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Eco-friendly Management of Early Blight Disease caused by *Alternaria solani* (Ellis and Martin) in Tomato (*Lycopersicon esculentum* Mill.)

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The present studies were made to evaluate the efficacy of two bio-agents - *Trichoderma viride* and *Pseudomonas fluorescens*, four botanicals- crude extract of Garlic (*Allium sativum*) bulb, Eucalyptus (*Eucalyptus globulus*), Neem (*Azadirachta indica*) and Datura (*Datura stramonium*) leaves and compost + charcoal powder product against *Alternaria solani* causing early blight disease of tomato *in vitro* and *in vivo* glass house pots trials during *Rabi* season (2022-23). The experiment was carried out by Complete Randomized Design (CRD). *In vitro* experiments were carried out with nine treatments and further investigation were carried out in *in vivo* glass house pots trials. Results showed that minimum radial growth (mm) of *Alternaria solani* was recorded with garlic bulb extract @ 15% (13.33 mm) followed by *T. viride* @ 2×10v CFUs/ml (23 mm). Under *in vivo* glass house pots trials, garlic bulb extract @ 15% recorded the maximum plant height (28.51 cm, 56.42 cm and 69.26 cm at 30, 60 and 90 DAT respectively) and the minimum disease intensity (9.86%, 16.94%, 25.74% and 32.78% at 45, 60, 75 and 90 DAT) respectively.

Keywords : *Alternaria solani,* bio-agents, botanicals,charcoal powder,compost,early blight, tomato

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill. is regarded as one of the most significant and widely consumed vegetables in the world. The tomato is a member of the genus *Lycopersicon* and the family *Solanaceae*.The Mexican Indians have been growing tomatoes since prehistoric times, and their term for the plant is "*tomati*", which is likely where the name "tomato" originates (Dhaliwal, 2012). This herbaceous sprawling plant has a weak woody stem and can reach a height of 1 to 1.5 meters.Tomato is regarded as a protective food due to its unique nutritional content and extensive global production in temperate, tropical, and sub-tropical Climates It is grown in natural conditions as well as in greenhouses or other protective constructions.

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It holds second position in the world's vegetable production and is the most consumed vegetable crop after the potato crop and ranks first among the processing crops in the world (Jewaliya *et al*. 2021). Tomato originated in South America and is now grown extensively in 140 nations. In terms of tomato production, China leads the world (31%), followed by the United States and India, which rank second and third, respectively (Shamurailatpam and Kumar, 2020**).** More than one-third of the world's production came from China, the greatest producer (52.6 million tons), followed by India (18.7 million tons) and the United States of America (14.5 million tons).Andhra Pradesh, Karnataka, Madhya Pradesh, Uttar Pradesh, Telangana, Orissa, Gujarat, Maharashtra, West Bengal, Bihar, Chhattisgarh and Himachal Pradesh are the major tomato growing states in India. These states account for 91% of the total production of the country. During 2021-22, the area under tomato cultivation in Uttar Pradesh was10.40 thousand hectares (Singh *et al.*2023). In India it is cultivated on 841 thousand

ha areas with an annual production of 20336 thousand metric tonnes during 2021-22 (Ghalawat *et al*. 2024**)**.

Tomato holds 95.3% of water, 0.07% calcium, phosphorus, iron, amino acids, sugars, dietary fibres and niacin (vit B3), all of which are crucial in metabolic activities of humans. It is referred to as "Poor man's orange" as it provides a balanced source of Vitamin A, C and E (Dhaliwal, 2012)as well as other antioxidants.They grow best in well-drained, fertile, light sandy loam soil with a pH of 6.0 to 7.0 and a good capacity to retain moisture (Dhaliwal, 2012).

The reduction in yield of tomato occurs due to various fungal, bacterial, viral diseases and disorders. Some important diseases of tomato are early blight, fusarium wilt, damping off, late blight, tomato mosaic virus, verticillium wilt, and bacterial wilt. The air-borne soil-inhabiting fungus *Alternaria solani* (Ellis and Martin) Jones and Grout causes early blight (EB) in tomatoes, which is known to be one of the most damaging diseases of the crop and can cause crop losses of up to 80% under a variety of climatic regions. Conidia or mycelia of the pathogen can overwinter in plant waste or soil, and it can serve as a source of primary inoculum when the favourable conditions prevail, including humidity (70–80%), frequent rainfall, heavy dew deposition, and a suitable temperature (27–32°C) (Rasool *et al*. 2021). When *A. solani* spores germinate, they can enter susceptible tissue directly or through wounds, producing additional conidia that are then dispersed by wind, rain, and other factors (Agrios, 2005).

Disease symptoms appear on foliage (leaf blight), stems (collar rot) and fruits (fruit rot) of tomato. Irregular, brown to dark brown coloured necrotic lesions with concentric rings giving target like appearance ("bull eye"), first appear on the older leaves of tomato plant which become enlarged with the progression of infection and cover the whole leaf. The age of the host plant determines its susceptibility to infection.It has been advised to use broad-spectrum fungicides, such as captan and mancozeb, to reduce early blight in tomato.Since the chemicals has negative impacts on the environment and human health and develops resistance in pathogen thus emphasis is now given on the use of indigenous sources and bio-agents for the management of plant diseases which is cost effective, eco-friendly, sustainable and doesn't affect public health and environment. Maintaining existing levels of agricultural production while lowering the amount of fungicides and other harmful chemicals released into the environment is possible with biological control. The use of very well-known plant growth promoting rhizobacteria (PGPR), *Pseudomonas fluorescens* and fungal antagonist like *Trichoderma* sp. is promising biological control strategies used for the disease management. Bio-agents and plant extracts represent a natural and ecological approach for managing diseases and provide many distinctive benefits to farmers, as these are non-phytotoxic, systemic, easily biodegradable and reduce the risk of residual effects on food. Considering the above mentioned strategies, this study was aimed to evaluate the efficacy of selected treatments on *Alternarias olani in vitro* and growth parameter and early blight disease intensity in tomato crop *in vivo* under glass house conditions.

MATERIALS AND METHODS

The current investigation was performed in *in vitro* and *in vivo* glass house pots using Complete Randomized Design (CRD) at Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj on early blight disease of tomato crop during *Rabi* season of 2022-2023. Geographically, Prayagraj district of U.P. is situated at 25.87°N latitude and 81.25°E longitudes at an elevation of 98 m above the sea level. Temperature extremes in the summer and winter define the mostly semi-arid and sub-tropical climate. Summer temperatures peak around 47°C, while winter temperatures fall to 2-3°C.

All laboratory experiments were carried out under aseptic conditions using sterilized glass goods and culture media.

Pathogen isolation

The diseased samples were collected from infected tomato plants for isolation of pathogen. The infected leaves were cut into 5 mm long and

2 to 3 mm breadth pieces in such a way that it contains both infected and healthy portions. A pinch of *Streptomycin* powder, an antibiotic was added to prepared PDA before use to inhibit probable bacterial growth. Surface sterilization of these pieces was done with 0.1% $HgCl_2$ solution for 30 sec followed by two changes in sterilized distilled water. Sterilized blotting paper was used to dry those pieces and then transferred those aseptically to sterilized Petri plates containing cooled solidified potato dextrose agar (PDA) medium and incubated at 25±2^{0}C and examined for the association of fungi after 5 days at frequent intervals. The fungal colonies appeared in the incubated plates were sub-cultured into fresh medium and then pure cultures were obtained.

Identification of the pathogen

Further examination of the fungal colony characteristics was done through compound microscope to confirm its identity. The culture was taken in small portions and put on a sterile glass slide using a sterile needle. Cotton blue and lactophenol were used to tint it. After then, the isolated fungus was identified using morphological traits in comparison to reference materials. Under microscope, characters of the pathogen were observed including mycelia branching, colour of mycelia, septa of mycelia, spores etc.

Culture Maintenance

The fungus was sub-cultured under aseptic conditions by transferring a small piece of the fungal growth to PDA slants and PDA plates and allowed to grow at 28±1°C for 15 days. Such mother culture was preserved at 4°C in refrigerator. Additionally, once in a month, these cultures were subcultured. Following the hyphal tip culture, pure culture of the fungus was aseptically obtained and used for further study.

Collection of bio-agents, botanicals, compost and charcoal powder

Leaves of all the three plants, *viz.*,Eucalyptus (*Eucalyptus globulus*), Neem (*Azadirachta indica*) and Datura (*Datura stramonium*) were collected from Central Research Farm, SHUATS, Prayagraj. Garlic bulb (*Allium sativum*), *Psf* (*Pseudomonas fluorescens*) powder and *Trichoderma viride* powder were purchased from Alopi bag market, Prayagraj. Compost and charcoal powder were collected from livestock unit, Department of Animal Husbandry and Dairying, SHUATS, Prayagraj.

Preparation of botanical extracts and extracts of compost and charcoal powder

The botanicals used for the experiment are Garlic (*Allium sativum*), Eucalyptus (Eucalyptus globulus), Neem (*Azadirachta indica*) and Datura (*Datura stramonium*). The bulb of garlic and leaves of neem, datura and eucalyptus were used to prepare crude extracts. All these botanical extracts were used in 15% concentration level. Extracts were prepared by grinding 100g fresh, washed leaves and bulbs with the help of a clean blender with 100 ml distilled water (1:1 W/V). Three layers of muslin cloth were used to filter the macerate and the resulting extract was regarded as a standard extract (100%) (Shekhawat and Prasada, 1971).The water extracts of compost (20%) and charcoal powder (6%) were prepared by mixing those materials with distilled water and kept overnight for complete soaking. Next day three-fold of muslin cloth was used to filter the solution and filtrate was used as extract.

Measurement of the radial growth of test pathogen

The efficacy of bio-agents *viz.*, *Pseudomonas fluorescens* and *Trichoderma viride* were screened *in vitro* against isolated pathogen *Alternaria solani* following dual culture technique (Dennis and Webster, 1971)andthe efficacy of four botanicals *viz.*, crude extract of Neem leaf, Eucalyptus leaf, Datura leaf, Garlic bulb at 15% concentration each and mixture of Compost (20%) and Charcoal powder (6%) were screened *in vitro* against *Alternaria solani* following poison food technique (Nene and Thapliyal, 1982) for further evaluation in pot. After every 24 hrs, the observations were recorded.

The efficacy of bio-agents, botanicals and compost + charcoal powder was expressed as per cent inhibition of mycelial growth over control

and calculated using the following formula (Vincent, 1947**).**

$$I = \frac{C - T}{C} x100$$

Where, I = Per cent inhibition of mycelial growth
C = Colony diameter in control (mm)
T = Colony diameter treatment (mm)

The further experiment was directed*in vivo* glass house pots at Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj.

Pots and soil preparation

Almost 100 kg sandy loam soil was collected from the Central Research Farm, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj. First soil was made weed free and cleaned. Then soil and pots were sterilized by spraying 1.5 ml formaldehyde/ liter of water. Thereafter, pots were kept in sunlight for drying and soil was kept airtight in big sized sacks or polythene bags for 48 hrs after which the pots were filled with the soil.

Cultivation of tomato

A local variety of tomatocrop was chosen for the experiment and tomato seedlings were bought from local nursery, Prayagraj.Transplanting was done in the afternoon in 45 earthen pots (10 inches) with 5 replications and 9 treatments including control using Complete Randomized Design (CRD) as given in Table 1. Three weeks old healthy tomato seedlings having uniform size were planted to pots carefully. The transplants were watered as and when required. However, light irrigations were given at 2 to 3 days interval in winter.In pots, weeding was done as and when required to provide proper soil aeration to encourage good crop growth. Staking was provided for good crop stand and better fruit quality. Every pot of 5 replications were tagged for observation and recording the data.

Inoculations of the pathogen

Spore suspension containing inoculum load of 10^4 spores/ml was applied as foliar spray at 30 DAT (Day After Transplanting) of tomato crop in the pots at dusk and the pots were kept covering with polythene bags overnight. The polythene bags were removed on the next morning.

Observations recorded

The height of tomato crops was measured in centimeter (cm) from ground level to the top of the main shoot of all the replications at 30, 60 and 90 DAT.Percent disease intensity (%) was recorded after appearance of *Alternaria* blight of tomato at 45, 60, 75 and 90 days after transplanting (DAT). The formula for calculating the disease intensity was given by **Wheeler (1969).** Disease intensity (%) is calculated by using the following formula :

$$PDI\ (\%) = \frac{Total\ Disease\ ratings}{Total\ No.\ of\ leaves\ assessed\ x\ Maximum\ disease\ rating\ observed} \times 100$$

Disease rating scale from 0-5 was used as given by Pandey *et al.* (2003) for tomato early blight in details (Pandey *et al*. 2003)

Statistical Analysis

In the present study, complete randomized design (CRD) was applied. The analysis of variance (ANOVA) and critical difference techniques were applied for drawing conclusion from the data. The value was calculated at 5% level of probability for the appropriate degree of freedom.

Standard error of difference, *S.E(d)* was calculated by the following formula ;

$$S.E(m) = \sqrt{MESS / r}$$

$$S.(d) = \sqrt{2xS.E(m)}$$

Where, *S.E(m)* = Standard error of mean

MESS = Mean sum of square due to error
r = No. of replications
t = No. of treatments

The following formula was used to calculate Critical difference (C.D.) :
C.D. at 5% **=** *S.E(d)* × t(5%) edf.
Where, df = Degree of freedom

RESULTS AND DISCUSSION

Effect of the treatments on radial growth of Alternaria solani in vitro

The experimental data as presented in Table 3 and Fig.1 revealed the minimum radial growth (mm) in treatment T_9: Garlic bulb extract (13.33) followed by T_4:*Trichoderma viride* @ 2×10^6 CFUs/ ml (23.00), T_7: Eucalyptus leaf extract (29.66), T_5: *Trichoderma viride* @ 0.25% (37.33) and maximum radial growth (mm) was recorded in T_1: control (90.00). All the treatments are found statistically significant compared to untreated control but the treatments (T_2 and T_3), (T_3 and T_8) and (T_8 and T_6) are statistically non-significant with each other and T_9, T_4, T_7, T_5 are statistically significant over all other treatments. Among the treatments T_9: Garlic bulb extract is found superior over all other treatments.

Effect of the treatments on plant height (cm) at 30, 60 and 90 DAT on tomato crop

Results indicatethat maximum plant height (cm) at 30,60 and 90 DAT were observed in treatment T_9: Garlic bulb extract (28.51, 56.42, and 69.26) respectively followed by T_4:*Trichoderma viride* @ 2×10^6 CFUs/ml (26.72, 52.40 and 65.12) and T_7: Eucalyptus leaf extract (25.04, 51.16 and 62.27). The minimum plant height (cm) was recorded in control (T_1) 19.31, 34.15 and 48.29 at 30, 60 and 90 DAT respectively.All the treatments are found statistically significant compared to control and the treatment T_9: Garlic bulb extract is found superior over all the treatments (Table 4, Fig. 2).

Table 1 : Treatment details with the bioinoculants

Treatments	Details
T_1	Control (foliar spray of distilled water)
T_2	20% Compost + 6% Charcoal Powder
T_3	*Pseudomonas fluorescens* inoculation @ 10g *Psf* powder/L of water
T_4	*Trichoderma viride* @ 2×10^6 CFUs/ ml
T_5	*Trichoderma viride* @ 0.25%
T_6	*Azadirachta indica* (Neem) leaf extract @ 15% conc.
T_7	*Eucalyptus globulus* leaf extract @ 15% conc.
T_8	*Datura stramonium* leaf extract @ 15% conc.
T_9	*Allium sativum* (Garlic) bulb extract @ 15% conc.

Table 2: Disease rating scale for tomato early blight in details (Pandey *et al*. 2003)

Ratings	Details
0	No infection
1	1-10% leaf area infected
2	11-25% leaf area infected
3	26-50% leaf area infected
4	51-75% leaf area infected
5	More than 75% leaf area infected

Effect of the treatments on early blight disease intensity

Minimum disease intensity (%) at 45, 60, 75 and 90 DAT were observed in treatment T_9: Garlic bulb extract (9.86%, 16.94%, 25.74% and 32.78%) followed by T_4: *Trichoderma viride* @ 2×10^6 CFUs/ml (10.79%, 20.74%, 29.47% and 36.06%) andT_7: Eucalyptus leaf extract (11.33%, 22.49%,

Table 3: Effect of the treatments on radial growth of *Alternaria solani*

Treatments	Details	Radial growth? (mm)	Percent Inhibition(%)
T_1	Control (foliar spray of distilled water)	90	0.00
T_2	20% Compost + 6% Charcoal Powder	51	43.33
T_3	*Pseudomonas fluorescens* inoculation @ 10g *Psf* powder/L of water	49.33	45.18
T_4	*Trichoderma viride* @ 2×10^6 CFUs/ ml	23	74.44
T_5	*Trichoderma viride* @ 0.25%	37.33	58.52
T_6	*Azadirachta indica* (Neem) leaf extract @ 15% conc.	42.33	52.96
T_7	*Eucalyptus globulus* leaf extract @ 15% conc.	29.66	67.03
T_8	*Datura stramonium* leaf extract @ 15% conc.	46	48.88
T_9	*Allium sativum* (Garlic) bulb extract @ 15% conc.	13.33	85.18
C.D. (p= 0.05)		4.490	-
S.Em. (±)		1.511	-

*Data are the mean of five replicates.

Table 4 : Effect of the treatments on plant height (cm) at 30, 60 and 90 DAT of tomato crop

Treatments	Details	Plant height*(cm) 30 DAT	60 DAT	90 DAT
T_1	Control (foliar spray of distilled water)	19.31	34.15	48.29
T_2	20% Compost + 6% Charcoal Powder	21.09	40.66	52.60
T_3	*Pseudomonas fluorescens* inoculation @ 10g *Psf* powder/L of water	21.73	44.21	57.59
T_4	*Trichoderma viride* @ 2×10^6 CFUs/ ml	26.72	52.40	65.12
T_5	*Trichoderma viride* @ 0.25%	24.54	49.96	59.36
T_6	*Azadirachta indica* (Neem) leaf extract @ 15% conc.	23.51	48.56	58.60
T_7	*Eucalyptus globulus* leaf extract @ 15% conc.	25.04	51.16	62.27
T_8	*Datura stramonium* leaf extract @ 15% conc.	22.77	48.54	57.83
T_9	*Allium sativum* (Garlic) bulb extract @ 15% conc.	28.51	56.42	69.26
C.D. (p=0.05)		1.615	3.276	3.763
S.Em (±)		0.563	1.142	1.312

*Data are the mean of five replicates.

30.16% and 38.18%).The maximum disease intensity (%) was observed in T_1: control 22.27%, 35.10%, 42.51% and 53.89% at 45, 60, 75 and 90 DAT respectively. All the treatments are found statistically significant compared to control and the treatment T_9: Garlic bulb extract is found superior over all the treatments(Table 5, Fig.3).

Similar findings were reported by Devi *et al*. (2017) and Kumar *et al*. (2018), who observed that garlic bulb extract resulted in the lowest radial growth (mm) of *Alternaria solani*. Consistent results were also documented by Nashwa and Abo-Elyousr (2012), Bhagat and Zacharia (2018), and Chakma *et al*. (2023), indicating that spraying tomato crops with garlic bulb extract led to the highest plant height (cm) and the lowest disease intensity (%),

Table 5: Effect of the treatments on early blight disease intensity (%) at 45, 60,75 and 90 DAT of tomato crop

Treatments	Details	Disease Intensity*(%)			
		45 DAT	60 DAT	75 DAT	90 DAT
T_1	Control (foliar spray of distilled water)	22.27 (28.12)	35.10 (36.32)	42.51 (40.67)	53.89 (47.21)
T_2	20% Compost + 6% Charcoal Powder	18.42 (25.25)	31.58 (34.17)	38.66 (38.42)	49.09 (44.46)
T_3	*Pseudomonas fluorescens* inoculation @ 10g *Psf* powder/L of water	15.21 (22.79)	28.26 (32.08)	36.63 (37.21)	46.59 (43.02)
T_4	*Trichoderma viride* @ 2×10^6 CFUs/ ml	10.79 (19.09)	20.74 (27.06)	29.47 (32.84)	36.06 (36.88)
T_5	*Trichoderma viride* @ 0.25%	12.93 (20.99)	23.82 (29.17)	31.02 (33.82)	39.53 (38.93)
T_6	*Azadirachta indica* (Neem) leaf extract @ 15% conc.	13.29 (21.32)	24.95 (29.92)	32.72 (34.86)	40.99 (39.79)
T_7	*Eucalyptus globulus* leaf extract @ 15% conc.	11.33 (19.63)	22.49 (28.27)	30.16 (33.28)	38.18 (38.14)
T_8	*Datura stramonium* leaf extract @ 15% conc.	14.57 (22.39)	25.17 (30.08)	34.18 (35.75)	44.15 (41.61)
T_9	*Allium sativum* (Garlic) bulb extract @ 15% conc.	9.86 (18.25)	16.94 (24.27)	25.74 (30.45)	32.78 (34.91)
C.D.(p=0.05)		2.897	1.812	2.071	1.814
S.Em.(±)		1.006	0.629	0.719	0.630

*Data are the mean of five replicates.

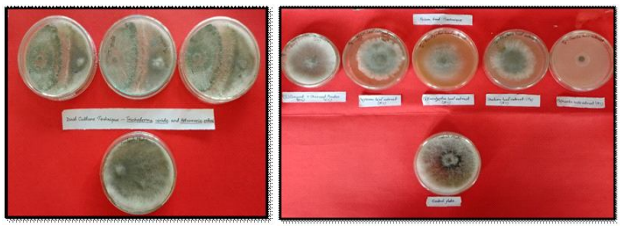

Fig.1: Effect of the treatments on radial growth of *Alternaria solani*

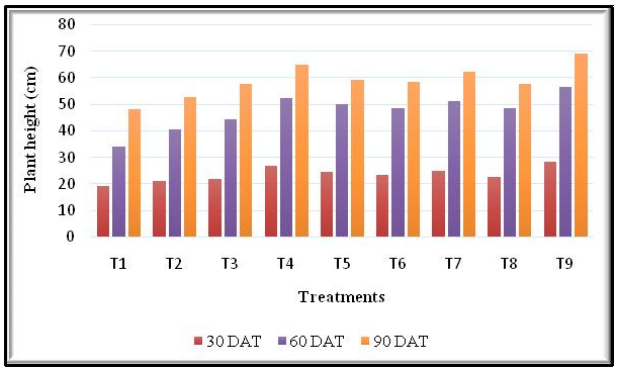

Fig.2: Effect of the treatments on plant height (cm)at 30, 60 and 90 DAT of tomato crop

outperforming other treatments. The garlic bulb extract contains various phytochemicals, including alkaloids, flavonoids, saponins, allicin, and sulfur-containing compounds, which exhibit broad-spectrum antimicrobial activity against harmful pathogens. Overall, the present finding demonstrated that three foliar applications of

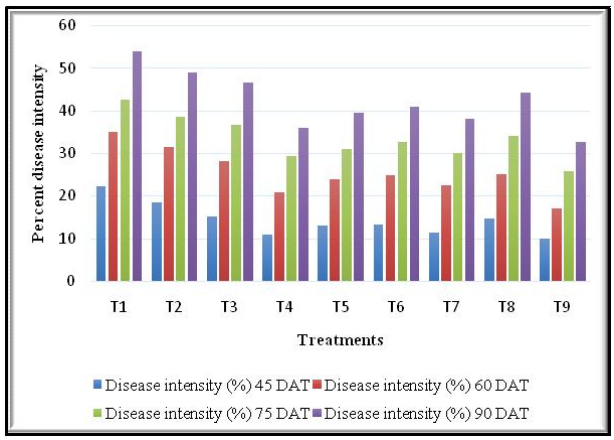

Fig. 3: Effect of the treatments on early blight disease intensity (%) at 45, 60,75 and 90 DAT of tomato crop

garlic bulb extract @15% at 15 days interval could be serve as an eco-friendly alternative to chemical fungicides for enhancing tomato plant growth and managing early blight effectively.

CONCLUSION

The results of experiment concluded that among all treatments, Garlic bulb extract @ 15% The effectiveness of garlic bulb extract may be due to presence of antimicrobial properties like diallyl-disulphide and diallyl-tri-sulphide and due to suppression of fungal growth which leads to the

increase in plant growth. The current experiment proved that without using any chemical, management of early blight disease in tomato can be profoundly possible by the use of different bio-agents and botanicals. However, the present studies are restricted to one crop season and significantly effective under Prayagraj agro climatic condition, therefore for substantiation of current result, more trials over many seasons should be conducted in future for further recommendations.

DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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