Environment and Ecology 41 (4B) : 2652—2655, October—December 2023
Article DOI: https://doi.org/10.60151/envec/TTBV4593
ISSN 0970-0420

In vitro Antagonistic Potential of Native *Trichoderma* Species against *Alternaria solani* (Ellis and Martin) Causing Early Blight of Potato

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Received 31 May 2023, Accepted 4 September 2023, Published on 29 November 2023

ABSTRACT

An experiment was conducted *in vitro* to evaluate the antagonistic potential of six native *Trichoderma* species viz., *T. ovalisporum* (KU904456), *T. harzianum* (KU933468), *T. atroviride* (KU933472), *T. harzianum* (KU933474), *T. asperellum* (KU933475), *T. koningiopsis* (KU904460) against *Alternaria solani* (Ellis and Martin) causing early blight of potato through dual culture technique. Six native *Trichoderma* species were tested, and NCIPMCAU-69 (*T. harzianum*), which was found to be the most effective and recorded significantly higher mycelial growth suppression of *A. solani* by 71.37% followed by NCIPMCAU-131 (*T. harzianum*) by 70.98%, NCIPMCAU-96 (*T. ovalisporum*) by 69.80%, NCIPMCAU-18 (*T. koningiopsis*) by 69.02% and NCIPMCAU-118 (*T. atroviride*) by 68.62% respectively. The growth of *A. solani* was significantly suppressed by all native species of *Trichoderma*. The results indicate the ability of *Trichoderma* species as potential antagonists and capable of reducing the growth of *A. solani* causing early blight of potato.

Keywords *Alternaria solani*, Early blight, Potato, *Trichoderma*.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is an important food crop worldwide. The potato, also known as the white or Irish potato, is a member of the Solanaceae family. Potato is grown almost in all the states of India. Major potato growing states are Uttar Pradesh, West Bengal, Bihar, Gujarat, Madhya Pradesh, Punjab, Assam, Chhattisgarh, Jharkhand and Haryana (Chakraborty *et al.* 2022). Potato is a popular source of carbohydrates that may be used in both processed foods and meals. 79% of a raw potato's weight is made up of water, 17% of which is made up of carbs, 88% of which is starch, 2% of which is protein, and very little fat. More than 75% of the dry matter is made up of starch, although there also consists of some protein, fiber, and traces of fatty acids (Prokop and Albert 2008). The potato plant is known to be susceptible to several kinds of bacterial, viral and fungal diseases when suitable conditions are present, which dramatically lower crop yields. One of

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the most prevalent foliar fungal diseases affecting potatoes is early blight, which is caused by *Alternaria solani*, significantly lowers the crop's potential yield. Depending on the nature of didease, season and cultivar type, the average annually yield loss of potatoes caused by this disease is around 75% of the total production (Dey and Chakraborty 2012). Due to concerns regarding cost, exposure risks, and the harmful effects of its residues, fungicides cannot be considered a long-term solution. *Alternaria solani* has low sensitive with fungicides because of its development of melanin, a dark brown to black pigment that increased the pathogen's capacity for survival and competition in particular environmental conditions. Moreover, the emergence of pathogenic fungi that are resistant to synthetic pesticides is a great problem that can significantly affect the efficacy of chemical fungicides. In recent years, biological agents have emerged as a potential plant pathogen management approach. The most commonly used fungi biocontrol agent is *Trichoderma* and have long been known as effective antagonists against plant pathogenic fungi for the management of crop diseases, bio agents are used as seed and soil treatments as well as foliar sprays, which is a new, effective, and environment friendly method (Chand 2005). Different species of *Trichoderma* produce compounds at varying rates, and each has a distinctive mechanism of action against pathogens. Hence, the present investigation was undertaken to study the *in vitro* Antagonistic potential of native *Trichoderma* species against *Alternaria solani* (Ellis and Martin) causing early blight of Potato.

MATERIALS AND METHODS

Collection of the diseased samples and isolation of the causal pathogen

Potato leaves showing early blight symptoms were collected from farmer's fields and infected leaf samples were brought to laboratory and cut into small pieces measuring about 2 mm. Surface of spaceman was sterilized with 0.1% sodium hypochlorite solution for 1 minute and washed thrice with sterilized distilled water. Then one piece of specimen was transferred on potato dextrose agar (PDA) medium in the center of Petri plate and incubated at 27±1°C in BOD incubator and was observed periodically for the fungal growth. Pure culture of the *Alternaria solani* was obtained using hyphal tip culture technique and during the experimental period, kept at 5°C in the refrigerator and occasionally subcultured to fresh media.

In vitro efficacy of plant extracts against growth of *Alternaria solani*

In vitro antagonistic effect of six isolates of *Trichoderma* spp. viz., *T. ovalisporum* (KU904456), *T. harzianum* (KU933468), *T. atroviride* (KU933472), *T. harzianum* (KU933474), *T. asperellum* (KU933475), *T. koningiopsis* (KU904460) were evaluated against *A. solani*. All the *Trichoderma* species were collected from the Department of Plant Pathology, COA, CAU, Imphal. During the course of the experiment, each *Trichoderma* species was evaluated against *A. solani* using the dual culture technique (Dennis and Webster 1971). Under aseptic condition, 20 ml of sterilized PDA was poured into each of the 90 mm sterilized petri plates. After solidification, from seven days old culture of *A. solani* five mm disc was taken and placed at the petri plate's one end and additionally respective *Trichoderma* species (5 mm disc) was also inoculated at the petri plate's opposite side. A set of control plates were inoculated with only test fungus and growth inhibition was measured by comparison. Each set of treatment replicated three times. At a temperature of 27±1°C inoculated petri plates were incubated. Observations were taken on the inhibitory effect of the Trichoderma species over the growth of the *A. solani.* The lists of *Trichoderma* species are given in Table 1. The percent inhibition of radial growth was calculated following the equation described by Vincent (1947).

Per cent inhibition of radial growth = (C-T)/C × 100

Table 1. List of bio control agents with their accession number used in this experiment.

Sl. No.	Isolation code	*Trichoderma* species	Accession no.
1	NCIPMCAU-96	*T. ovalisporum*	KU904456
2	NCIPMCAU-69	*T. harzianum*	KU933468
3	NCIPMCAU-118	*T. atroviride*	KU933472
4	NCIPMCAU-131	*T. harzianum*	KU933474
5	NCIPMCAU-7	*T. asperellum*	KU933475
6	NCIPMCAU-18	*T. koningiopsis*	KU904460

Where, C=Radial growth of the fungus in control

T=Radial growth of the fungus in treatment

Based on the growth and mycoparasitic nature, bio control agents were grouped into various categories as per the scale given by Bell *et al.* (1982) with slight modifications as described below :

Class I - The antagonist completely overgrew the pathogen.

Class II - The antagonist overgrew at least 2/3rd of the pathogen's surface.

Class III - The antagonist colonizes on half of the growth of the pathogen.

Class IV - The pathogen and the antagonist locked at the point of contact.

Class V - The pathogen overgrew the mycoparasite.

Class VI - Formation of inhibition zone between pathogen and antagonist.

RESULTS AND DISCUSSION

Six native *Trichoderma* species were evaluated to check their efficacy against *A. solani* through dual culture technique and the results of the experimental data are presented in Table 2 and Fig.1. When compared to controls, all of the tested *Trichoderma* species significantly inhibited the test pathogen's mycelial growth. Among the six tested *Trichoderma* species, NCIPMCAU-69 (*T. harzianum*) resulted in maximum mycelial growth inhibition by 71.37%. However, NCIPMCAU-131 (*T. harzianum*) by 70.98%, NCIPMCAU-96 (*T. ovalisporum*) by 69.80%, NCIPMCAU-18 (*T. koningiopsis*) by 69.02%, and NCIPMCAU-118 (*T. atroviride*) by 68.62% accordingly showed a significant mycelial growth inhibition. While, NCIPMCAU-7 (*T. asperellum*) showed minimum mycelial growth inhibition of 66.66% and according to Bell's scale the antagonistic nature of all *Trichoderma* spp. were classified under Class II where the antagonist overgrew at least two third of the pathogen surface.

Table 2. *In vitro* evaluation of bio control agents against growth of (*Alternaria solani*) AS12 isolate. *Mean of three replications, values in parenthesis are angular transformation.

Sl. No.	*Trichoderma* species	Per cent inhibition over control (%)*
1	NCIPMCAU-96 (*T. ovalisporum*)	69.80 (56.67)
2	NCIPMCAU-69 (*T. harzianum*)	71.37 (57.65)
3	NCIPMCAU-118 (*T. atroviride*)	68.62 (55.93)
4	NCIPMCAU-131 (*T. harzianum*)	70.98 (57.41)
5	NCIPMCAU-7 (*T. asperellum*)	66.66 (54.74)
6	NCIPMCAU-18 (*T. koningiopsis*)	69.02 (56.18)
	SE (d)±	0.60
	CD (5%)	1.30

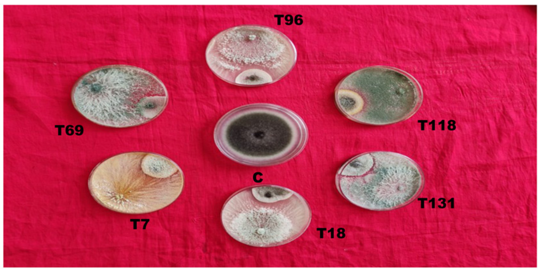

Fig 1. Inhibition of *Trichoderma* species on the growth of *Alternaria solani* (AS12) *in vitro* (T96-NCIPMCAU-96, T69-NCIPMCAU-69, T118-NCIPMCAU-118, T131- NCIPMCAU-131; T7- NCIPMCAU-7; T18-NCIPMCAU-18; C- Control).

The inhibitory effect of *Trichoderma* species against *A. solani* may be due to its greater ability to compete with *A solani* for food, nutrients, space, mycoparasitism, and antibiosis. The *Trichoderma* species also suppress the pathogen by generating both volatile and non-volatile chemicals (Tapwal *et al.* 2011, Sumana and Devaki 2012). *Trichoderma* species can be potentially employed as efficient biological control agents due to strong mycoparasitism of fungal pathogens and fast growth potential (Whipps and Lumsden 2001). The result of present findings are in confirmation with the finding of Babu *et al.* (2000) where they reported the highest mycelial growth inhibition (50.22%) in *T. harzianum* over control followed by *T. viride* when six *Trichoderma* species were compared for their efficacy against tomato early blight. The present findings are also in conformity with Singh *et al.* (2018) where they reported highest mycelial inhibition of *A. solani* in *T. harzianum* (80.37%) followed by *T. viride* (71.48%)

and *T. koningii* (77.41 %) and least in *T. hamatum* (27.41%). The antagonistic nature of *Trichoderma* species against many fungi is mainly due to production of acetaldehyde and carbonyl compound. This may also be the reason for its inhibitory effect on *A. solani.* The effectiveness of *T. harzianum* against *A. solani* was also reported by several workers (Devi *et al.* 2017, Rani *et al.* 2017, Naik *et al.* 2020).

CONCLUSION

The results obtained from this study shows that among the six tested *Trichoderma* species, NCIPMCAU-69 (*T. harzianum*) was found to be most effective by exhibiting maximum mycelial growth inhibition of 71.37% under *in vitro* condition against *A. solani*. These findings proved that *Trichoderma* species can be utilized as a bio control agent for the management of *A. solani.* Therefore, further research into these prospective biological agents and the bioactive substances they contain that are effective against *A. solani* can be exploited under field condition, in order to limit the number of pesticides and pollution hazards.

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