5 (152 mg) and in pH 7 it was 142 mg. The least dry weight of the fungus (120 mg) was observed in pH 4. The fungus grows well at the pH 6. Every organism has minimum, maximum and optimum pH for the growth. The results indicated that the maximum growth and sporulation of *A. brassicicola* was observed at pH 6.0, followed by pH 6.5 and the minimum was recorded at pH 4.0 and at pH 10. Similar findings have been reported by Padmanabhan and Narayaswamy (1977) who found pH 5 to 7 to be optimum for the growth of *A. macrospora*. Mahabaleswarappa (1981) observed that *A. carthami* made fairly good growth between pH range of 5.3 to 8.1 and maximum growth of the fungus was at pH 6.0

## 5.2 *In vitro* studies

### 5.2.1 Evaluation of fungicide against *A.solani*

Considering the economic importance and serious nature of the disease and in the absence of suitable immune or resistant genotype of the crop, chemical method of control is a dependable method to control disease.

The efficacy of fungicides on the inhibition of mycelia growth of *Alternaria solani* was studied by Poison food technique. The fungicide, Tebuconazole was found to be highly effective (100 per cent inhibition) at all the concentrations. Based on per cent inhibition of fungus over control. The fungicides Tebuconazole, Hexaconazole + Zineb and Trifloxystrobin + Tebuconazole found to be effective against *A.solani* at 100, 250, 500 and 1000 ppm concentrations. These results were supported by Amaresh and Nargund (2004) reported Hexaconazole as most effective each at 200 ppm among systemic fungicides against *Alternaria helathi* plant blight of sunflower. Suralirajan and Janki (2003) were reported, the efficacy of Propiconazole, Tebuconazole, and Hexaconazole were effective against *Alternaria solani* under *in vitro* condition.

Sujoy *et al.* (2014) reported the best inhibitor of the fungal growth was exhibited by the test fungicide mixture (Trifloxystrobin 25 % + Tebuconazole 50 %) at 350 mg kg<sup>-1</sup>, that is 75.1 % and this was at on par with the same at 300 mg kg<sup>-1</sup> (74.87 %). Nandi *et al.* (2012) the test Nativo fungicide formulation even at lower concentrations (100 and 250 mg kg<sup>-1</sup>) recorded significant higher inhibition of the mycelia growth of *A. solani* that is 61.95 and 65.33 per cent. respectively as compared to control.

### 5.2.2 Evaluation of bio-agents against *A.solani*

Several possibilities of existence of microbial interactions have been evidenced as, stimulating, inhibiting, mutual intermingling, antibiosis, hyperparasitism etc. The inhibitory effect of those fungi against *A.solani* due to competition and/or antibiosis was evaluated. The maximum inhibition of mycelial growth (69.63 %) was observed in *T.viride*-21-IIHR which was followed by *T.viride*-22-IIHR (66.30 %) whereas *T.viride*-52-IIHR recorded least (46.67 %) in *Trichoderma viride*- 52 (IIHR) followed by *T.viride*-58-IIHR (48.56 %). *Pseudomonas fluorescens* (27.41 %) and *Bacillus subtilis* (20.45 %) showed least inhibition of mycelial growth of *A. solani*. The bacterial antagonist, *Pseudomonas fluorescens* and *Bacillus subtilis* collected from GKVK showed least inhibition effect on *A. solani*. This may be to our isolates are not effective against this

fungus. However, these strains, the further confirmation is required. These results were supported by the findings of Rudresh *et al.* (2005) who noticed 72.1 and 77.0 per cent growth inhibition of *A. solani* and *F. oxysporum*, respectively by *T. viride*. Extensive studies on the efficacy of three different species of *Trichoderma* against *A. porri* revealed *T. harzianum* and *T. viride* are most effective in inhibiting the pathogen (Imtiaz and Lee, 2008).

Das *et al.* (1996) Impact of dual culture on population of bio agents observed that, the sclerotial production of *Rhizoctonia* was drastically reduced in the presence of *T. viride* –52 (IIHR), *T. viride* - 56 (IIHR) and *T. viride* - 27 (IIHR) recording 2.97, 2.37 and 2.08 sclerotia per microscopic field. Whereas, least number of sclerotia 0.48 produced in *T. harzianum* – 55 (NBAII). Significant reduction of sclerotial growth of *R. solani* by *T. viride*, *T. harzianum* was examined under *in vitro* trails.

### **5.2.3 Evaluation of plant extracts against *A. solani***

Due to increasing awareness of environment pollution by way of continuous use of chemical pesticides and their residual toxicity on crops like effort are being made to use some eco-friendly substitutes like plant extracts. In view of their nonphytotoxicity and systemic actions, they have gained the attention. Several plant extracts known to possess antifungal activities are being exploited to manage fungal plant diseases. The efficacy of nine plant extracts were evaluated and observed the radial growth of the mycelium. Among the plant extract, Simarouba inhibited 44.81, 49.63 and 54.44 per cent inhibition at 1:3, 1:2 and 1:1 dilute concentrations respectively, Nagadhale 42.96, 49.26 and 54.07 per cent inhibition at 1:3, 1:2 and 1:1 concentrations respectively), and Lantana plant extracts were found to be effective against *A. solani*. The results were similar with findings Dushyent *et al.* (1997) of the effect of 11 different halophytic plant extracts (root, stem, plant and bark) on the mycelial growth of *A. solani* were studied *in vitro* tests. Vijayan (1989) reported that the bulb extract of *A. sativum*, plant extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*. Babu *et al.* (2000) reported the effect of plant extracts, oils and Neem products (Neem leaf, neem seed kernel and neem cake) on early blight. Among the plant products, *Acacia concinna* pod extract resulted in the lowest percent disease severity (23.1 %) followed by neem oil (30.9 %). Lemon grass leaves extract gave the best result to inhibit the spore germination and reduce the mycelia growth of two pathogenic fungi. *In vivo* tests, detached plant technique showed that the TAMPE decreased the disease infection with both *P. infestans* and *A. solani*. It is clear that, the efficacy of plant extracts in controlling the late and early blight (Stephan and Koch, 2002)

### **5.3 Effect of fungicide, bioagents and sea weed extracts on growth parameters and yield of potato affected by early blight disease under green house conditions**

Among different treatments, the maximum plant height (97.55 cm), fresh weight (140.75 g), dry weight (22.83g) and tuber weight (138g/plant) obtained from Tebuconazole@0.1 per cent treated pots followed by Tebuconazole > *T. viride* > *P. flourosense* > Sea weed extracts treated plants. Least growth and yield parameters were recorded from control plants. The results similar with findings of the spray program trials confirmed the importance of fungicide use for the control of *A. solani* in the production

of potato. Yield increases were primarily a result of controlling early blight and prolonging green plant areas, which increases the period of tuber bulking. (Stevenson and James 2005; Stevenson and James 2007; Franc and Stump 2008). Pyraclostrobin significantly reduced the early blight and increased the yield in tomato and potato has reported by many workers (Ganeshan and Chethana, 2009 and McDonald *et al.*, 2007). In addition to disease control, strobilurin fungicides are known to have beneficial physiological/growth-promoting effects on plants, including delaying of plant senescence (Bertelsen *et al.* 2001), increased chlorophyll content (Butkute *et al.* 2008) and greater stress tolerance (Jabs *et al.* 2002). These physiological effects may contribute to greater yield, even in the absence of disease. Application of difenoconazole is likely to be more effective than a protective fungicide, as it has both protective and post-infection activity (Dahren and Staub 1992) and can provide greater efficacy and potential yield/economic response.

The minimum disease severity of 7.56, 14.65, 29.25 and 48.33 per cent was recorded at 45, 60, 75 and 90 DAP respectively from Tebuconazole @ 0.1 per cent treated plants followed by Tebuconazole > *T. viride* > *P. flourosense* > Sea weed extracts treated plants. Maximum disease severity was observed in untreated plants. The results similar with findings of Jambhulkar *et al.* (2012) reported spray of Azoxystrobin showed promising results by reducing disease severity by 38.9 per cent as compare with control. Kumar *et al.* (2007) reported that Hexaconazole (0.05 %) and Azoxystrobin (0.2 %) was very effective in managing early blight of tomato.

#### **5.4 Integrated management of early blight disease under field condition**

The effective fungicides identified *in vitro* were used in field for the management of early blight disease of Potato condition (CV Kufri Jyothi). Among the different treatment with weekly interval spray were taken and recorded the observation on severity and growth and yield parameters. The minimum disease severity of 0.91, 8.63, 23.86 and 41.10 per cent was recorded at 45, 60, 75 and 90 DAP respectively in Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin followed by Folicur treated plants. Yield of 61.33 kg/plot and 40.36 ton/ha followed by Folicur treated plots with yield of 60.66 kg/plot and 39.68 ton/ha. The maximum disease severity was noticed from control plants. This result agreed with the findings of Soltanpour and Harrison (1994) who reported that the disease incidence of Early Blight was reduced in plots treated with the chemicals application. Sujoy *et al.* (2014) reported that the individual components, that is Trifloxystrobin 50 WG and Tebuconazole 250 EC (at both doses) were able to control the disease, but their combination proved better in the same. The yield of fruit was also highest in the treatment of the test fungicide at 350 gHa<sup>-1</sup>, that is 26.24 per cent and 35.35 per cent more as compared to the control in two seasons, respectively.

#### **5.4 Growth and yield parameters in integrated management of early blight disease of potato under field condition.**

Among different treatments Folicur treated plots showed maximum plant height (88.66 cm) and dry weight (23.30 g/plant). The maximum fresh weight (133.33g/plant) and tuber yield (61.33 kg/plot) per plot obtained from Mancozeb > Avtar > Folicur > Metalaxyl > Nativo > Sectin treated plots. The results similar with Bhardwaja (1991)

reported that sequential application of Captofol, Mancozeb and Copper oxy chloride 40, 55, 70 days after transplanting, increased yield by 50.5 per cent by reducing the incidence of *A. solani*. Pyraclostrobin alternated with Maneb and Pyraclostrobin + Boscalid alternated with Maneb significantly reduced the anthracnose incidence in bell pepper as compared to control. Best disease control with highest yields and fruit quality was reported in combination product of Pyraclostrobin+Metiram effective against both early blight and late blight has reported by Capriotti *et al.* (2005).

In order to avoid chemical resistance problem in Potato, the individual fungicides with sequence at weekly intervals gave good control. The fungicide Folicur was found effective against early blight of potato. This combination of fungicides spray for the management of early disease will be useful for the farming community.

### **Future line of work**

1. The foliar spray of bio control agents would be strengthened for the management of blight diseases.
2. Combination of fungicides with plant extract in field condition would be strengthened for the management of blight disease of potato.

## VI SUMMARY

An investigation on the studies on early blight of potato was carried out with reference to evaluation of fungicides, plant extracts and bio-agents against the disease under laboratory conditions, effect of fungicides, bioagents and plant extracts on growth and yield parameters of potato under green house conditions and evaluation of chemicals, bioagents and plant extracts against the disease under field conditions.

- ❖ Cultural studies of the pathogen revealed that the radial growth of the fungus after 7 days was maximum in Potato dextrose agar (78.11mm) followed by Oat meal agar (73.65mm) The mean dry mycelial weight of the fungus was maximum in Potato dextrose broth (188 mg), followed by Oat meal broth (180 mg) after seven days incubation.
- ❖ Growth was good at temperature range of 20 to 35 °C and maximum growth was at 30 °C (73.83 mm), followed by 35 °C (65.67 mm). Hence the optimum temperature for the growth and sporulation of the pathogen was found to be between 27 to 32 °C
- ❖ Maximum growth of *A. solani* was at pH of 6 as indicated by dry mycelial weight (157 mg) and the fungus was grown poorly at pH 4 and 10 (dry mycelial weight was 120 mg and 128 mg, respectively).
- ❖ Among the eight fungicides evaluated *in vitro* conditions against *A. solani*, Tebuconazole, Hexaconazole+Zineb and Tebuconazole+ Trifloxistrubin gave maximum inhibition of the fungal growth at 100,250,500 and 1000 ppm concentrations.
- ❖ The antagonist *T.viride*-21-IIHR found effective against *A.solani* and showed maximum inhibition of the pathogen, on par with *T.viride*-22-IIHR where as *Pseudomonas fluorescens* and *Bacillus subtilis* showed least inhibition of mycelial growth of *A. Solani*.
- ❖ Among nine botanicals evaluated in *in vitro* conditions against *A. solani*, Simarouba, Nagadhale and Lantana gave maximum inhibition of the mycelial growth at all the tested concentrations.
- ❖ Among different treatments to test their effect on growth and yield parameters of potato under green house condition. Tebuconazole was found very effective by reducing early blight by increasing plant height ,fresh weight, dry weight ,number of tubers and tuber weight of the potato crop which was followed by Tebuconazole>*T.viride*>*P.flourosense*>sea weed extracts treated pots .
- ❖ The field evaluation of fungicides, bioagents and sea weed extracts indicated that Mancozeb @0.2 %> Avatar@0.2 %>Folicur @0.1 % >Metalaxyl@0.2 % > Nativo@0.1 % >Sectin@0.2 % and spraying of Folicur> Folicur> Folicur > Folicur > Folicur@0.1 % were most effective in reducing severity of the early blight disease and increasing tuber yield over control during 2015.

- ❖ Among different treatments Folicur treated plots showed maximum plant height, number of tubers and dry weight (23.30 g/plant) and . The maximum fresh weight (133.33 g/plant), tuber yield (61.33 kg/plot) per plot and 40.36 ton/ha obtained from Mancozeb>Avtar>Folicur>Metalaxyl>Nativo>Sectin treated plots.

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