Antagonistic Activities of Homeopathic Medicines And Bioagents Against *Alternaria Solani* Causing Early Blight of Tomato

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Alternaria solani(Ellis and Martin)causing early blight of tomato is a destructive disease which causes an economic yield losses ranges from 34-79per cent. The present study is carried out to assess inhibitory effect of homeopathic medicines and bioagents against *Alternaria solani*. Among homeopathic medicines, *Arnica montana* at 0. followed by Sulphur (69.94%) and *Silicea terra* (66.45%). Among bioagents, maximum inhibition (71.04%) was observed in *Trichoderma harzianum* followed by *Trichoderma viride* (68.00%), *Pseudomonas fluorescence* (64.97%) and *Bacillus subtilis* (52.18%) in *in vitro* 20 per cent concentration and was found highly effective and inhibit the growth by 72.21per cent respectively condition.

Keywords: Homeopathic medicines; Antifungal activities; Plant pathogenic fungi; Bioagents.

1. INTRODUCTION

The tomato (L*ycopersicon esculentum* Mill), a member of the Solanaceae family, stands as one of the most profitable and extensively cultivated vegetable crops globally. It ranks second only to potatoes among all vegetables in terms of processed crop production. This plant is primarily grown for its edible fruits, which are consumed both fresh and cooked, providing a rich source of vitamins, organic acids, essential amino acids, and dietary fibbers. In the years 2021–22, tomato cultivation in India reached a production level of 20 MMT witha productivity of 24.4 metric tons per hectare, spanning an area of 840,000 hectares.

Among the various fungal diseases affecting tomato crops, early blight, caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, stands out as one of the most devastating, resulting in significant qualitative and quantitative losses. Reports from India, Canada, USA, and Nigeria indicate yield losses ranging from 48% to 80 percent due to early blight damage [1, 2, 3, 4, 5, 6]. Annual economic losses attributable to early blight have been estimated at 79percent [7].

Mancozeb is commonly recommended for managing *Alternaria* spp., but due to the adverse effects of fungicides,however, highly effective alternatives for disease management may not always be available or cost-effective. There are limited reports on the use of homeopathic medicines and

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bioagents in controlling various plant pathogen. Some studies have demonstrated the inhibitory effects of homeopathic drugs such as *Lycopodium*, *Thuja, Arsenicum*, *Arnica montana*, and *Zincum* against *Alternaria solani, Alternaria alternata, Fusarium moniliforme, Gloeosporiumpsidii, Colletotrichum gloeosporioides*and certain fruit rot pathogens [8,9,10]. Sulfur is another effective homeopathic medicine against some plant pathogenic fungi such as Fusarium wilt (caused by *F. oxysporum*), Septoria leaf spot (caused by *S. lycopersici*), and Phomopsis blight (caused by *P. vexans*) [11]. Research has demonstrated that sulfur can totally prevent Aspergillus parasiticus from growing[12], and at 200 ppm, sulfur has been found to totally prevent the growth of 22 different fungal genera [13]. Moreover, silicea-induced inhibition of A. solani's mycelial proliferation [14]. Similarly, several bioagents such as *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluroresenes,* and *Bacillus subtillis* are being explored for eco-friendly management strategies against early blight of tomatoes. These bioagents are harmless bacterial and fungal species found in nature that protect plant roots from diseases [15, 16]. Previous studies have reported the suppressive effects of several biocontrol agents, including *Pseudomonas* species and *Trichoderma harzianum*, on fungi [17, 18, and 19]. Furthermore, these bioagents are easily biodegradable, non-phytotoxic, systemic, and environmentally safe [20]. The objective of this study is to document and assess the effectiveness of three homeopathic medicines at appropriate concentrations and four bioagents, namely *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens,* and *Bacillus subtillis*, on inhibitingthegrowth of *Alternaria solani*.

2. MATERIAL AND METHODS

2.1 Isolation of pathogen

This pathogen was isolated from the infected plant that showed typical indications of blight. To ensure that there were no dust particles left, the leaves of the sick plant were meticulously cleaned with tap water and then rinsed with distilled water. Then, with a sterile knife, the diseased sections of the leaves were finely sliced such that every piece had a combination of healthy and diseased tissues. After being properly rinsed three times with distilled water to eliminate any remaining $HgCl_2$, these cut pieces were briefly submerged in a 0.15% solution of mercuric chloride for thirty seconds. For the purpose of eliminating extra water, the pieces were aseptically placed between two folds of sterilized blotter paper. Prepared autoclaved potato dextrose agar (PDA) was put into each of the sterilized Petri plates after they had been heated to 165°C for two hours in a hot air oven and moved to a laminar airflow chamber. Using sterilized forceps, the surface-sterilized leaf pieces were carefully placed in each plate once the media had set. After that, the plates were sealed with Parafilm tape and kept at 25±1°C in an incubator. Every day, the proliferation of mycelia surrounding the leaf fragments was observed.

2.2 Identification of the pathogen

The identification of the pathogen was carried out by examining its morphological and cultural traits, along with its pathogenicity towards the host plant. *A. solani* was distinguished from other

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genera by its characteristic transversely and longitudinally septate (muriform) conidia. These conidia exhibited a noticeable beak, which could vary in length from short to very long. Typically, the conidia were arranged in chains and appeared dark brown, with an obclavate shape in the present case [21].

2.3 Evaluation of homeopathic medicines against *Alternaria solani*

Using a potato dextrose agar (PDA) medium and the poisoned food technique, four homeopathic remedies like *Arnica montana*, *Thuja occidentalis*, Sulfur, and *Silicea terra*were assessed for their ability to effectively combat *A. solani*. For every homeopathic medication, a 0.20% concentration was prepared. Each necessary dose of a homeopathic remedy was aseptically added to autoclaved PDA at a concentration of 0.2% after being inoculated with *A. solani*. After that, the plates were incubated for eight days at 26 ± 2°C. After being incubated for 2, 4, 6, and 8 days, the mycelium's growth was monitored. The control group consisted of Petri plates devoid of any homeopathic medication.

2.4 Evaluation of bioagents against *Alternaria solani*

All the bioagents (*Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens,* and *Bacillus subtilis*) were evaluated using the dual culture technique. A mycelial disc (5 mm in diameter), obtained from the peripheral region of a 5-7 day old culture of the pathogens grown on potato dextrose agar (PDA), was placed on a fresh PDA plate, positioned 3 cm from the centre. Then, a 5 mm mycelial disc, obtained from the periphery of a 5-7 day old culture of fungal bioagents, was placed 3 cm away from the pathogen inoculums. For bacterial bioagents, streaking was done 3 cm away from the pathogen inoculums. Three replicates of each treatment were maintained, with one control set without the inoculation of bioagents. The plates were then incubated at 26±2°C and measurements were taken on different days. At the end of the incubation period, the radial growth of mycelium was measured.Radial growth reductions were calculated in relation to growth of the control using the following formula [22]

$$\text{Growth inhibition \%} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

3. ResultsAnd Discussion

In vitro evaluation of homeopathic medicines and bio-agents on against *A. Solani*

The evaluation of all homeopathic medications resulted in the inhibition of pathogen mycelial proliferation. Among the homeopathic medicines,*A. monta* at 0.20 per cent conc. was significantly

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inhibits the growth (72.21 per cent) of test pathogen with maximum per cent inhibition over control of 72.21 per cent. Which was followed by sulphur @ 0.20 per cent recorded the radial growth of 69.94 per cent. While, the minimum radial growth (66.45 per cent)of test pathogen was recorded with *Silica terra* @ 0.20 per cent. Among the bioagents tested, *Trichoderma harzianum* was found to be most effective with highest mycelial growth inhibition of the test pathogen (25.43 mm) followed by *Trichoderma viride* (28.10 mm) and highest mycelial growth inhibition (71.04%)ofthe test pathogen followed by *Trichoderma viride* (68.00%), respectively. *Bacillus subtilis w*as found comparatively less effective with maximum mycelial growth (42.00 mm) and minimum mycelial growth inhibition (52.18%). All the treatments were found significantly superior over the control.The antifungal activity of homeopathic medications, such as *T. occidentalis, sulfur, A. montana, A. phosphoricum, Spongia tosta*and *Chelidonium majus,* were described by [23, 24], which supported the comparable results. The Potato Dextrose Agar (PDA) medium was used to test these therapies separately and in combination with mancozeb against the mycelial development of *A. alternata* using the food poisoning method. The effect of four homeopathic medicines like *T. occidentalis, sulfur, A. montana* and silicea, against *A. solani* was tested at different concentrations (50, 100, 150, and 200 ppm) in an *in vitro* setting. The results showed that *T. occidentalis* at 200 ppm (76.73%) had the highest mycelial growth inhibition over control, followed by *A. montana* at 200 ppm (75.35%) and sulfur at 200 ppm (74.29%). At 50 ppm (39.42%), silicea exhibited the lowest mycelial growth inhibition in comparison to the control, resulting in a 47.13 mm mycelia growth at 8 DAI [25].

Table1: Effect of homeopathic medicines and bio-agents on mycelial growth of *Alternaria solani*

S. No.	Treatments details	Mycelial growth (mm/days)				Per cent inhibition over control
		2nd Day	4th Day	6th Day	8th Day	
T1	*Silica terra* 0.20%	13.40	16.00	24.10	29.46	66.45
T2	*Arnica Montana 0*.20%	11.16	14.80	19.03	24.40	72.21
T3	Sulphur 0.20%	12.03	15.53	21.70	26.40	69.94
T4	*Trichoderma harzianum*	12.70	15.03	20.20	25.43	71.04
T5	*Trichoderma viride*	13.33	16.05	22.86	28.10	68.00
T6	*Bacillus subtilis*	14.10	21.66	29.40	42.00	52.18
T7	*Pseudomonas fluorescens*	13.40	19.43	25.40	30.76	64.97
T8	Control	16.66	38.50	69.60	87.83	-
	CD at 5%	1.26	2.16	3.57	2.78	
	SE (m)	0.41	0.71	1.18	0.92	

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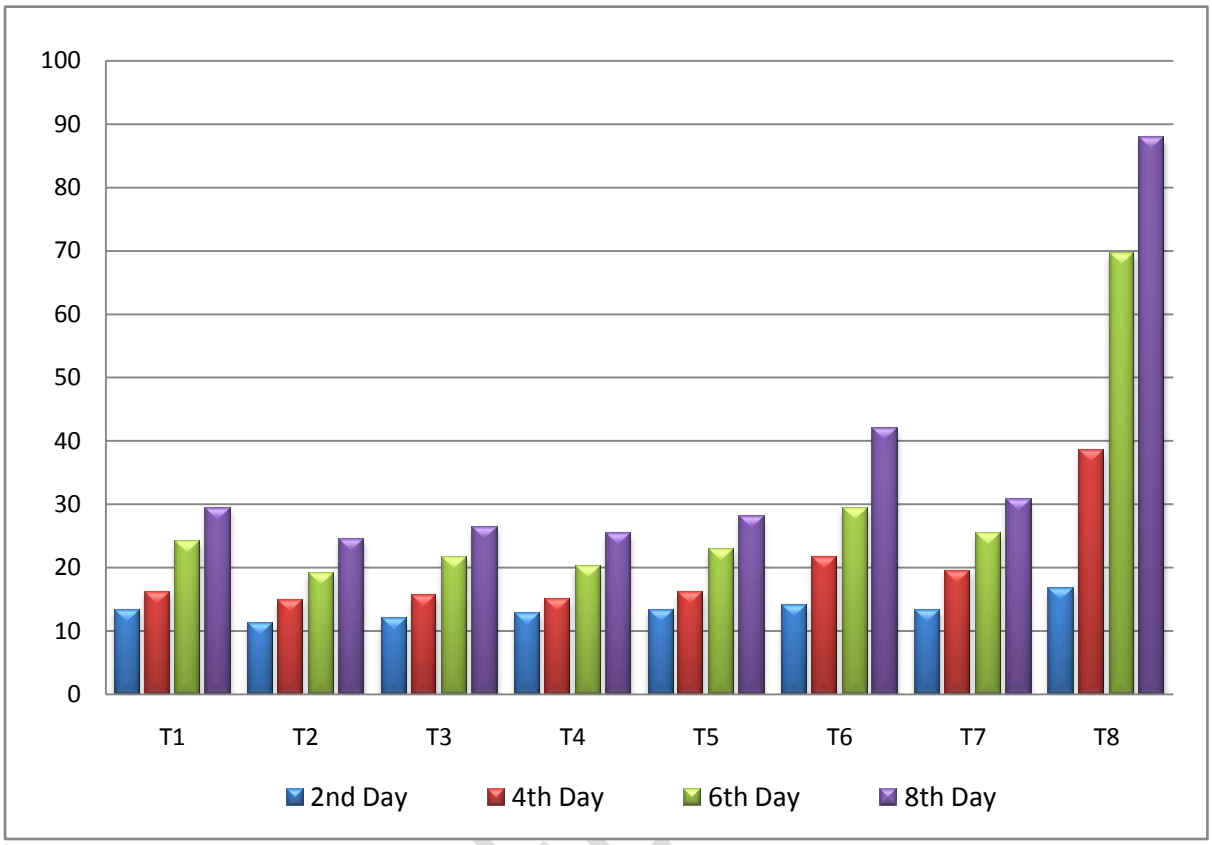
Fig. 1.Effect of homeopathic medicines and bio agents on mycelial growth of *Alternaria solani* at different day's interval (*in-vitro*)



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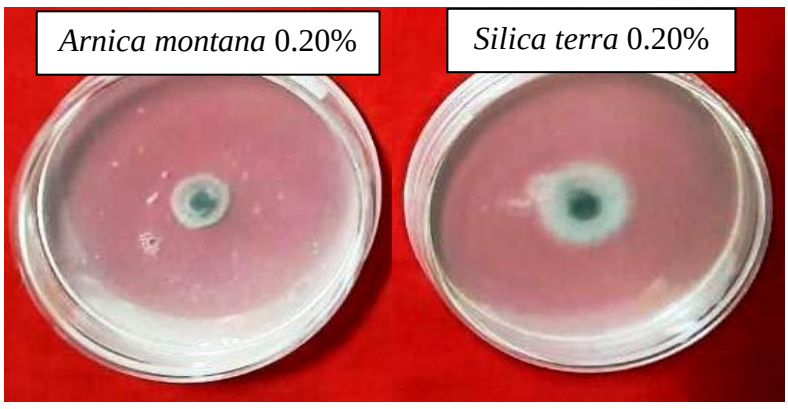
Sulphur 0.20%



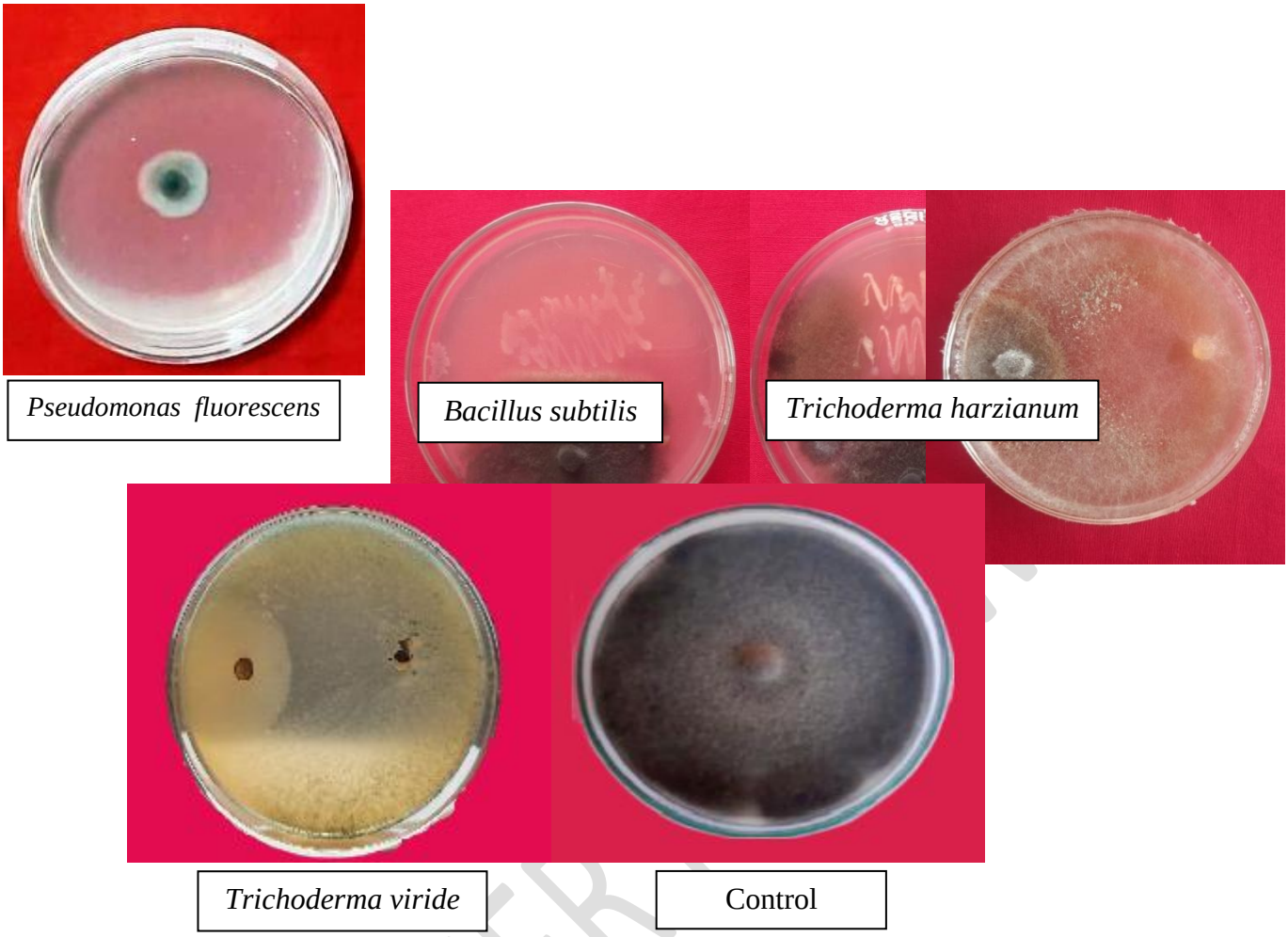


Plate 1: Efficacy of homeopathic medicines and bioagents on radial mycelial growth of early blight disease of tomato caused by *A. Solani*

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4. CONCLUSION

The current study assessed various homeopathic medicines (Sulphur, *Arnica montana*, and *Silicea terra* at 0.20%) and bioagents (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*, and *Trichoderma harzianum*) against the pathogen *A. solani*. *Arnica montana* demonstrated high effectiveness, followed by Sulphur, among the homeopathic medicines. Regarding bioagents, *Trichoderma harzianum* exhibited the highest percentage inhibition, followed by *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. All evaluated homeopathic medicines and bioagents significantly controlled the disease, suggesting their potential for managing the disease under natural field conditions. Future strategies should focus on investigating the antimicrobial potential of these homeopathic medicines and bioagents, formulating them for better shelf life, and utilizing them for eco-friendly and sustainable disease management without harming the environment.

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