



***In-vitro* Evaluation of Antagonistic Activity of Native *Trichoderma* spp. and *Pseudomonas fluorescens* Isolates against *Alternaria solani* Causing Early Blight of Tomato**

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Authors' contributions

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

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ABSTRACT

The present study was carried out in 2018-2019 at Department of Plant pathology, SASRD, Nagaland University to evaluate the *in vitro* efficacy of 24 native isolates of *Trichoderma* and 18 isolates of *Pseudomonas* against *Alternaria solani* by dual culture technique method. The test pathogen was isolated from disease infected tomato plants collected from an experimental field of Department of Plant pathology, SASRD, Medziphema campus. The results showed that all the isolates had significantly inhibited the mycelial growth of the pathogen. The highest inhibition of mycelial growth of *A. solani* was shown by T-5 (73.34%) followed by T-11(70.23%). The lowest inhibition was shown by T-24 (51.55%). Among the *Pseudomonas* isolates, highest inhibition in mycelial growth of *A. solani* was shown by P-7 (77.73%) followed by P-12(76.00%) respectively.

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The lowest inhibition was shown by P-17 (53.78%). The results indicate that different local isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* were effective against the tested pathogenic fungi which provides their potential in biological management of early blight disease of tomato.

Keywords: *Trichoderma*; tomato; *Alternaria solani*; dual culture.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.), native to the Andean region of South America is one of the most common horticultural crops cultivated throughout the world. They are important source of vitamins and important cash crop for both small holders and medium scale commercial farmers [1]. The fruit also contains plenty of antioxidant carotenoid lycopene that can prevent cancer, heart disease and muscular degeneration [2]. Among several diseases of tomato, early blight disease caused by *Alternaria solani* has become one of the most destructive diseases all over the world with yield losses up to 80% [3]. *Alternaria solani* (Ellis and Martin) Jones and Grout, is a soil inhabiting air-borne pathogen responsible for leaf blight, collar and fruit rot of tomato [4]. *A. solani* contains enzymes such as cellulases which degrade the host cell wall and also contain pectin methyl galacturonase which facilitate host colonization [5]. The disease in severe cases can lead to complete defoliation and it is favoured by heavy dew, rainfall, high humidity, and fairly high temperatures. As all the above ground parts of the plant can be attacked by the pathogen, failure to control this disease can highly reduce the yield [6].

Currently control strategies for managing early blight of tomato include cultural practices, fungicide application, and breeding for resistant varieties [7,8]. Because of issues such as lack of major resistance genes for early blight in tomato cultivars with market desirable traits and the complex nature of inheritable quantitative resistance in tomato cultivars, fungicide application remains the major approach to combat this disease. Protective fungicides such as Mancozeb and systemic fungicides such as azoxystrobin with multisite mode of action are widely used around the world. Several round of fungicide applications are required during tomato growing season to achieve a satisfactory level of disease control [9]. There have been increasing reports on loss of efficacy of commercial fungicides against the pathogen [10,11,12]. "Management of plant diseases by chemical

pesticides mostly concerned environmental damage since pesticides accumulate in soils as toxic residues, as well as the development of resistance by pathogens resulting from pesticides overuse and single-site fungicides, which enhances the development of specific resistance [13,14,15].

Biological control using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals" [16,17]. Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means and is known to be a cheap and effective eco-friendly method for the management of crop diseases [15,18,19]. Environmentally, they are also more efficient as they do not release toxic compounds, and it decreases the negative effects of plant pathogens and increases positive responses by the plants [20]. Additionally, they usually have several modes of action, thus reducing the development of resistance [21]. These mutualistic bioagents are considered eco-friendly and have no negative effects on non-targeted organisms, including humans, the useful microflora and host plants. In such tripartite interactions between the host plant, fungal pathogen and mutualistic bioagents, different mechanisms of action were reported and considered responsible for protecting the host plants from pathogens and parasites [22,23,24]. *Trichoderma* spp. is the most widely studied biocontrol agent (BCA) against plant pathogens because of their ability to reduce the population of soil borne plant pathogens. *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots [25].

Therefore, the objective of the present investigation was to assess the efficacy of local isolates of *Trichoderma* and *Pseudomonas* under *in vitro* condition against *A. solani*. The experiment was conducted in Department of Plant Pathology, SASRD, Medziphema campus, Nagaland University.

2. MATERIALS AND METHODS

The dual culture technique described by Dennis and Webster [26] was followed for the evaluation of antagonistic activities of native *Trichoderma* isolates against *A. solani*. Briefly 20 ml of sterilized PDA medium was poured into each of the sterilized Petri dishes under aseptic condition. After the media gets solidified in the plates, the test fungal pathogen was inoculated at one end of each Petri plate and the antagonists on the opposite end. A set of control plates inoculated with the test fungal pathogen was maintained. Each set of treatments were replicated three times and incubated at a temperature of $28\pm 1^{\circ}\text{C}$ for 10 days. The per cent inhibition of growth was calculated following the equation given by Vincent [27].

$$\text{Per cent inhibition} = \text{PI} = \frac{C-T}{C} \times 100$$

Where, C= Radial growth of pathogen in control, T= Radial growth in of pathogen in dual culture plates.

The dual culture technique described by Maurya et al. [28] was followed for the evaluation of antagonistic activities of native bacterial isolates against *A. solani*. *Pseudomonas* isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 10 mm mycelial disc from seven days old PDA culture of the pathogen was placed at the opposite side of Petri dishes perpendicular to the bacterial streak respectively and incubated at $28\pm 1^{\circ}\text{C}$ for 5-7 days. Petri dishes inoculated with fungal pathogen alone served as control. Three replications were maintained for each isolate. The per cent inhibition of growth will be calculated following the equation given by Vincent [27].

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically.

3. RESULTS AND DISCUSSION

Altogether 24 native isolates of *Trichoderma* spp. and 18 isolates of *Pseudomonas* spp. were screened for their inhibitory action on the radial

growth of *P. infestans* by adopting dual culture technique [29] and the data obtained are presented in Tables 1 & 2 and Figs. 1 & 2. All isolates screened against *A. solani* were significantly superior over control plate. It was found that the growth of the pathogen in dual culture plates progress until they came in contact with the leading edges of the antagonist. Among the different isolates of *Trichoderma* spp. least radial mycelial growth of the pathogen was recorded in T-5 (2.00 cm) followed by T-11 (2.10 cm), T-20 (2.23 cm) respectively. The per cent inhibition over control showed that T-5 was the most promising isolate against *A. solani* with 73.34 per cent inhibition followed by T-11 (70.23%) and T-20 (69.34%). The least antagonistic effect was observed in T-24 (51.55%). Among the different isolates of *Pseudomonas* spp. least radial mycelial growth of the pathogen was recorded in P-7 (1.67 cm) followed by P-12 (1.80 cm), P-14 (1.90 cm) respectively. The per cent inhibition over control showed that P-7 (77.73%) was the most promising isolate against *A. solani* followed by P-12 (76.00%) and P-14 (74.67%). The least antagonistic effect was observed in P-17 (53.78%). Our findings are in agreement with the findings of earlier workers [30,31].

Trichoderma spp. are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity [32]. Mechanism used by *Trichoderma* spp. for control of plant pathogen includes competition, mycoparasitism, antibiosis and induced resistance of the plant host [33]. Fluorescent *pseudomonas* also produced anti fungal compounds such as pseudobactin, HCN, salicylic acid and 2- hydroxy phenazine to suppress plant pathogenic fungi [34]. The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of the pathogen and *P. fluorescens* were known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents [35]. Moreover, the difference in their potential may probably be correlated with the differences in levels of hydrolytic enzymes produced by each species or isolates when they attack the mycelium of the pathogens [36,37]. They are soil borne fungi and show significant activity against a wide range of plant pathogenic fungi [33].

Table 1. Antagonistic activity of native *Trichoderma* isolates against *A. solani*

Treatment	Inhibition of <i>Alternaria solani</i> growth		
	Radial growth (cm)	Radial growth inhibited (cm)	Inhibition %
T ₀ (Control)	07.50	00.00	00.00 (4.05)
T ₁ (<i>A.solani</i> + T-1)	02.86	04.63	61.78 (51.81)
T ₂ (<i>A.solani</i> + T-2)	02.36	05.14	65.78 (54.21)
T ₃ (<i>A.solani</i> + T-3)	03.10	04.40	60.89 (51.30)
T ₄ (<i>A.solani</i> + T-4)	03.03	04.46	59.55 (50.50)
T ₅ (<i>A.solani</i> + T-5)	02.00	05.50	73.34 (58.91)
T ₆ (<i>A.solani</i> + T-6)	02.80	04.76	62.67 (52.34)
T ₇ (<i>A.solani</i> + T-7)	02.83	04.67	62.23 (52.08)
T ₈ (<i>A.solani</i> + T-8)	03.26	04.24	56.89 (48.95)
T ₉ (<i>A.solani</i> + T-9)	02.86	04.64	61.78 (51.82)
T ₁₀ (<i>A.solani</i> + T-10)	02.73	04.77	63.56 (52.86)
T ₁₁ (<i>A.solani</i> + T-11)	02.10	05.40	70.23(56.93)
T ₁₂ (<i>A.solani</i> + T-12)	02.36	05.14	65.78 (54.21)
T ₁₃ (<i>A.solani</i> + T-13)	03.23	04.27	56.89 (48.95)
T ₁₄ (<i>A.solani</i> + T-14)	03.40	04.10	54.67 (47.67)
T ₁₅ (<i>A.solani</i> + T-15)	02.30	05.20	68.00 (55.57)
T ₁₆ (<i>A.solani</i> + T-16)	03.56	03.94	52.45 (46.40)
T ₁₇ (<i>A.solani</i> + T-17)	03.00	04.50	60.00 (50.76)
T ₁₈ (<i>A.solani</i> + T-18)	03.44	03.86	63.11 (52.60)
T ₁₉ (<i>A.solani</i> + T-19)	03.26	04.24	56.89 (48.95)
T ₂₀ (<i>A.solani</i> + T-20)	02.23	05.27	69.34(56.41)
T ₂₁ (<i>A.solani</i> + T-21)	02.50	05.00	65.34 (53.93)
T ₂₂ (<i>A.solani</i> + T-22)	03.40	04.10	54.66 (47.67)
T ₂₃ (<i>A.solani</i> + T-23)	03.03	04.47	59.55 (50.50)
T ₂₄ (<i>A.solani</i> + T-24)	03.63	03.87	51.55 (45.89)
Sem +-	0.01	0.02	0.39
C.V. (%)	2.53	1.72	3.27
CD (p=0.01)	0.16	0.17	4.23
CD (p=0.05)	0.12	0.13	3.17

* Data in the parentheses are angular transformed values

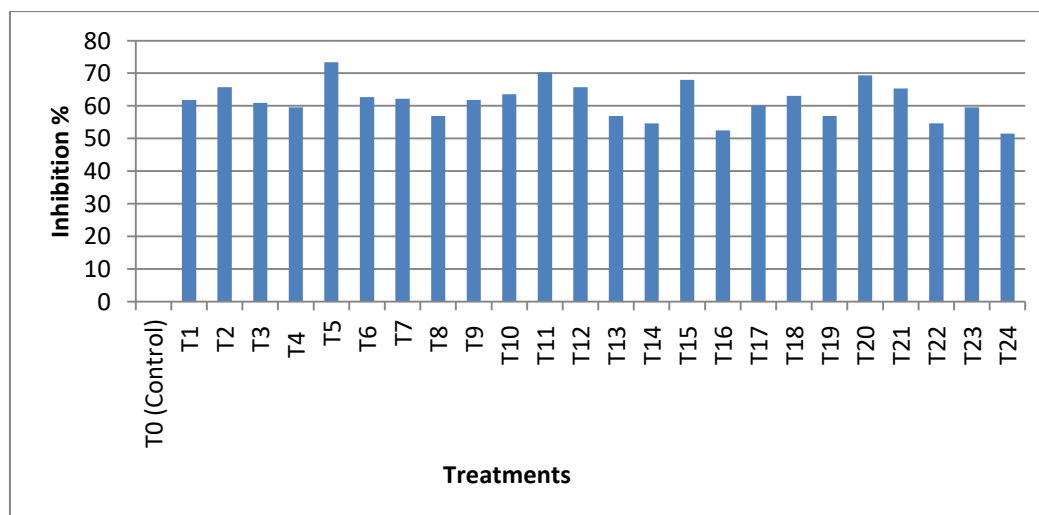
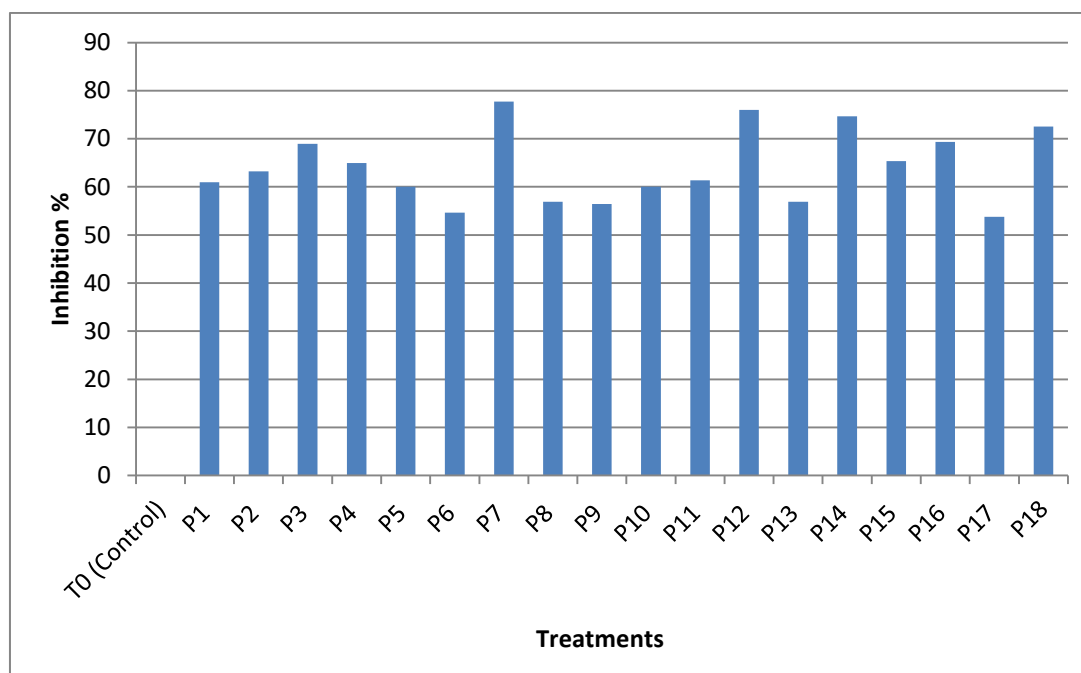
**Fig. 1. Antagonistic activity of native *Trichoderma* isolates against *A. solani***

Table 2. Antagonistic activity of native *Pseudomonas* isolates against *A. solani*

Treatment	Inhibition of <i>Alternaria solani</i> growth		
	Radial growth (cm)	Radial growth inhibited (cm)	Inhibition %
T ₀ (Control)	07.50	00.00	00.00 (4.05)
P ₁ (<i>A. solani</i> +P-1)	02.93	04.57	60.93 (51.29)
P ₂ (<i>A. solani</i> +P-2)	02.76	04.74	63.20 (52.62)
P ₃ (<i>A. solani</i> +P-3)	02.33	05.17	68.94 (56.10)
P ₄ (<i>A. solani</i> +P-4)	02.63	04.87	64.93 (53.67)
P ₅ (<i>A. solani</i> +P-5)	03.00	04.50	60.00 (50.78)
P ₆ (<i>A. solani</i> +P-6)	03.40	04.10	54.67 (47.68)
P ₇ (<i>A. solani</i> +P-7)	01.67	05.83	77.73 (61.89)
P ₈ (<i>A. solani</i> +P-8)	03.23	04.27	56.88 (48.95)
P ₉ (<i>A. solani</i> +P-9)	03.26	04.23	56.44 (48.95)
P ₁₀ (<i>A. solani</i> +P-10)	03.00	04.50	60.00 (50.78)
P ₁₁ (<i>A. solani</i> +P-11)	02.90	04.60	61.34 (51.55)
P ₁₂ (<i>A. solani</i> +P-12)	01.80	05.70	76.00 (60.67)
P ₁₃ (<i>A. solani</i> +P-13)	03.23	04.27	56.88 (48.95)
P ₁₄ (<i>A. solani</i> +P-14)	01.90	05.60	74.67 (59.78)
P ₁₅ (<i>A. solani</i> +P-15)	03.10	04.40	65.34 (51.30)
P ₁₆ (<i>A. solani</i> +P-16)	02.30	05.20	69.34 (56.37)
P ₁₇ (<i>A. solani</i> +P-17)	03.46	04.03	53.78 (47.14)
P ₁₈ (<i>A. solani</i> +P-18)	02.06	05.44	72.54 (58.34)
SEm+-	0.02	0.02	0.47 (0.28)
C.V. (%)	4.71	3.09	3.41 (2.47)
CD (p=0.01)	0.31	0.31	4.57 (2.75)
CD (p=0.05)	0.23	0.23	3.41 (2.06)

*Data in the parentheses are angular transformed values

**Fig. 2. Antagonistic activity of native *Pseudomonas* isolates against *A. solani***

4. CONCLUSION

From the present study, it is observed that all the local isolates of *Trichoderma* and *Pseudomonas* inhibited the growth of the test pathogen, *A. solani* at different ranges. Although, the mechanisms employed by the selected native isolates were not mentioned, our findings indicate that these native isolates can be adopted for biological management of early blight of tomato which ultimately will increase the quality as well as quantity of productivity. Moreover, disease suppression by combination of compatible bioagents could be more effective than the individual ones possibly by the existence of synergism among the metabolites of the strains. However, field trials aimed at understanding the potential use of indigenous isolates of *Trichoderma* and *Pseudomonas* for management of early blight of tomato in agricultural system are suggested for future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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