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Efficacy of bio-agents and botanical extracts against early blight (*Alternaria solani*) of Tomato (*Solanum lycopersicum* L.)

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Abstract
To evaluate the efficacy of bio-agents, *viz*., *Trichoderma viride* 2% plant extracts, *viz*, neem leaf extract, Dathura leaf extract Garlic bulb extract, *Aloe vera* leaf extract and Tulsi leaf extract, fungicide, *viz*., carbendazim 50 WP against *A. solani in-vivo* by growth parameters and disease intensity. The field experiment was laid out in a complete randomized block design with seven treatments and three replication. All the treatments significantly inhibited the disease intensity of *A. solani* as compared to control Experiments were also carried out in randomized block design with seven treatments and three replications. Neem leaf extract 20% was found to be the most effective treatment and recorded minimum disease intensity (27.72%) followed by *Trichoderma viride*, Dhatura leaf extract, garlic bulb extract, *Aloe vera* leaf extract and tulsi leaf extract as compare to treated check carbendazim 50 WP and untreated control. recorded at 45, 60 and 75 DAT respectively).

Keywords: *Alternaria solani*, carbendazim, early blight, bio-agent, plant extract, tomato, *Trichoderma viride*, efficacy

1. Introduction
Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops and is known as protective food both because of its special nutritive value and wide spread production Tomato is said to be the native of tropical America. From tropical America it spreads to other parts of the world in the 16th century and it became popular in India with in the last six decades. It is the world's largest vegetable crop after potato and sweet potato. Globally, tomatoes are grown on an area of 4528519 hectares with a production of 124748292 metric tonnes. In India, tomato is a popular crop, grown on 540000 hectares, producing 7600000 tonnes of tomatoes. (Ganeshan and Chethana, 2009) [3]. The leading tomato growing states are Orissa, Andhra Pradesh, Karnataka, Maharashtra, Bihar, West Bengal, Uttar Pradesh, Haryana, Punjab and Gujarat. Tomato has multipurpose uses in food industry. It is used both for fresh consumption as well as for processing purpose as soups, salad, pickles, sausages, ketchup, puree, chutney, jam and many other products (Thompson and Kelley, 1957) [6].Tomato has high medicinal value. It acts as a promoter of gastric secretion and blood purifier. It is a mild and natural stimulant, which helps to reduce the concentration of poison in the blood system. It is popular because it supplies vitamin C and adds variety of colour and flavour to food. It has special significance because of its dietary importance and nutritive value. Early blight of tomato (*A. solani*) is a soil inhabiting fungus and it can also come from other host through air and splashing rain. The germinating spores of *A. solani* penetrate susceptible tissue directly or through wounds and soon produce new conidia that are further spread by wind, splashing rain etc. (Agrios, 2005) [1]. The disease can occur over a wide range of climatic conditions, but it is most prominent in areas where received heavy dew deposition, heavy rainfall precipitation and high relative humidity. In severe rainfall, high humidity and fairly high temperatures 24-29 Care more favourable for disease development (Peralta *et al*. 2005) [4]. Spraying of broad spectrum fungicides like mancozeb and captan has been recommended for the control of early blight of tomato by several workers (Ramakrishnan *et al.*, 1971 and Stevenson, 1977). While the numbers of applications of these chemicals are more, they are less persistent on foliage. Thus, the controls achieved by these chemicals are inadequate. One of the reasons attributed for the low sensitivity of *A. solani* to fungicides mentioned above is the production of dark brown to black pigment called melanin by the fungus, which enhanced survival and competitive abilities of the pathogen, under certain environmental conditions (Bell and Wheeler, 1986) [2].

Therefore, in the present study it is thought worthwhile to test the efficacy of more promising chemicals like iprodione, propic onazole, difenconazole, pyraclostrobin, benlate, ridomil against early blight fungus. Not much light has been shed on the biological control, botanicals which are effective against *A. solani*. Hence, an attempt has been made to test some of commonly available bio-agents and botanicals against the pathogen.

2. Materials and Methods

The present study was conducted field condition at Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, during the *Kharif* season of 2019-20. Field experiment was laid-out in Randomized block design with three replications.

2.1 Experimental site

The present investigation was carried out in the Central Research Farm, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj

2.2 Location

Field experiments were conducted at the Central Research Far m, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj during *Kharif* season2019. The site selected was uniform, cultivable with typical sandy loam soil having good drainage.

2.3 Topography

Prayagraj is situated at an elevation of 98 m above the sea level at 25.87°N latitude and 81.25 °E longitudes.

2.4 Climate

The region is located in the south-eastern part of Uttar Pradesh and has subtropical climate with extreme of summer and winter. During the winter season especial in month of December and January the temperature drops down to as low as 1°C while during the summer temperature reaches up to 48-50°C. The average rainfall in area is around 1013.4 mm annually and maximum concentration during July to September with a few occasions of shower and drizzles in winter also. The soil type of experimental site was sandy loam, low in organic carbon, nitrogen and phosphorus.

2.5 General laboratory procedures

2.5.1 Glassware cleaning

For all laboratory experiments, Borosil and Corning glasswar were used. The glassware's were kept for 24 hours in the cleaning solution containing 60.0 g of potassium dichromate, 60.0 ml of concentrated sulphuric acid in 1000 ml of water. They were washed with detergent solution followed by rinsing with tap water and finally with distilled water.

1.5.2 Sterilization

The Petri plates and pipettes were wrapped in clean paper and sterilized in hot air oven at a temperature of 160°C for two hours**.** Sterilization of both solid and liquid media was achieved by autoclaving at 1.1 kg/cm^2 (121.6°C) pressure for 20 minutes for all the laboratory studies. All cultural studies were conducted in aseptic condition under laminar air flow. The tips of inoculation needle, forceps and cork borers were sterilized under flame.

1.5.3 Potato Dextrose Agar (PDA) media

Potato Dextrose Agar medium was used to isolate and maintain the culture of the pathogen *Alternaria Solani* from the diseased plant parts. The composition of PDA used is given below.

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

200 grams of peeled potatoes were cut into pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. 20 gram each of dextrose and agar-agar was dissolved in potato extracts and the final volume was made up to 1000 ml by adding distilled water. The flask containing dispensed medium was sterilized at 1.1 kg/cm^2 pressure for 20 minutes.

2.5.4 Early blight of Tomato

The species of *Alternaria* attack cruciferous plants such as mustard, rapeseed (toria or lahi), Eruca, cauliflower, knol-kohl and radish. Two species, *A. brasscae* and *A. brassicicola*, attack all the plants these fungi are seed-borne and cause shrivelling of the seed low germination. Spore and mycelium in diseased plant debris also serve as means of perennation.

2.5.5 The causal organism:

The conidiophores of *Alternaria solani*. Wiltshine are olivaceous, septet, branched 5-7.5 x 35-45 microns. Conidia are linear to obligate, borne in chains of 8-10, septet, muriform and on maturity, measure 11-17x50-75 microns. In *Alternaria Solani* (Berk.) Sacc. the conidiophores arise in fascicles and the conidia are dark, obclavate, muriform, 125-225x16-28 microns, borne singly or in short chains.

2.5.6 Characteristics of the pathogen

The morphological studies of *Alternaria Solani* were made on host and in medium (PDA) by using compound microscope. The taxonomy of *A. solani* has been based principally on morphology and sometimes host plant association of each of the species occurring (*A. solani*) has a distinct morphology considering the diversity of conidium shapes and sizes among *Alternaria* spp.

3.1 Isolation of the pathogen

The pathogen was isolated from the disease infected plants and it was identified as the *Alternaria Solani.* Early blight of Tomato infected leaves was collected. The infected leaves were cut into small pieces (0.5cm^2) surface sterilized with mercuric chloride (0.1%) for 15-30 seconds, rinsed with three changes of sterile distilled water to remove the disinfectant and blotted dry. The sterilized pieces were plated (4 pieces/dish) on Potato Dextrose Agar (PDA) medium in Petri dishes under aseptic conditions and incubated at 25^{0}C for 2 weeks. For obtaining sufficient quantity of inoculums, pure cultures were obtained by sub culturing. For this purpose, small bits of the fungus were taken at the tip of a sterilized needle and transferred aseptically to the centre of fresh PDA medium in Petri dishes. The dishes were incubated for 2 weeks at 25°C in the dark.

3.1.1 Identification of the Pathogen

Morphological studies of the pathogen were conducted from pure culture. Spore suspension in sterilized distilled water was made from pure culture of the pathogen grown on PDA.

One drop of the spore suspension was placed on a slide and morphological characters were noted with the help of microscope.

3.1.2 Maintenance of the culture
The fungus was sub cultured on PDA slants and allowed to grow at 27 ± 1° C for 15 days. Such slants were preserved in refrigerator at 5° C and sub cultured once in fort nightly. This pure culture was used for further study.

4.1 Experimental details
4.1.1 Field Preparation
The selected field area was well prepared and plot marked as per the lay out plan. The selected field was ploughed, cleaned and the soil was well pulverized after which the total area was divided into sub-plots.

4.1.2 Cultivation of Tomato
A F1 hybrid variety PKM-1 is chosen for the experiment. All the packages of practices were followed as per the general agronomic practices.

4.1.3 Raising of seedlings
Three trays each having size of 2.5 x 1.5 ft. were prepared for obtaining seedlings for transplanting and gap filling in the field for experimentation. The seed sowing was done on 23th July 2019. The seed were so on the raise bed. On the raise bed they were covered with the soil, raise bed has superior water holding capacity, excellent air space and high nutrient contents. The seed rate utilized was 500 g/ha (*i.e.* seedlings required for transplanting one hectare area field.). These raise bed were irrigated whenever required with the help of sprayer**.**

4.1.4 Transplantation of seedling
The experimental plot was laid out as per statistical design and necessary marking of the hills was done for transplanting the seedling. The healthy seedling of about 20-25 days old having uniform size were used for transplantation one these marked hills. The transplanting was done on 15th August 2019.

4.1.5 Gap filling
Gap filling were done where ever seedlings are not germinated in order to avoid the loss of land. It was done immediately after 3 days of transplanting. I.e. on 18th August 2019.

4.1.6 Intercultural operation
In a nursery beds, frequent weeding was done for keeping the beds weed free in order to get healthy seedlings. In field, weeding and hoeing were done as and when required to keep the plot weed free and to provide soil aeration.

4.1.7 Protective irrigations
Protective irrigations were given in nursery as well as in field when required.

5.1 Details of layout

Design	Randomized Block Design
Replications	3
Treatments	8
Plot size	2m x 1m
Total number of plots	24
Main irrigation channel	1m
Sub irrigation channel	0.5m
Width of bund	0.3m
Gross cultivated area	177.76m^2
Net cultivated area	96m^2
Crop	Tomato
Variety	PKM-1
Seed rate	450-500gm/ha
Spacing	60x45cm
Row to row distance	60cm
Plant to plant	45cm
N:P:K	120:80:80, NPK kg ha^{-1}
FYM	20 tons/ha

Table 1: Details of treatments on field management of Early blight of Tomato:

Sr. No	Treatments	Treatments name	Concentration
1	T_0	Control	-
2	T_1	Neem leaf extract	20%
3	T_2	Garlic bulb extract	20%
4	T_3	Dhatura leaf extract	20%
5	T_4	Tulsi leaf extract	20%
6	T_5	*Aloe vera* leaf extract	20%
7	T_6	*Trichoderma viride*	0.5%
8	T_7	Carbendazim 50WP	0.2%

A) Standard error of mean:
Standard error of means was calculated by following formula:

$$S.Em = \sqrt{\frac{2MSSE}{r}}$$

B) Critical Difference
Critical difference was calculated by following formula:

$$CD = \sqrt{\frac{2MSSE}{r}} \times t\alpha$$

Where,
tα = "t" table value at error degree of freedom at 5% level of significance
r = number of replications.
MSSE = mean sum of square due to error.
Significant "F" value indicates that, there is significant difference among the treatments.

4.1 Results and Discussion
The results of study entitled, **"**Efficacy of bio-agents and

botanical extracts against early blight (*Alternaria solani*) of tomato (*Solanum lycopersicum* L.)"Under field condition w ere conducted at the Department of Plant Pathology, Sam Hig ginbottom University of Agriculture Technology and Sciences , Prayagraj.

Plate 1: Early blight symptom on leaves

Plate 2: Early blight symptom on stem

Plate 3: Early blight symptom on fruits

Plate 4: Conidia of *Alternaria solani* on PDA

Plate 5: Pure culture of *Alternaria solani*

Table 2: Effect of treatments on Plant height at 45, 60 and 75 DAT

Treatments no.	Treatments	Plant height (cm)			
		45 DAT	60 DAT	75 DAT	Mean
T_0	Control	23.22	32.30	41.46	32.33
T_1	Neem leaf extract	28.26	39.36	46.38	38.00
T_2	Garlic bulb extract	25.40	38.38	45.34	36.31
T_3	Dhatura leaf extract	26.22	38.74	45.26	36.74
T_4	Tulsi leaf extract	24.42	35.34	43.26	34.34
T_5	*Aloe vera* leaf extract	25.22	37.46	44.30	35.72
T_6	*Trichoderma viride*	27.34	38.62	46.28	37.41
T_7	Carbendazim 50WP	30.24	40.44	48.44	39.71
S. Ed		0.19	0.18	0.21	0.69
CD (p=0.05)		0.34	0.33	0.38	1.21

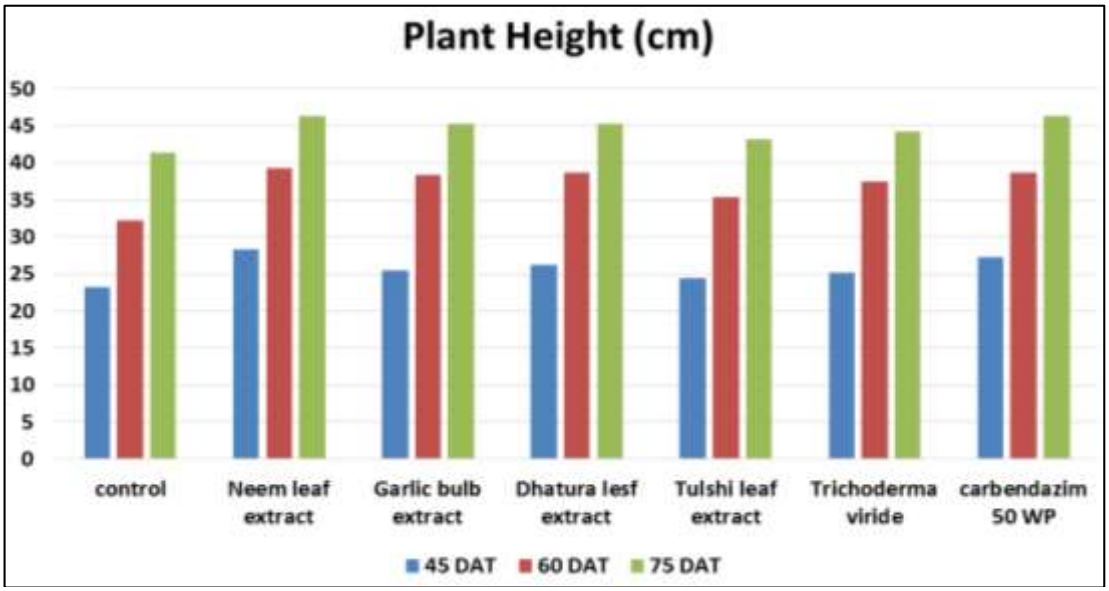

Fig 1: Effect of treatments on Plant height at 45, 60 and 75 DAT.

Table 2: Effect of treatments on per cent disease intensity of early blight of tomato at 45 DAT

Treatments		Disease Intensity %
T_0	Control	24.50
T_1	Neem leaf extract	20.20
T_2	Garlic bulb extract	22.23
T_3	Dhatura leaf extract	21.37
T_4	Tulsi leaf extract	22.33
T_5	*Aloe vera* leaf extract	22.37
T_6	*Trichoderma viride*	21.40
T_7	Carbendazim 50WP	17.36
F-test		S
SE.d		0.78
CD (5%)		0.43

4.5.1 Disease intensity at 45 DAT: Effect of plant extract and bio-agents on mean disease intensity percentage at 45 days after transplanting (DAT) is given in table 4.2 and depicted in figure 4.2

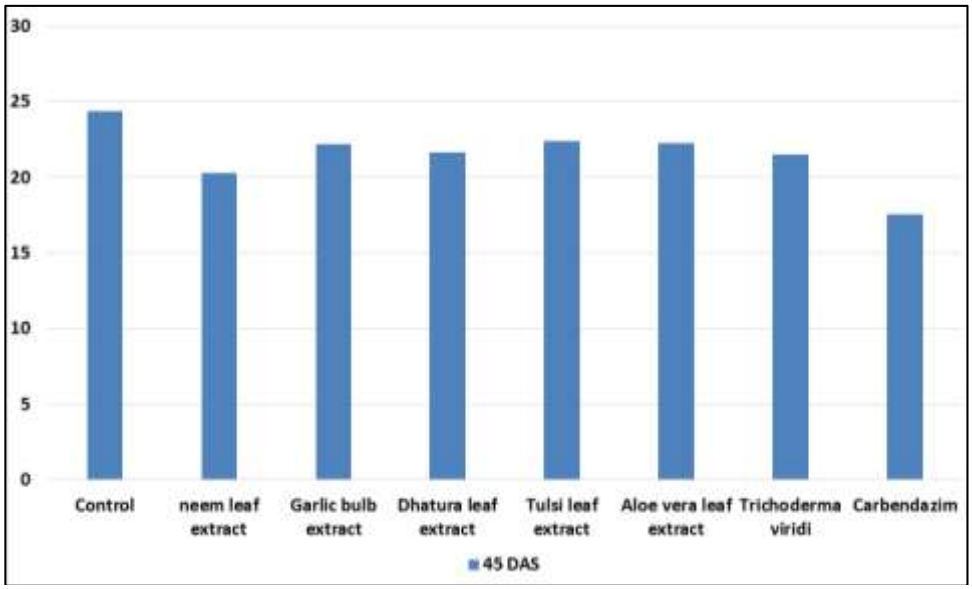

Fig 2: Effect of treatments on per cent disease intensity of early blight of tomato at 45 DAT

4.5.2 Disease intensity at 60 DAT: Effect of plant extract and bio-agents on mean disease intensity percentage at 60 days after transplanting (DAT) is given in table 4.3 and depicted in figure 4.3.

Table 3: Effect of treatments on per cent disease intensity of early blight of tomato at 60 DAT

Treatments		Disease Intensity %
T_0	Control	39.36
T_1	Neem leaf extract	23.24
T_2	Garlic bulb extract	26.30
T_3	Dhatura leaf extract	25.36
T_4	Tulsi leaf extract	29.63
T_5	*Aloe vera* leaf extract	27.50
T_6	*Trichoderma viride*	25.40
T_7	Carbendazim 50WP	20.73
F-test		S
S. Ed		0.70
CD (5%)		0.39

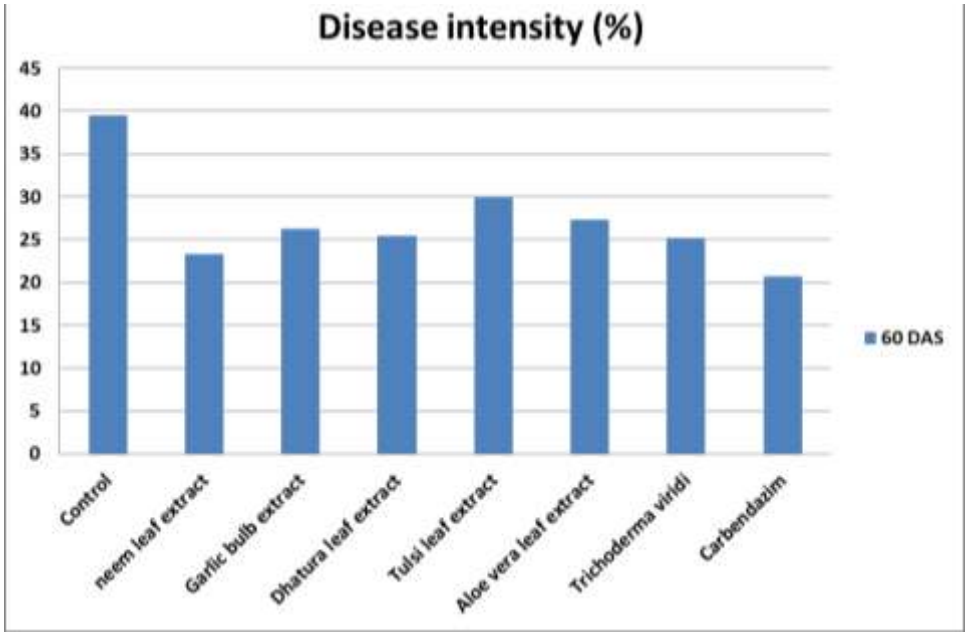

Fig 3: Per cent Disease Intensity of early blight disease of tomato at 60 DAT

4.5.3 Disease intensity at 75 DAT: Effect of plant extract and bio-agents on mean disease intensity percentage at 75 days after transplanting (DAT) is given in table 4.4 and depicted in figure 4.4

Table 4: Effect of treatments on per cent disease intensity of early blight of tomato at 75 DAT

Treatments		Disease Intensity %
T_0	Control	46.23
T_1	Neem leaf extract	27.60
T_2	Garlic bulb extract	31.53
T_3	Dhatura leaf extract	30.43
T_4	Tulsi leaf extract	38.36
T_5	*Aloe vera* leaf extract	33.60
T_6	*Trichoderma viride*	30.57
T_7	Carbendazim 50WP	23.56
F-test		S
S. Ed		0.63
CD (5%)		0.35

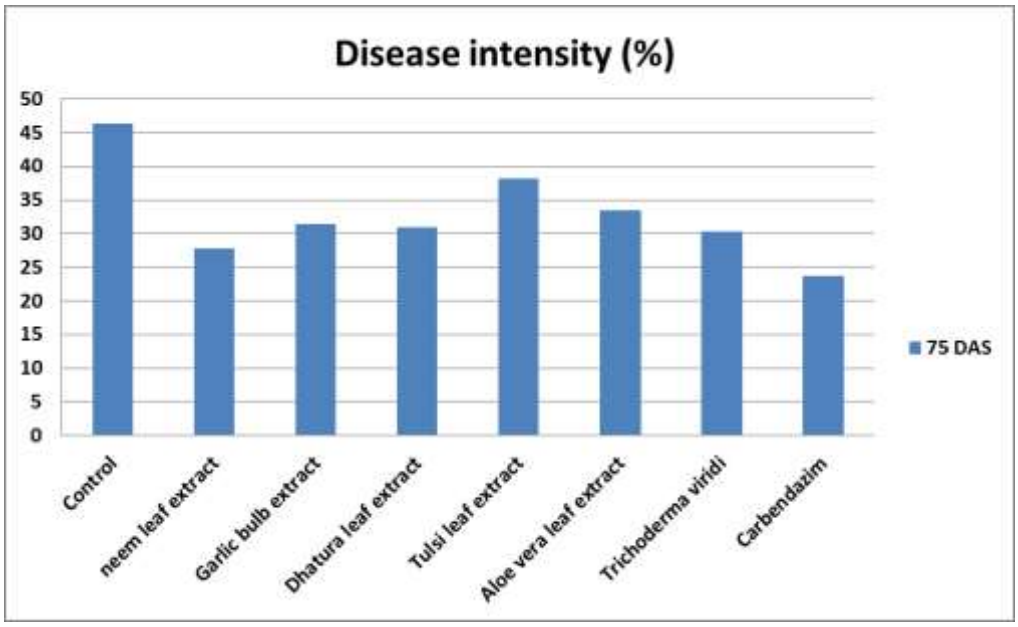

Fig 4: Per cent Disease Intensity of early blight disease of tomato at 75 DAT

5.1.2 Effect of treatments on Plant height at 45, 60 and 75 DAT

Data recorded in Table 4.1 showed that, among all treatments, maximum plant height (cm) at 45 DAT was recorded with T_1 Neem leaf extract (28.26) followed by T_6 Trichoderma *viride* (27.34), T_3 Dhatura leaf extract(26.22), T_2 Garlic bulb extract (25.40), T_5 *Aloe vera* leaf extract(25.22) and T_4 Tulsi leaf extract (24.42) as compare to treated check T_7 Carbendazim 50 WP (30.24) and minimum plant height was recorded with T_0 Control (23.22). At 60 DAT Data recorded in Table 4.1 showed that, among all treatments, maximum plant height (cm) at 45 DAT was recorded with T_1 Neem leaf extract (39.36) followed by T_6 *Trichoderma viride* (38.62), T_3 Dhatura leaf extract(38.74), T_2 Garlic bulb extract(38.38), T_5 *Aloe vera* leaf extract(37.46) and T_4 Tulsi leaf extract (35.34) as compare to treated check T_7 Carbendazim 50 WP (40.44) and minimum plant height was recorded with T_0 Control (32.30). At 75 DAT Data recorded in Table 4.1 showed that, among all treatments, maximum plant height (cm) at 45 DAT was recorded with T_1 Neem leaf extract (46.36) followed by T_6 *Trichoderma viride* (46.28), T_3 Dhatura leaf extract(45.26), T_2 Garlic bulb extract(45.34), T_5 *Aloe vera* leaf extract(44.30) and T_4 Tulsi leaf extract (43.26) as compare to treated check T_7 Carbendazim 50 WP (48.44) and minimum plant height was recorded with T_0 Control (41.46).

5.1.3 Disease Intensity of early blight disease of tomato at 45, 60 and 75 DAT

The data presented in table 4.2 and depicted in figure 4.2 reveals the response of plant extract and bio-agents against early blight of tomato under field conditions. At 45 DAT, among the treatments minimum percentage of disease intensity was recorded in treatments T_1 foliar spray of neem leaf extract (20.20) followed by T_6 *Trichoderma viride* (21.40), T_3 Dhatura leaf extract (21.37), T_2 Garlic bulb extract (22.23), T_5 *Aloe vera* leaf extract (22.37) and T_4 Tulsi leaf extract (22.33) as compare to treated check T_7-Carbendazim 50WP (17.36) and maximum percentage disease intensity was recorded with T_0 Control (24.50). All the treatments were found statistically significant from T_0 Control and the all the treatments were found significant to each other. The data presented in table 4.3 at 60 DAT, the results indicated that after first application of treatments, the least percentage of disease intensity was recorded in treatments T_1 foliar spray of neem leaf extract (23.24) followed by T_6 *Trichoderma viride* (25.40),T_3 Dhatura leaf extract (25.36),T_2 Garlic bulb extract (26.30), T_5 *Aloe vera* leaf extract (27.50) and T_4 Tulsi leaf extract (29.63) as compare to treated check T_7 Carbendazim 50WP (20.73) and maximum percentage disease intensity was recorded with T_0 Control (39.36).All the treatments were found statistically significant from T_0 Control and all the treatments were found significant to each other. The data presented in table 4.4 at 75 DAT, the results indicated that after second application of treatments, the least percentage of disease intensity was recorded in treatments T_1 foliar spray of neem leaf extract (27.60) followed by T_6 *Trichoderma viride* (30.57),T_3 Dhatura leaf extract (30.43),T_2 Garlic bulb extract (31.53), T_5 *Aloe vera* leaf extract (33.60) and T_4 Tulsi leaf extract (38.36) as compare to treated check T_7 Carbendazim 50WP (23.56) and maximum percentage disease intensity was recorded with T_0 Control (46.23).All the treatments were found statistically significant from T_0 Control and all the treatments were found significant to each other.

6. Summary and Conclusion

A field experiment was conducted in RBD by selecting the best treatments from in-vivo study, with five botanicals (neem leaf extract @ 20%, Garlic bulb extract@ 20%, Dhatura leaf extract @20%, Tulsi leaf extract @ 20%, *Aloe vera* leaf extract @20%), one bio-agents *Trichoderma viride*, one fungicide (carbendazim 50 WP) with untreated control (water spray) as foliar sprays against Early blight of tomato. From the yield (t/ha) study of the treatments the most effective treatment was neem leaf extract (16.40 t/ha) followed by *Trichoderma viride* (15.90 t/ha), Dhatura leaf extract (14.90 t/ha), Garlic leaf extract (14.30 t/ha), *Aloe vera* leaf extract (13.90 t/ha) and Tulsi leaf extract (13.40 t/ha) as compared to treated control carbendazim 50 WP (20.70 t/ha) and untreated control (water spray) (7.9 t/ha). However, all treatments were superior to the untreated control with respect to the yield in managing early blight of tomato. A field experiment was conducted in RBD by selecting the best treatments from in-vivo study, with five botanicals (neem leaf extract @ 20%, Garlic bulb extract@ 20%, Dhatura leaf extract @20%, Tulsi leaf extract @ 20%, *Aloe vera* leaf extract @20%), one bio-agents *Trichoderma viride*, one fungicide (carbendazim 50

WP) with untreated control (water spray) as foliar sprays against Early blight of tomato. From the economic study of the treatments the most effective treatments was treatment was neem leaf extract (1:4.41) followed by *Trichoderma viride* (1:4.29), Dhatura leaf extract (1:4.02), Garlic leaf extract (1:3.86), *Aloe vera* leaf extract (1:3.76) and Tulsi leaf extract (1:3.63) as compared to treated control carbendazim 50 WP (1:5.53) and untreated control (water spray) (1.2.17). However, all treatments were superior to the untreated control with respect to the cost benefit ratio in early blight of tomato.

7. References

1. Agrios GN. Plant Pathology, 5th edition. Elsevier Academic Press, London, 2005, 454-455.
2. Bell AA, Wheeler MH. Biosynthesis and Functions of Fungal Melanins. Annual Review of Phytopathology 1986;24:411-451.
3. Girija Ganeshan, Chethana BS. Bioefficacy of Pyraclostrobin 25%EC against Early Blight of Tomato. World Applied Sciences Journal 2009;7(2):227-229.
4. Peralta IE, Spooner DM. Classification of wild tomato. A festschrift for william g. Darcy. Kurtziana 2005;28:45-54.
5. Ramakrishna A, Desai S, Uma Devi G, Uma Maheswari, T. Antifungal Effect of Chitosans and Chito-oligosaccharides against Early Blight of Tomato. International Journal Current Microbiology Science 2018;7(07):61-66.
6. Thompson HC, Kelly WC. Vegetable crops. 5th Education McGraw Hill Book Company, 1957, 471-500.
7. Wheeler BEJ. An introduction to plant disease. John Wiley Sons Limited London 1969.