

Evaluation of Neem cake and bio-agents on early blight (*Alternaria solani*) of tomato (*Lycopersicon esculentum* L.)

Abstract

This study was conducted during the *Rabi* season 2022-23 at the Central research farm, Department of Plant Pathology, Naini Agricultural Institute, SHUATS, Prayagraj (U.P) to conclude the Evaluation of Neem cake and bio-agents on early blight (*Alternaria solani*) of tomato (*Lycopersicon esculentum* L.) by soil application with Neem cake @ 250kg/ha and seedling treatment with bio agents in single and combination viz. *Pseudomonas fluorescens* @10g/lit, *Bacillus thuringiensis* @10g/lit, *Trichoderma viride* @10g/lit, *Pseudomonas fluorescens* @5g/lit + *Bacillus thuringiensis* @5g/lit, *Pseudomonas fluorescens*@5g/lit + *Trichoderma viride* @5g/lit and *Trichoderma viride* @ 5g/lit + *Bacillusthuringiensis* @5g/lit both *in-vivo* and *in-vitro* for their effectiveness to manage early blight of tomato caused by *Alternaria solani* .All the treatments were found significantly reduced the severity of the disease and increased growth parameters, Among all the treatments Neem cake@250kg/ha+ *Pseudomonas fluorescens* @5g/lit + *Trichoderma viride* @ 5g/lit followed by Neem cake@250kg/ha + *Trichoderma viride* @5g/lit + *Bacillusthuringiensis* @5g/lit were significantly superior over other treatments in reducing early blight infection and also in increasing growth parameters of the crop. For *in-vitro* studies only bio agents were selected among the treatments *Pseudomonas fluorescens* + *Trichoderma viride* showed highest mycelia inhibition (89.3%) followed by *Trichoderma viride* + *Bacillusthuringiensis* (86.6%). The highest cost benefit ratio was obtained with Neem cake 250kg/ha + *Pseudomonas fluorescens* @5g/lit + *Trichoderma viride* @5g/lit treatment (1:3.85). while other treatments also showed significantly effective for the checking of disease intensity (PDI) and yield over control in the field condition.

Key words: *Alternaria solani*, tomato, soil application, seedling treatment, neem cake, bio-agents, *in-vivo*, *in-vitro*, cost benefit ratio.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most significant vegetable crop in the world which is a member of the Solanaceae family. Tomato is referred to as the "poor man's apple". It is a very versatile plant that is used in both natural (raw material) and as an ingredient in other products. Tomato ranks first world wide among crops used for processing. Products made from tomato are an important part of the human diet. It is a plentiful source of minerals, carbohydrates, vitamins, and amino acids (A, C & K). Tomato contains lycopene, a very potent antioxidant that prevents cancers (Agarwal and Rao, 2000)^[2]

The crop is cultivated across all continents in the fields as well as in protected conditions. The annual production of fresh tomatoes accounts to approximately 180 million tonnes (FAO, 2019). India the total production of tomato is 20.70 million tonnes from 796.87 thousand hectares area (FAOSTAT 2019-2020)^[9], The major three tomato producing states in the country are Madhya Pradesh, Andhra Pradesh and Karnataka. In Uttar Pradesh the tomato is cultivated in 23000Ha and the production is 902000 tonnes (Anonymous, 2022)^[5].

There have been reports of more than 200 diseases that affect tomatoes worldwide (Sharma *et al.*, 2022)^[18]. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones *et al.*, 1991)^[11]. Symptoms of early blight are small, dark, necrotic lesions that usually appear on the older leaves and spread upward of the plants. As lesions enlarge, they commonly have concentric rings with a target-like appearance, and they are often surrounded by a yellowing zone (Sherf and MacNab, 1986)^[19]. It directly harms the plant and reduces both the quantity and quality of the economic yield. It has a significant impact on crop growth at all stages during both *Kharif* and *Rabi* season. This disease, which can cause severe defoliation in severe condition, is most damaging to tomato in areas with heavy rainfall, high humidity and fairly high temperatures 24-29°C (Peralta *et al.*, 2009)^[15].

According to morphological characters and phylogenetic analysis, *Alternaria solani* bear large, long-beaked and non-catenated spores (Simmons, 2000)^[20]. The mycelium consisted of septa, branched, light brown hyphae which turned darker with age. The conidiophores are short, 50-90µm long and dark in colour. Conidia are 120-296 × 12-20 µm in size. It has beaked, muriform, dark colour and born singly. Conidia contained 5-10 transverse septa and 1-5 longitudinal septa (Seyoun *et al.*, 2021)^[17]. *A. solani* was most destructive causing heavy losses in yield of tomato sometimes as high as 78 percent of fruit loss (Datar and Mayer, 1981)^[8].

For the management of early blight of tomato, there is need to incorporate alternative control components that are effective in field. The use of bio agents and botanicals are the best alternatives for management of *Alternaria solani*. Use of indigenous sources and bio-agents for the management of the plant disease which is less costly and doesn't affect public health and environment. Considering the effects of this disease, the present paper discusses the efficiency of neem cake and bio-agents for the management of early blight disease in tomato.

2. MATERIALS AND METHODS

2.1 Experiment Details

The field experiment was conducted in randomized block design (RBD) with plot size 2x1m² and there were three replication for each treatment. Treatments used for the experiment were T1 Neem cake @ 250kg/ha +

Pseudomonas fluorescens @10g/lit ,T2 Neem cake @250kg/ha + *Bacillus thuringiensis* @10g/lit, T3 Neem cake @250kg/ha+ *Trichoderma viride* @10g/lit,T4 Neem cake @250kg/ha+ *Pseudomonas fluorescens* @5g/lit+ *Bacillus thuringiensis* @5g/lit,T5 Neem cake @250kg/ha+*Pseudomonas fluorescens*@5g/lit +*Trichoderma viride* @5g/lit,T6 Neem cake @250kg/ha + *Trichoderma viride* @5g/lit+ *Bacillus thuringiensis* @5g/lit in which Neem cake was treated as soil application and bio agents were treated as seedling dip.

2.2 Isolation and purification of fungal pathogen.

Alternaria solani can be isolated from the infected leaves and infected plant parts are cut into small pieces and are surface sterilized with 0.5% of sodium hypochlorite rinsed with sterile distilled water and the sterile pieces are placed on the Petri dishes these are incubated under aseptic conditions for 2 weeks for obtaining sufficient quantity of inoculum from the subculture and pure culturing for sub culturing a sterile needle was taken to transfer the fungal bits at the tips aseptically in a fresh PDA media and cultured under 25⁰C for 2 weeks in dark condition.

2.3 Source of bioagents and Tomato Seedling Treatment .

Commercial based Powder formulations of *Trichoderma viride* (2×10^7 c.f.u /g), *Pseudomonas fluorescens* (2×10^8 c.f.u. /g) and *Bacillus thuringiensis* (2×10^8 c.f.u. /g) obtained from Yash Green Land Pvt. Limited, Allahabad, was used as slurry seedling treatment for this experiment. All the formulations contained 0.5% w/w Carboxymethyl cellulose (CMC) as an adhesive material. In single bio-agent treatments, 10g of formulation was mixed in 1liter water for seedling dip. However, in mixtures of bio-agents, combinations of the two powders were formed in ratio 1:1 to make the same quantity as recommended by the manufacturer (Suleiman *et al.*,2016)^[21]

2.4 Isolation of bioagents from Talc formulation

Serial dilutions and spread plate techniques were adopted on the talc formulations to obtain the pure cultures of the bio-agents. For *T. viride*, PDA was used as medium and incubated at 25⁰ C for 3 days while *Pseudomonas fluorescens* and *Bacillus thuringiensis* is KB and NA were used respectively at 37⁰C for 48 hours.



Plate .1 Pure culture of bio-agents

2.5 Dual culture technique

Dual culture method was applied to evaluate the inhibition (%) of the pathogen by the bio-agents *in vitro*. A mycelia plug from an actively growing *A. solani* on PDA was taken with a cork borer (7mm diameter) and kept at 1cm from one side of the agar medium contained in 90mm diameter petri dish. Another mycelia disc of *T. viride* was kept at the other side. The bacterial isolates were streaked on the other side of the pathogen. In consortial treatments, however, both mycelial plug and bacterial streaks were used. The experimental design was complete randomized design (CRD) with three replications. PDA medium was used in the experiments in order to favor the growth of *A. solani* and the potential antagonists. Petri plates without antagonist served as control. After that plates were incubated at temperature $28 \pm 1^\circ\text{C}$ for next five days. The data was analysed statistically. The efficacy of biocontrol agents was expressed as percentage inhibition of mycelia growth over control and calculated as (Vincent, 1927).

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

Where,

I = Percent Inhibition,

C = Radial growth in control,

T = Radial growth in treatment.

2.6 Assessment of disease symptoms and plant growth parameters in the field

Plants were observed weekly for the symptoms of early blight, and plant growth promoting activity. Percent disease intensity was recorded at 60, 75 and 90 DAT. It is calculated by using the following formula: (Wheeler, 1969)

$$\text{Disease Intensity} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total number of rating} \times \text{Maximum diseases grade}}$$

Also, disease severity was estimated by scoring individual plants on a 0-5 visual scale described by (Vincent, 1927). 0-Free from infection, 1-One or two necrotic spots on a few lower leaves of plants, covering nearly 1-10% 2-A few isolated spots on leaves, covering nearly 11-25% 3-Many spots coalesced on the leaves, covering 26-50% 4-Irregular, blighted leaves and sunken lesion with prominent Concentric rings on the stem petiole, fruit, covering 51-75% leaf area of the plant 5- Whole plants blighted, leaf and fruits starting to fall, covering more than 75% leaf area of plant. Yield was also recorded in respect to challenged control. The experiment was designed in Randomized Block Design (RBD). Data were analyzed statistically using analysis of variance according to SAS procedure for a completely randomized design.

3. RESULTS AND DISCUSSION

Table .1 Effect of Treatments on Disease intensity (%) on tomato at different days of intervals

Treatments		Mean of three replicates of Disease intensity(%)		
		60 DAT	75 DAT	90 DAT
T0	Control	20.95	31.83	50.47
T1	Neem cake @250kg/ha + <i>P.fluorescens</i> @10g/lit	16.18	26.72	43.07
T2	Neem cake @ 250kg/ha + <i>B.thuringiensis</i> @10g/lit	17.18	27.40	43.63
T3	Neem cake @ 250kg/ha+ <i>T. viride</i> @ 10g/lit	15.15	25.94	39.67
T4	Neem cake @ 250kg/ha + <i>P. fluorescens</i> @ 5g/lit+ <i>B. thuringiensis</i> @ 5g/lit	14.08	24.12	33.57
T5	Neemcake@250kg/ha+ <i>P.fluorescens</i> @5g/lit+ <i>T. viride</i> @5g/lit	11.76	20.42	32.21
T6	Neemcake@250kg/ha+ <i>T.virid</i> @5g/lit+ <i>B. thuringiensis</i> @5g/lit	13.39	22.62	33.20
C.D. (5%)		0.96	0.71	0.73
C.V.		3.45	1.56	1.04
SE(d)		0.44	0.33	0.33

Effect of Treatments on early blight intensity of tomato under field conditions

The Data presented in table .1 reveals the response of different treatments on disease intensity of tomato at 60,75 and 90 DAT under field condition. The statistical analysis of data showed that all treatments were significantly reduced from control. Results showed that among the treatments T₅ - Neem cake + *Trichoderma viride* + *Pseudomonas fluorescens* (32.20%) significantly reduced the disease intensity of *Alternaria solani* as compared with T₆ - Neem cake + *Trichoderma viride* + *Bacillus thuringiensis* (33.20%), T₄ - Neem cake + *Pseudomonas fluorescens* + *Bacillus thuringiensis* (33.56%), T₃ - Neem cake + *Trichoderma viride* (39.66%), T₁- Neem cake + *Pseudomonas fluorescens* (43.06%), T₂ - Neem cake +*Bacillus thuringiensis* (43.63%) and non - significant results were found in between the treatments (T₂, T₁) and (T₄, T₆).

Table .2 Effect of treatments on Plant height(cm) and yield of tomato under field conditions

Treatments		Mean of three replicates of Plant height (cm)			Yield(t/ha)
		30 DAT	60 DAT	90 DAT	Total
T₀	Control	18.67	37.07	48.40	5.66
T₁	Neem cake @250kg/ha + <i>P. fluorescens</i> @10g/lit	24.33	41.93	58.93	10.0
T₂	Neem cake @ 250kg/ha + <i>B. thuringiensis</i> @10g/lit	23.80	40.83	57.40	9.83
T₃	Neem cake @ 250kg/ha+ <i>T. viride</i> @ 10g/lit	25.30	42.60	63.20	10.5
T₄	Neem cake @ 250kg/ha + <i>P.fluorescens</i> @5g/lit + <i>B. thuringiensis</i> @5g/lit	27.00	45.33	71.97	14.6
T₅	Neemcake@250kg/ha+ <i>P. fluorescens</i> @5g/lit+ <i>T. viride</i> @5g/lit	28.67	52.67	74.13	16.1
T₆	Neemcake @250kg/ha+ <i>T. viride</i> @5g/lit+ <i>B. thuringiensis</i> @5g/lit	27.60	46.67	72.73	15.1
C.D. (5%)		0.72	0.97	1.61	2.19
C.V.		1.62	1.25	1.42	0.18
SE(d)		0.33	0.45	0.74	1.16

Effect of treatments on Plant height (cm) of tomato under field condition

The data presented in table .2 reveals the response of different treatments on plant height of tomato at 30,60,90 DAT under field condition. The statistical analysis of data showed that all treatments were significantly increased plant height from control. Among the treatments T₅ – Neem cake + *Trichoderma viride* +*Pseudomonas fluorescens* (74.13), significantly increased plant height followed by T₆– Neem cake + *Trichoderma viride* + *Bacillus thuringiensis* (72.73), T₄– Neem cake + *Pseudomonas fluorescens* + *Bacillus thuringiensis* (71.96), T₃ - Neem cake + *Trichoderma viride* (63.20), T₁- Neem cake + *Pseudomonas fluorescens* (58.93) and T₂- Neem cake+ *Bacillus thuringiensis* (57.40) Among the treatments (T₅,T₆),(T₆,T₄) and (T₁,T₂) were found non- significant to each other

Fruit Yield (t/ha) of tomato at harvest

The analysis of data presented in table .4 and graphically shown in fig.1 revealed that all the treatments significantly increased in fruit Yield t/ha from the control .Among the treatments T₅ - Neem cake + *Trichoderma viride* +*Pseudomonas fluorescens* (16.1 t/ha) significantly increased in fruit yield followed by T₆– Neem cake + *Trichoderma viride* + *Bacillus thuringiensis* (15.1 t/ha), T₄– Neem cake + *Pseudomonas*

fluorescens + *Bacillus thuringiensis* (14.6 t/ha), T₃ - Neem cake + *Trichoderma viride* (10.5), T₁- Neem cake + *Pseudomonas fluorescens* (10.0 t/ha) and T₂ - Neem cake + *Bacillus thuringiensis* (9.8 t/ha). Among the treatments (T₁, T₂ and T₃) (T₅, T₆) found non - significant to each other.

Table 3 *In vitro* evaluation of bioagents on the radial growth (mm) of *Alternaria solani* at 72 hrs after incubation

Tr.No	Treatments	Mean colony diameter(mm) of <i>Alternaria solani</i>	Inhibition percentage(%)
T ₁	<i>Pseudomonas fluorescens</i>	25	66.6
T ₂	<i>Bacillus thuringiensis</i>	28	62.6
T ₃	<i>Trichoderma viride</i>	20	73.3
T ₄	<i>P.fluorescens</i> + <i>B.thuringensis</i>	16	78.6
T ₅	<i>T. viride</i> + <i>P. fluorescens</i>	8	89.3
T ₆	<i>T.viride</i> + <i>B.thuringensis</i>	10	86.6
T ₀	Control	75	00.0
	CD(5%)	1.62	
	SE(d)	0.75	

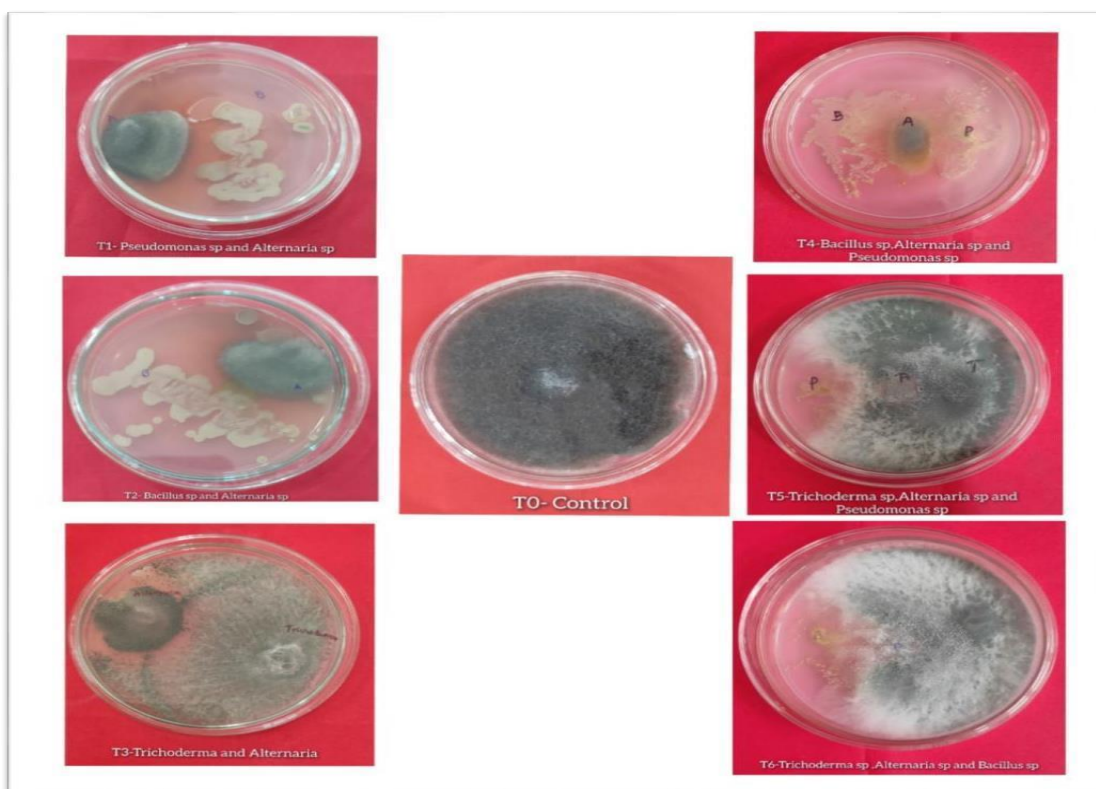


Plate 2 Antagonistic potential of bioagents on *Alternaria solani*

3.2 *In-vitro* evaluation of antagonistic potential of bioagents on radial growth (mm) of *Alternaria solani*

Following the dual culture technique between the *A. solani* and the biological control agents, *in vitro* analysis revealed that the six treatments, except control, inhibited the growth of *A. solani* significantly. The results obtained (Table .4) showed that T₅ - *T. viride* + *P. fluorescens* inhibited the growth of *A. solani* most by 89.3% with only 8.00 mm growth in diameter of the pathogen. This is followed by T₆ - *T. viride* + *B. thuringiensis* with inhibition of 86.6 and 10.00mm diameter of pathogen growth, and then T₄ - *P. fluorescens* + *B. thuringiensis* with 78.6% inhibition and pathogen diameter of 16.00mm, T₃ - *Trichoderma viride* with 73.3% inhibition and pathogen diameter of 20.00mm, T₁ - *P. fluorescens* with 66.6% inhibition and pathogen diameter of 25.00mm and the least inhibition is on T₂ - *B. thuringiensis* with 62.6% inhibition and pathogen diameter of 28.00mm. Based on this *in vitro* study, the mixture between the three bioagents gave higher inhibition (%) of *A. solani* in comparison with single bio-agents. This is in agreement with the work of (Suleiman *et al.*, 2016)^[58].

4. CONCLUSION

Based on the results obtained from present investigations it was found that Soil application of Neem cake along with combination of bio-agents *Trichoderma viride* + *Pseudomonas fluorescens* seedling treatment was found most effective against *Alternaria solani* which cause early blight disease in tomato therefore it may be recommended for the better management of early blight of tomato. In field conditions Neem cake + *Trichoderma viride* + *Pseudomonas fluorescens* showed significant reduction of disease intensity (%) and significant increase in plant height, number of branches and yield.

In *in-vitro* condition dual culture method *Trichoderma viride* + *Pseudomonas fluorescens* showed highest growth inhibition percentage as compared to other treatments including control

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