

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

ABSTRACT

Alternaria solani(Ellis and Martin), causing early blight of tomato is a destructive disease which causes an economic yield loss ranges from 34-79%. The present study is carried out to check 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 fluorescense* (64.97%) and *Bacillus subtilis*(52.18%) in *in vitro* 20 per cent concentration and was found highly effective and inhibit the growth by 72.21%, respectively condition.

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

1. INTRODUCTION

The tomato (*Lycopersicon 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 fibers. In the years 2021–22, tomato cultivation in India reached a production level of 20 million metric tonnes with a 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% due to early blight damage [1, 2, 3, 4, 5, 6]. Annual economic losses attributable to early blight have been estimated at 79%[7].

Mancozeb is commonly recommended for managing *Alternaria* spp., but due to the adverse effects of fungicides, growers are increasingly interested in integrating disease management methods into tomato production. 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 pathogens. 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 [8,9,10,11]. 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*). Studies have shown that sulfur can fully inhibit the growth of *Aspergillus parasiticus* [12], and it has been observed to inhibit the growth of 22 fungal genera when used at a concentration of 200 ppm [13]. Additionally, inhibited mycelial growth of *A. solani* under the influence of silicea [14]. Similarly, several bioagents such as *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* 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 subtilis*, in inhibiting the growth of *Alternaria solani*, the primary pathogen responsible for tomato early blight, under laboratory conditions.

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2. MATERIAL AND METHODS

2.1 Isolation of pathogen

The diseased plant exhibiting typical blight symptoms was utilized for isolating the pathogen. Leaves from the diseased plant were carefully washed with tap water and then rinsed with distilled water to eliminate all dust particles. Subsequently, the diseased portions of the leaves were finely chopped using a sterilized knife, ensuring that each piece contained a mixture of diseased and healthy tissues. These chopped pieces were then briefly immersed in a 0.15% solution of HgCl₂ (mercuric chloride) for 30 seconds and thoroughly rinsed with distilled water three times to remove any residual HgCl₂. The pieces were placed between two folds of sterilized blotter paper under aseptic conditions to remove excess water. Sterilized Petri plates were heated at 165°C for 2 hours in a hot air oven. Once sterilized, these plates were transferred to a laminar airflow chamber, and prepared autoclaved potato dextrose agar (PDA) was poured into each plate. After the media solidified, the surface-sterilized leaf pieces were carefully positioned in each plate using sterilized forceps. Finally, the plates were sealed with Para film tape and placed in an incubator at 25±1°C. Daily observations were made to monitor the growth of mycelia around the leaf bits.

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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 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].

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2.3 Evaluation of homeopathic medicines against *Alternaria solani*

The effectiveness of four homeopathic medicines (*Arnica montana*, *Thuja occidentalis*, Sulfur, and *Silicea terra*) against *A. solani* was evaluated using the poisoned food technique on potato dextrose agar (PDA) medium in the laboratory. A concentration of 0.20% for each homeopathic medicine was prepared. The required amount of each homeopathic medicine was inoculated with *A. solani* and incorporated aseptically into autoclaved PDA at 0.2%. The plates were then incubated at $26 \pm 2^\circ\text{C}$ for 8 days. The growth of mycelium was measured after 2, 4, 6, and 8 days of incubation. Petri plates without any homeopathic medicine served as controls.

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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 $26 \pm 1^\circ\text{C}$, 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]

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

Where,

I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

3. Results And Discussion

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

All the homeopathic medicines evaluated caused inhibition of mycelial growth of pathogen. Among the homeopathic medicines, the minimum radial growth of mycelium was found in *Arnica montana* @0.20% with 72.21 per cent inhibition was observed in treatment and it was followed by treatment Sulphur 0.20% (69.94%) While minimum inhibition was observed in treatment *Silica terra* 0.20% (66.45%). Among bioagents tested, *Trichoderma harzianum* was found most effective with significantly least mycelial growth and highest mycelial growth inhibition of the test pathogen (25.43 mm) followed by *Trichoderma viridae* (28.10 mm) and highest mycelial growth inhibition (71.04%) of the test pathogen followed by *Trichoderma viridae* (68.04%), respectively. *Bacillus subtilis* was 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 similar results were founded by [23, 24] reported the antifungal activities of homeopathic medicines, i.e., *T. occidentalis*, sulfur, *A. montana*, *A. phosphoricum*, *Spongia tosta*, and *Chelidonium majus*. These treatments were tested individually and in mixture with mancozeb against the mycelial growth of *A. alternata* using Potato Dextrose Agar (PDA) medium via food poisoning method. These findings are in close vicinity tested effect of four homeopathic medicine, i.e., *T. occidentalis*, sulfur, *A. montana*, and silicea against *A. solani* was evaluated at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition and result revealed that highest mycelial growth inhibition over control was observed with *T. occidentalis* at 200 ppm (76.73%), followed by *A. montana* at 200 ppm (75.35%), sulfur at 200 ppm (74.29%). The lowest mycelial growth inhibition compared to the control was attributed to silicea at 50 ppm (39.42%), corresponding to a 47.13 mm mycelia growth at 8 DAI [25].

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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
		2 nd Day	4 th Day	6 th Day	8 th Day	
T1	<i>Silica terra</i> 0.20%	13.40	16.00	24.10	29.46	66.45
T2	<i>Arnica Montana</i> 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	<i>Trichoderma harzianum</i>	12.70	15.03	20.20	25.43	71.04
T5	<i>Trichoderma viridae</i>	13.33	16.05	22.86	28.10	68.00
T6	<i>Bacillus subtilis</i>	14.10	21.66	29.40	42.00	52.18
T7	<i>Pseudomonas fluorescens</i>	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	

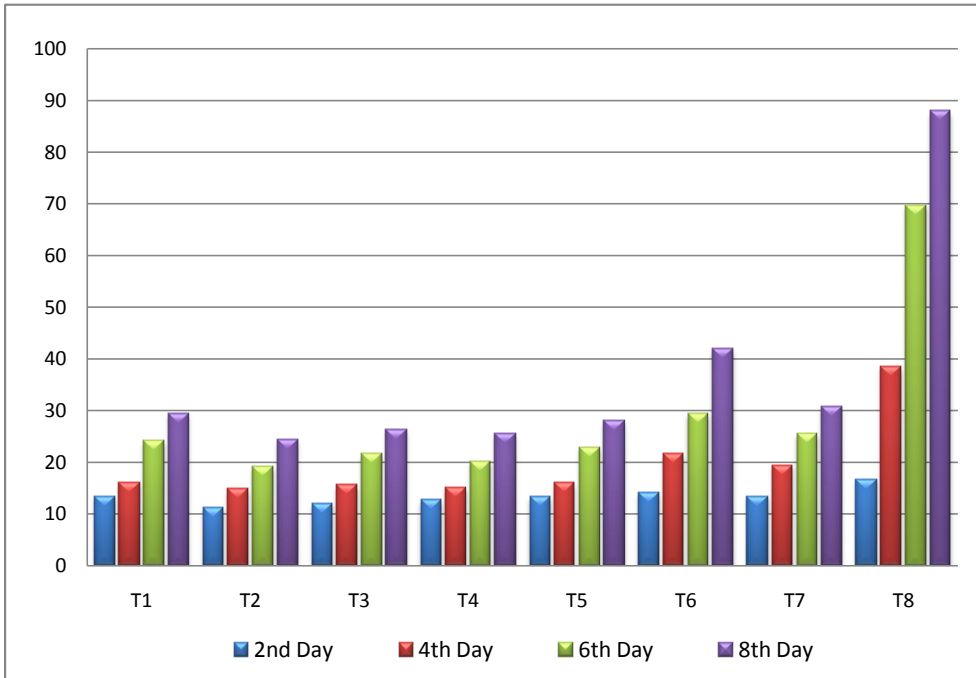
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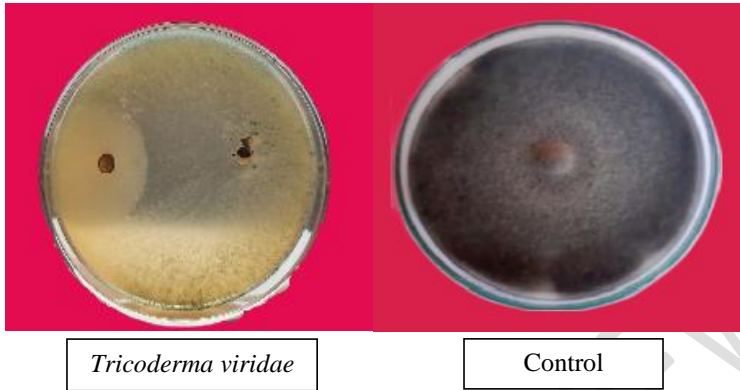
Fig. 1.Effect of homeopathic medicines and bio agents on mycelial growth of *Alternaria solani* at different day's interval (*in-vitro*)



Pseudomonas fluorescens

Bacillus subtilis

Trichoderma harzianum



Trichoderma viridae

Control

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

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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