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Management of Early Blight of Tomato through Neem Formulation and Bio-inoculants

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ABSTRACT: Early blight disease is painful nerve to tomato growing farmer because it causes huge economic loss to the farmer every year. *Alternaria solani* cause early blight of tomato and it is considered weed of field because of its wide adaptability under different environment. Use of fungicides for the management of disease in crop puts a large number of negative health and environmental effects therefore, the urgent need for a more sustainable and ecological approach to manage disease without fungicides. To avoid relying solely on chemicals and to identify a viable alternative component, five neem formulations and four bio-inoculants were evaluated by Poison Food Technique and Dual Culture Technique in the lab in the year of 2020-21 against *A. solani*. Among neem formulations, neem oil (71.91%) was found effective inhibiting mycelial growth of *A. solani* followed by multineem (58.49%), Neem excel (48.92%) and Repellent plus (38.00%) at the mean of two concentration (1000 & 1500 ppm). In case of bio-inoculants, *T. harzianum* was found most effective inhibiting mycelial growth 71.15% followed by *T. viride* (66.35%).

Keywords: *Alternaria solani*, Early blight, Neem Formulation, Bio-inoculants, Mycelial inhibition.

INTRODUCTION

The Portuguese introduced the tomato (*Solanum lycopersicum* L) to India in the 1700s (Kale and Kale, 1994). Tomato is also known as "Poor Man's Orange" in India because it is a nutrient-dense super food and an excellent source of antioxidants, which help to reduce the risk of heart disease and cancer. As a result, tomato fruits are in high demand throughout the year. The tomato is the world's fourth most cultivated crop, with a production of 130 million tons and an area of 5.2 million hectares (Anonymous, 2020). It is an inevitable vegetable crop world over and of course, for India.

India is the world's second largest tomato producer after China, with an area of 778 thousand hectares and a production of 19397 metric tons, accounting for 11% of global production (Anonymous, 2020). Cultivated tomatoes have a narrow genetic diversity as a result of intense selection and inbreeding during evolution and domestication, these species are more susceptible to disease epidemics during the growing season (Zhang *et al*., 2002).

Early blight disease is caused by several species of *Alternaria* including *Alternaria solani*, *A. alternata* as well as *A. tomatophila* (Adhikari *et al.*, 2017). Worldwide, early blight disease is one of the dreadful diseases of tomato resulting up to 78% yield and production loss (Datar and Mayee 1981; Bessadat *et al*., 2014). 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*., 2005). Epidemics can occur in semi arid climates where frequent and prolonged nightly dews occur (Vennila *et al.*, 2020).

Currently, the control of plant disease, pest in only through by spaying a large amount of synthetic fungicides (Cook, 2000). However, an increase use of synthetic fungicide can severely deteriorate the planet's health (Wang *et al.*, 2010). Different types of fungicides have been used for the control of Alternaria blight, but fungicide treatment is not economically feasible, nor environmentally sound. Fungicides are first applied 1–2 days after transplantation and then require routine application at the interval of 7 to10 days for effective control, thereby increasing production cost and environment pollution (Kemmitt 2002).

Neem oil has been the cure for many fungal diseases caused by *Alternaria solani*, *Aspergillus flavus*, *Alternaria alternate* (Girish and Shankara, 2008). Different neem formulation founds antifungal properties against *Alternaria spp.* (Saha *et al*., 2005; Guleria and Kumar, 2006). Kota, (2003) has reported that bioagents *T. harzianum* and *T. virens* were highly inhibiting mycelial growth of *A. alternata in vitro*. Kumar, (2008) evaluated effect of four bio-agents *Trichoderma spp*., namely *Trichoderma harzianum, T. konigii, T. viride* and *T. virens*, against growth of *A. alternata* under laboratory conditions. In his

experiments, *T. harzianum* has shown highest inhibitory effect on radial growth (RG) rate of *A. alternata*. *Trichoderma spp*. have ability to detoxify pesticides and herbicide have been revealed in several findings (Vazquez *et al*., 2015; Zafra *et al*., 2015).

The effects of systematic fungicides are inactivated by fungi enzymes (Golyshin, 1990). Under mutation impact, fungicides lose their inhibitory effect. Therefore, we urgent need to identify other viable alternative component to manage disease of plants. Keeping in view the importance of sustainable and ecological approach to manage disease, the present study was conducted to assess the *in vitro* efficacy of different neem formulation and bio-agents against *Alternaria solani.*

MATERIAL AND METHODS

Evaluation of neem formulations under in *in vitro* condition. A total five neem formulations (Table 1) along with control were used in present study in the year of 2020-21. Bio efficacy of different neem formulations were tested *in vitro* by "Poisoned Food Technique". The poisoned food technique was used for study of neem formulation at 1000 ppm and 1500 ppm concentration was mixed with potato dextrose agar medium. The experiment was conducted in the CRD with three replications. Percent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Vincent, (1947).

Poison Food Technique. Required quantity of each neem formulations under study was mixed thoroughly in sterilized 100 ml PDA media filled in 250 ml flask separately under aseptic condition. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The poisoned medium was then poured in sterilized petri plates (20 ml) and allowed it to solidify. Mycelium discs of 5 mm size from seven days old culture was cut by a sterile cork borer and one such disc was placed at the center of each agar plate. The plate without any neem formulations served as control. Three replications were maintained for each concentration. Such plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. Percent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Vincent, (1947).

Table 1: List of Neem formulations evaluated against *A. solani* by Dual Culture Technique (*in vitro*).

Sr. No.	Treatment	Doses in ppm	
1.	Multineem	1000	1500
2.	Neemix	1000	1500
3.	Repellent Plus	1000	1500
4.	Neem oil	1000	1500
5.	Neem Excel	1000	1500
6.	Control	—	—

***In vitro* evaluation of bio-inoculants.** *In vitro* evaluation was carried out with two fungal and two bacterial bio-agents (Table 2) along with control against *A. solani* by Dual Culture Technique (Denis and Webster, 1971) in the year of 2021-21. Both bio-agents and test fungus were cultured on potato dextrose agar media.

Dual Culture Technique. Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petri plates and allowed to solidify. For evaluation of fungal bio-agents, mycelial discs of test fungus were inoculated at one end of the petri plate and antagonistic fungus was placed opposite to it on the other end.

In case of bacterial bio-agents evaluation, the bacteria were streaked one day earlier at one end of the petri plate or at the middle of the petri plate and the test fungus placed at the opposite end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The colony diameter of the pathogen in control plates will be also recorded.

The experiment will be conducted in the CRD with four replications. Percent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Vincent, (1947).

Table 2: List of bio-agents evaluated against *A. solani* by Dual Culture Technique (*in vitro*).

Sr. No.	Bio-agent
1.	*Trichoderma viride*
2.	*Trichoderma harzianum*
3.	*Pseudomonas fluorescens*
4.	*Bacillus subtillis*
5.	Control

RESULTS AND DISCUSSION

Management of early blight of tomato through neem formulations under *in vitro* condition. Five neem formulations with two different concentrations (1000 & 1500 ppm) along with control were evaluated *in vitro* against *A. solani* by applying Poisoned Food Technique (Nene and Thapliyal, 1993). The observations on per cent growth inhibition (PGI) were recorded and the results were presented in Table 3 Fig. 1, Plate 1a &1b.

Table 3: Efficacy of neem formulations against *Alternaria solani in vitro* condition.

Sr. No.	Treatment	Percent mycelium growth inhibition*		
		Concentration (ppm)		
		1000	1500	Mean
1.	Multineem	52.19 (46.24)	64.79 (53.58)	58.49
2.	Neemix	36.04 (36.87)	46.15 (42.77)	41.09
3.	Repellent plus	34.15 (35.74)	41.85 (40.29)	38.00
4.	Neem oil	66.73 (54.79)	77.09 (61.39)	71.91
5.	Neem excel	40.96 (39.77)	56.87 (48.93)	48.92
6.	Control	0.00 (0.00)	0.00 (0.00)	0.00
Factor		**S. Em ±**	**CD (p = 0.05)**	
Neem formulation (N)		**0.460**	**1.349**	
Concentration (C)		**0.265**	**0.779**	
Interaction (N x C)		**0.650**	**1.908**	

*Average of three replications
Figures in parentheses are angular transformed values

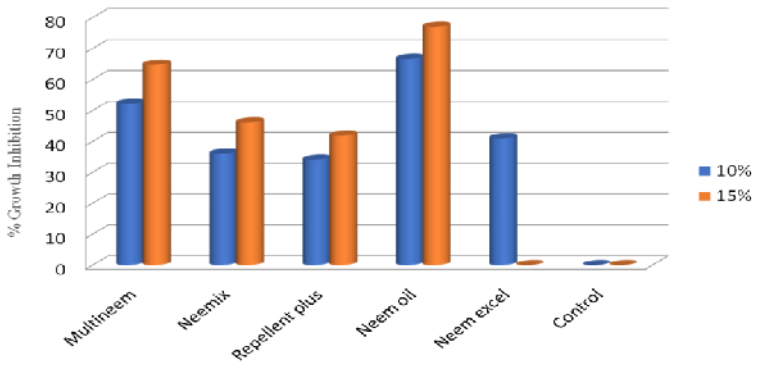

Fig. 1. Effect of neem formulations on mycelial growth inhibition (%) of *A. solani in vitro.*

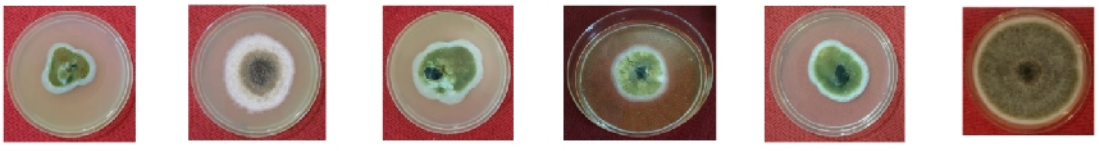

Plate 1a. Efficacy of neem formulations against *A. solani in vitro* 1000 ppm.

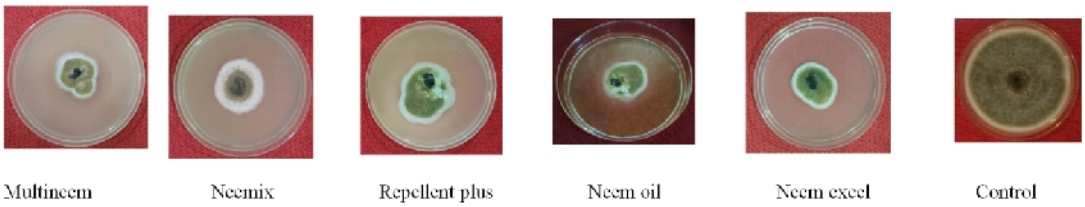

Plate 1b. Efficacy of neem formulations against *A. solani in vitro* 1500 ppm

The result presented data in Table 3 showed that neem oil (77.09 %) at 1500 ppm was found best and significantly superior over all other neem formulation concentrations in inhibiting mycelial growth of *A. solani* followed by multineem (64.79 %) at 1500 ppm concentrations. Neem oil at 1000 ppm concentrations was more effective as compare to multineem at 1500 ppm concentrations. Least mycelial inhibition was observed in repellent plus (34.15%) at 1000 ppm concentration. In case of neem excel, it inhibiting 56.87% mycelial growth of pathogen at 1500 ppm concentrations. Least inhibition i.e. 34.15% & 41.85 % was observed in repellent at both concentrations, respectively. Since, no precise information was available on the efficacy of neem formulation against *A. solani* caused early blight in tomato.

Management of early blight of tomato through bio-inoculants in *in vitro* conditions. Four antagonists' *viz., Trichoderma viride, T. harzianum, Pseudomonas fluorescence* and *Bacillus subtilis* (Plate 2) were studied under laboratory condition for their antagonism against *Alternaria solani by* dual culture technique.

The results revealed that all the antagonists significantly helped in inhibiting the mycelial growth of *A. solani* over control. Significantly highest per cent mycelial growth inhibition was observed in *T harzianum* (71.15%) followed by *T. viride* (66.35%) and *Pseudomonas fluorescence* (55.25%) after 7 day of incubation. While bio-inoculants *Bacillus subtilis* showed minimum per cent mycelial growth inhibition (51.57%) (Table 4, Fig. 2 and Plate 2).

Table 4: Effect of different bio-inoculants against *A. solani in vitro.*

Sr. No.	Bio-inoculants	Per cent inhibition of mycelial growth*
1.	*Trichoderma viride*	66.35 (54.52)
2.	*Trichoderma harzianum*	71.15 (57.49)
3.	*Bacillus subtilis*	51.57 (45.88)
4.	*Pseudomonas fluorescence*	55.25 (47.99)
5.	Control	0.00 (0.00)
	S.Em ±	**0.354**
	CD (p = 0.05)	**1.718**

*Average of four replications
Figures in parentheses are angular transformed values

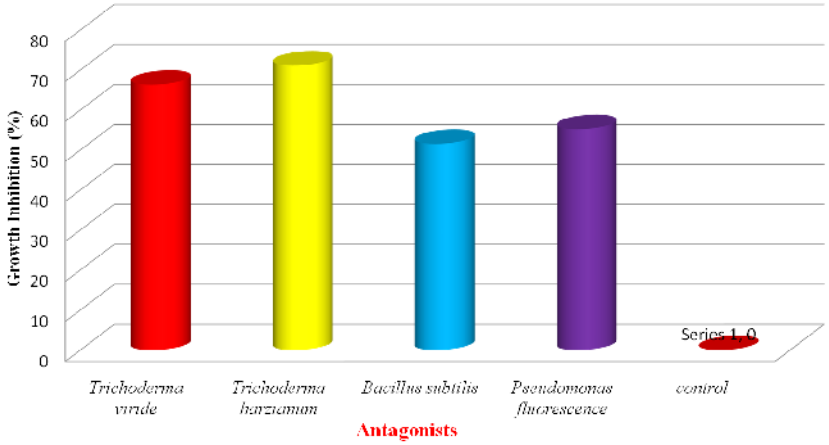

Fig. 2. Effect of antagonists on mycelial growth inhibition (%) of *A. solani in vitro.*

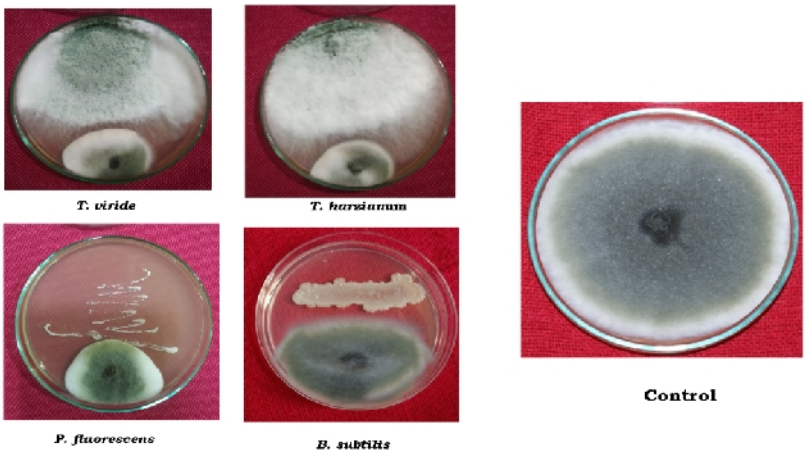

Plate 2. Bio-efficacy of Bio-inoculants against *A. solani in vitro.*

It is evident from the above results that neem leaf formulation protected from early blight pathogen (*Alternaria solani*) of the tomato plants. *In vitro* tests carried out by Chaudhary *et al*., (2003) reported that extracts of *A. indica* gave the second highest inhibition of *A. alternata* (54%). Neem formulation derived from the Neem tree (*Azadirachta indica*) have been found to be fetal against a broad spectrum of plant pathogenic fungi such as *Alternaria solani*, *Fusarium oxysporum* (Bhonde *et al*., 1999.) Since, no precise information was available on the efficacy of neem formulation against *A. solani* caused early blight in tomato.

Bio-inoculants *viz. T. viride*, *T. harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescence* were reported efficient antagonists against *A. solani* earlier by many workers (Bais *et al*., 2019; Naik *et al*., 2020; Sudarshan *et al*., 2020, Verma *et al*., 2020; Mohamed *et al.*, 2021). The species of *Trichoderma viz. viride* and

harzianum were reported as efficient antagonists against *Alternaria* spp (Pun *et al*., 2020; Zin and Badaluddin, 2020). The fungistatic antifungal action exerted by the species of *Trichoderma* and against *A. solani* and other species of *Alternaria* may be attributed to their production of volatile and non-volatile substances, cell wall degrading enzymes (glucanases, Bl, 3 glucanase), the phenomenon of competition, lysis and antibiosis (Contreras-Cornejo *et al*., 2015a, 2015b; Kubicek *et al*., 2001; Kullnig *et al*., 2000).

In conclusion, the study confirms that different neem formulation, neem oil (77.09%) at 1500 ppm and bio-agents, *T harzianum* (71.15%) and *T. viride* (66.35%) are very effective against early blight caused by *Alternaria solani* of tomato.

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Conflicts of Interest. The results furnished in this paper were from my own research and there were no any conflicts from other research scholars or scientists.

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