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Antagonistic Potential of *Trichoderma* spp., Botanicals and Fungicides against *Alternaria solani* Causing Early Blight of Tomato *in-vitro* Conditions

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Abstract
The tomato is the edible, often red berry of the plant Solanum lycopersicum, commonly known as a tomato plant. The species originated in western South America and Central America. The area under tomato cultivation in Manipur accounts for about 0.15 million hectares with an average production of 2.10 million tonnes and productivity of 12.02 tonnes ha^{-1} during 2016-17. The major constraints in production of tomato are biotic and abiotic stress. Among the biotic stress Early blight caused by *Alternaria solani* inflicts tremendous losses to the crop. The present research was carried out to study *in vitro* evaluations of native *Trichoderma* spp., botanicals and fungicides against *Alternaria solani* causing Early blight of tomato which induces losses in Manipur. Food poison technique and Dual culture were aided in this investigation. The investigated results revealed that among bio control agents tested Mix (*Trichoderma asperellum* + *Trichoderma harzianum*), *Trichoderma viride* and *Trichoderma asperellum* effectively controlled mycelial growth of the pathogen by 74.92% and 73% respectively. Botanicals used in this study significantly inhibited the growth of the fungus, among which garlic (*Allium sativum)* gave the best results by showing 74.17% of inhibition at 10% concentration followed by garlic 5% and ginger 10% showed 66.98 to 61.05% inhibition, among fungicides Propiconazole 13.9% + Difenoconazole 13% gave the best results by showing of 100% inhibition at 0.1% concentrations.

Keywords*: Alternaria solani*, Antagonistic Potential of *Trichoderma* spp, Botanicals, Fungicides and Early blight of Tomato

1. Introduction

The tomato is the edible, often red berry of the plant Solanum lycopersicum, commonly known as a tomato plant. The species originated in western South America and Central America. Tomato holds second rank next to potato in world acreage although it is first among processing crops. Tomato is an important source of nutrients as it has high nutritive value of vitamin A, B, C, E and other important nutrients *viz*., protein, carbohydrates, fiber, fat, biotin, malic acid, citric acid, oxalic acid etc. It contains fibers and is known as free of cholesterol. Lycopene is a very potent antioxidant that prevents cancers and it avert humans from free radicals that degrade many parts of the body. Tomatoes are utilized more in the developed countries than in the developing countries and hence it may be referred to as a luxury crop (Anon., 2013).

In India, it is grown in a wide range of climate across states of Andhra Pradesh, Odisha, Karnataka, Maharashtra, West Bengal, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh and Chhattisgarh and accounting a total production of 19.69 million tonnes from an area of 0.81 million hectares with an average productivity of 24.4 tonnes ha^{-1} during 2016-17 (FAO Stat 2016).

There are several diseases of tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors. Among the fungal diseases occurring on tomato early blight (*Alternaria solani),* wilt (*Fusarium oxysporum* f.*sp. lycopersici*), Septoria leaf spot (*Septoria lycopersici*) and late blight (*Phytophthora infestans)* are threatening the tomato production (Sokhi *et al*., 1991).

Early blight of tomato caused by *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 (Datar and Mayee, 1981). Fruit rot of tomato caused by *A. solani* is most destructive and causes fruit rot in storage, transportation and marketing. Infected tomato fruits reduced in their nutritional value and becomes unfit for consumption. The early blight was reported to be the most important disease, causing 50 to 80 per cent losses in fruit yield of Tomato (Mathur and Shekhawat, 1986). Every one per cent increase in disease intensity could reduce yield by 1.36 per cent, and complete crop failure occurred when the disease is severe (Sherf and MacNab, 1986). Saha and Das (2012) also reported losses in tomato yield to an extent of 0.75 to 0.77 tons ha^{-1} with 1 per cent increase in disease severity.

2. Materials and Methodologies

2.1 Isolation of fungus

Early blight infected tomato plant samples were collected from farmer's field of different locations and isolation, identification of the causal pathogen was carried out in the Department of Plant Pathology, College of Agriculture, CAU, Imphal. Diseased samples were lacerated to small pieces with the help of sterilized scalpel. The lacerated pieces were surface sterilized using 1% sodium hypochlorite solution for 1 minute followed by rinsing the pieces in three phases of sterile distilled water in order to remove the traces of sodium hyphochlorite. Later the pices were blot dried using blotting paper. The sterile pieces were aseptically transferred to sterilized petri dishes containing Potato dextrose agar (PDA). The petri dishes were incubated at 27 ± 1^{0}C in BOD incubator and were observed periodically for the fungal growth. Purified cultures of the fungus ware obtained by hyphal tip culture methods. Identification was done according to the key of (Leslie and Summerell, 2006).

2.2 *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Alternaria solani* causing Early blight of Tomato

In-vitro antagonistic effect of three isolates of *Trichoderma* spp. *viz., (T. harzianum, T. asperellum* and *T.viride*) were evaluated against the test fungus. All the bio-control agents were collected from the Department of Plant Pathology, COA, CAU. Antagonistic test of bio-control agent was done, following the dual culture technique (Bell 1982). The observations were recorded based on Bell's scale

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Bell's scale with slight modification:

Class I : The antagonist completely overgrew the pathogen (100%over growth)

Class II : The antagonist overgrew at least 2/3rd of the pathogen surface (75%over growth)

Class III: The antagonist colonized on half of the growth of the pathogen surface (50%over growth)

Class IV: The pathogen and the antagonist locked at the point if contact

Class V: The pathogen overgrew the mycoparasite

Class VI: The pathogen and antagonistic from inhibition

A chemical fungicide, mancozeb (0.3 %) will be used for the *in vitro* experiment as a check. Per cent inhibition will be calculated by using following formula suggested by Vincent (1927).

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

Where,

C = radial growth of fungus in control

T = radial growth of fungus in treatment

2.3 Effect of Botanicals against Growth of *Alternaria solani* causing Early blight of Tomato

Extracts of three locally available botanicals namely, Garlic (*Allium sativa*), Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale)* were studied *in vitro* for their effect on growth of the fungus. Each plant extract was tried at three different concentrations. Fresh plant parts were collected and washed thoroughly in running water and surface sterilized with 70% ethanol for few second then finally washed with sterile water. They were then crushed using mortar and pestle separately by mixing with sterile water at the ratio of 1:1 w/v. The extracts were filtered through muslin cloth and centrifuge at 1500 rpm for 15 minutes and the supernatants were separated. The prepared plant extracts were considered as 100% concentration. The required concentrations of plant extracts were added to hundred (100) ml Erlenmeyer conical flask containing sterilized 50 ml molten PDA medium to give the desired concentrations and shaken well and mixed thoroughly. The poisoned PDA medium were poured in petriplates @ 20 ml per plate and allowed to solidify. The plates were then inoculated aseptically by transferring 5 mm mycelial disc with the help of cork borer and sterilized needle. The plates were then kept inside BOD incubator (25+1°C) till the pathogen fully grows in the control plates. The PDA medium without plant extracts served as control. Each treatment was replicated three times. Per cent inhibition of the fungus was calculated by following the formula given by Vincent (1947) mentioned above

2.3 Effect of fungicides and a fungicidal combination against growth of *Alternaria solani* causing Early blight of Tomato

Fungicides and a fungicidal combination *viz*., Propiconazole 25%, Difenoconazole 25% and Propiconazole13.9%+Difenoconazole13% used in the current *in vitro* studies along with the particulars like trade name, chemical name and active ingredient of the chemical formulation. Food poison technique was used for this evaluation. The poisoned PDA medium were poured in petriplates @ 20 ml per plates and allowed to solidify. The plates were then inoculated aseptically by transferring 5 mm mycelial disc with the help of cork borer and sterilized needle. The plates were then kept inside BOD incubator (25+1°C) till the pathogen fully grows on the control plates. Each treatment was replicated three times. Per cent inhibition of the fungus was calculated by following the formula given by Vincent (1947) mentioned above.

3. Results and Discussions

3.1 *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Alternaria solani* causing Early blight of Tomato

The effect of different *Trichoderma* spp. and a chemical fungicide on radial growth of *Alternaria solani* are presented in Table 9, Figure 5. and Plate 9. revealed that all the species of *Trichoderma* spp exhibited different antagonistic potential against the *Alternaria solani*. Among three *Trichoderma* spp tested *Trichoderma harzianum* showed highest colony growth (3.5 cm) and inhibition percentage (55.55%) followed by *Trichoderma asperellum* (2.2 cm colony growth and 72.06% inhibition), *Trichoderma viride* (2.17 cm colony growth and 72.38% inhibition), mix (*Trichoderma harzianum* + *Trichoderma asperellum*) (1.95 cm and 74.92%) and mancozeb (no colony growth and 100% respectively).

Rini and sulochana in kerala studied the ability of *T. viride* and *P. fluorescence* in suppressing the population of *Alternaria solani* in chili and their effects on growth and yield of the crop they reported that *T. viride* isolates TR19 and TR22 when applied alone produced higher yield (294.5 g and 288 g per plant respectively) fresh and dry weights of the root at 90 days after planting recorded maximum in TR22 applied plots (7.4 and 2.2 g respectively) followed by TR19 and *P. flourscence* in year 2006.

3.2 Effect of Botanicals against growth of *Alternaria solani* causing Early blight of Tomato

In *Alternaria solani* best result showed by Garlic (10%) colony growth 2.03 cm and 74.14% inhibition, followed by Garlic(5%) showed (2.6 cm colony growth and 74.14% inhibition) and Garlic (2.5%) showed (3.13 cm and 60.21%) remaining plant extracts that's as Ginger (5%) (10%), Turmeric (2.5%) (5%) (10%) showed more are less same colony growth that's is 3.0 to 3.15 cm and inhibition is 60.10 to 61.05%, and lowest results was showed by Ginger (2.5%) colony growth is 3.5cm and inhibition 55.55%. findings are in conformity with the findings of Mishra and Gupta (2006).

3.3 Effect of fungicides and a fungicidal combination against growth of *Alternaria solani* causing Early blight of Tomato

Data revealed that *Alternaria solani* at 0.1% Propiconazole 13.9% + Difenoconazole 13% was found to be the best with no colony growth and 100% growth inhibition followed by Propiconazole 13.9% + Difenoconazole13% (0.05% and 0.025%) showed nearly same (0.1 cm colony growth and 98.2% inhibition respectively), Propiconazole 25% (0.1% and 0.05%) and Difenoconazole 25% shows more are less same (0.7 to 0.8 cm colony growth 85% to and 91 % inhibition respectively), Propiconazole 25% (0.025% and) and Difenoconazole 25% (0.05% shows nearly same results) (3-3.5 cm and 65%-55% respectively), and lowest result shown by Difenoconazole 25% (0.025%)with (4.8 cm and 38.20% respectively).

El. Nazar *et al*., (1970) found that mancozeb gave better protection than the cupravit and zineb against *Alternaria solani* and *Phytopthora infestans*. Mathur *et al*., (1971) reported that Dithane M-45 (0.2%), Brestan (0.05%), Antracol (0.2%) and Captan (0.2%) were found effective in their merits for controlling the Early blight disease of potato.

Table 1. *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Alternaria solani* causing Early blight of Tomato

Treatment No.	Treatment details	Dose (%)	*Alternaria solani*	
			Colony growth(cm)*	Inhibition % over control*
T1	*Trichoderma asperellum (25)*	10	2.2	72.06
T2	*Trichoderma harzianum (69)*	10	3.5	55.55
T3	*Trichoderma viride*	10	2.1	72.38
T4	Mix (T1+T2)	10	1.9	74.92
T5	Mancozeb	0.3	0	100
SE(d)				0.04
CD (0.05)				0.12

*Mean of three replications

Table 2. Effect of Botanicals against growth of *Alternaria solani* causing Early blight of Tomato

Botanicals (Parts used)	Concentration (%)	*Alternaria solani*	
		Colony growth(cm)*	Inhibition % over control
Garlic (Cloves)	2.5	3.13	60.21
	5.0	2.6	66.98
	10	2.0	74.17
Ginger (Rhizome)	2.5	3.5	55.55
	5.0	3.1	60.21
	10	3.0	61.05
Turmeric (Rhizome)	2.5	3.13	60.21
	5.0	3.1	60.63
	10	3.1	60.63
SE(d)±			0.04
CD (0.05)			0.13

*Mean of three replications

Table 3. Effect of fungicides and a fungicidal combination against growth of *Alternaria solani* causing Early blight of Tomato

Fungicide	Concentration	*Alternaria solani*	
		Colony growth	Inhibition over control
Propiconazole25%EC	0.1	0.7	91.11
	0.05	1.33	83.06
	0.025	2.8	64.44
Difenoconazole25% EC	0.1	0.83	89.41
	0.05	3.56	54.70
	0.025	4.86	38.20
Propiconazole13.9% + Difenoconazole13% EC	0.1	0	100
	0.05	0.13	98.29
	0.025	0.14	98.18
SE(d)±			0.04
CD(0.05)			0.12

*Mean of three replications

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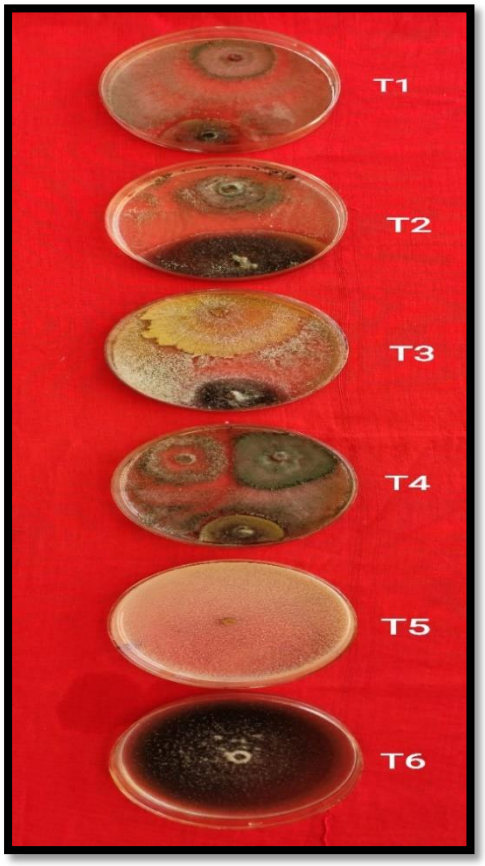


T1 - *Trichoderma asperellum*

T2 -*Trichoderma harzianum*

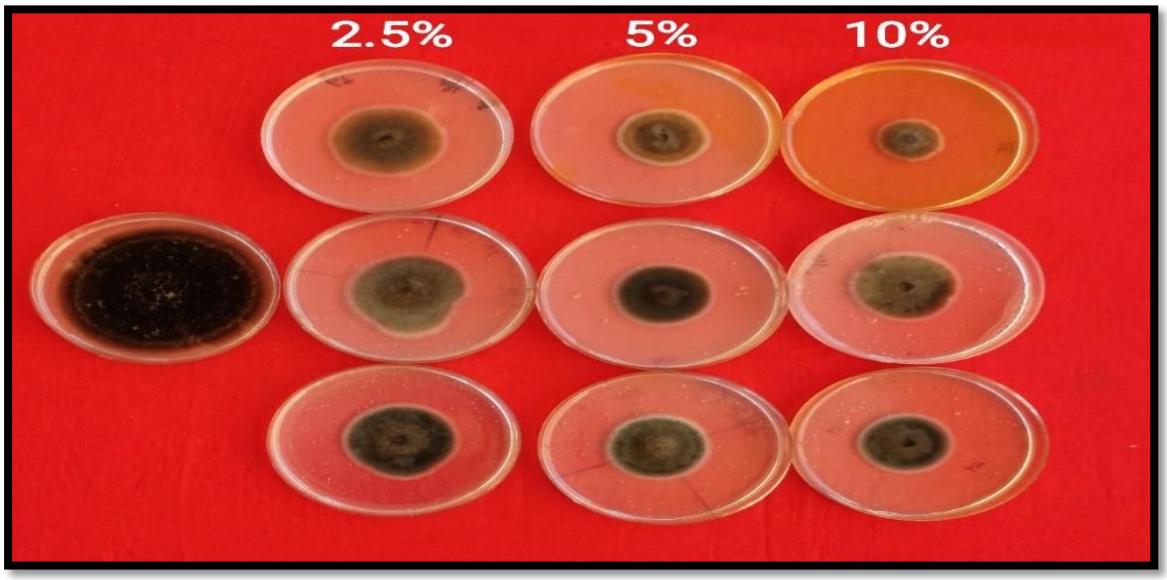
T3 -*Trichoderma viride,*

T4 - Mix (T1+T2)

T5- Mancozeb

T6 – control

Plate 1. *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Alternaria solani* causing Early blight of Tomato



1.Garlic 2.Ginger 3.Turmeric

Plate 2. Effect of Botanicals against growth of *Alternaria solani* causing Early blight of Tomato

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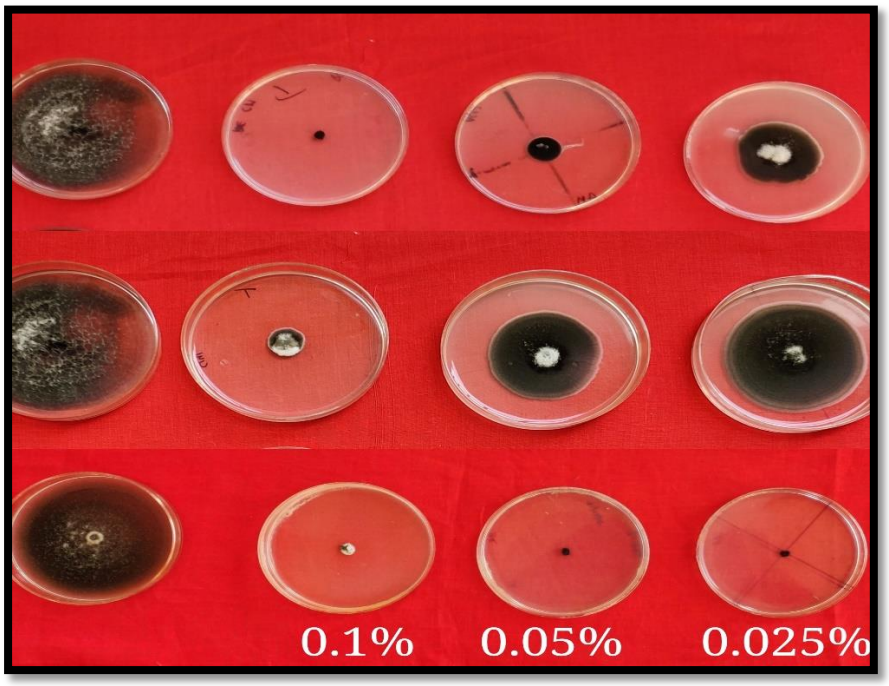

1. Propiconazole 25%

2. Difenoconazole 25%

3. Propiconazole13.9% + Difenoconazole 13%

Plate 3. Effect of fungicides and a fungicidal combination against growth of *Alternaria solani* causing Early blight of Tomato

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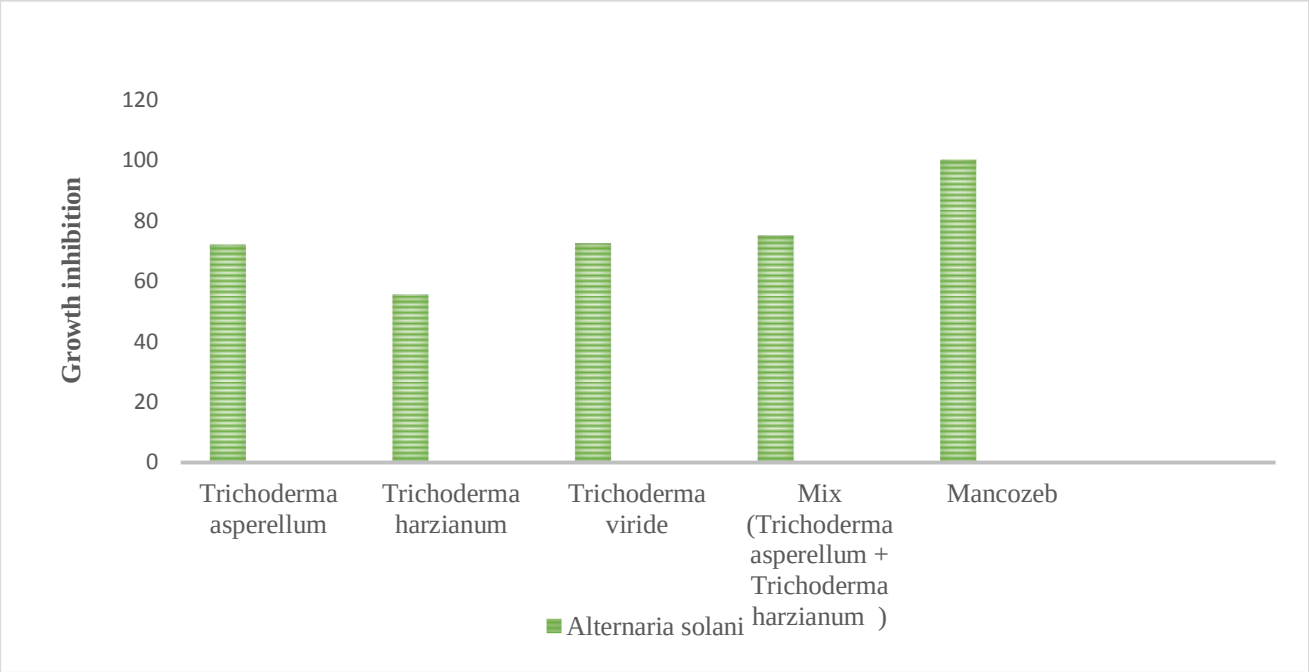
Graph 1. *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Alternaria solani* causing Early blight of Tomato



Graph 2. Effect of Botanicals against growth of *Alternaria solani* causing Early blight of Tomato

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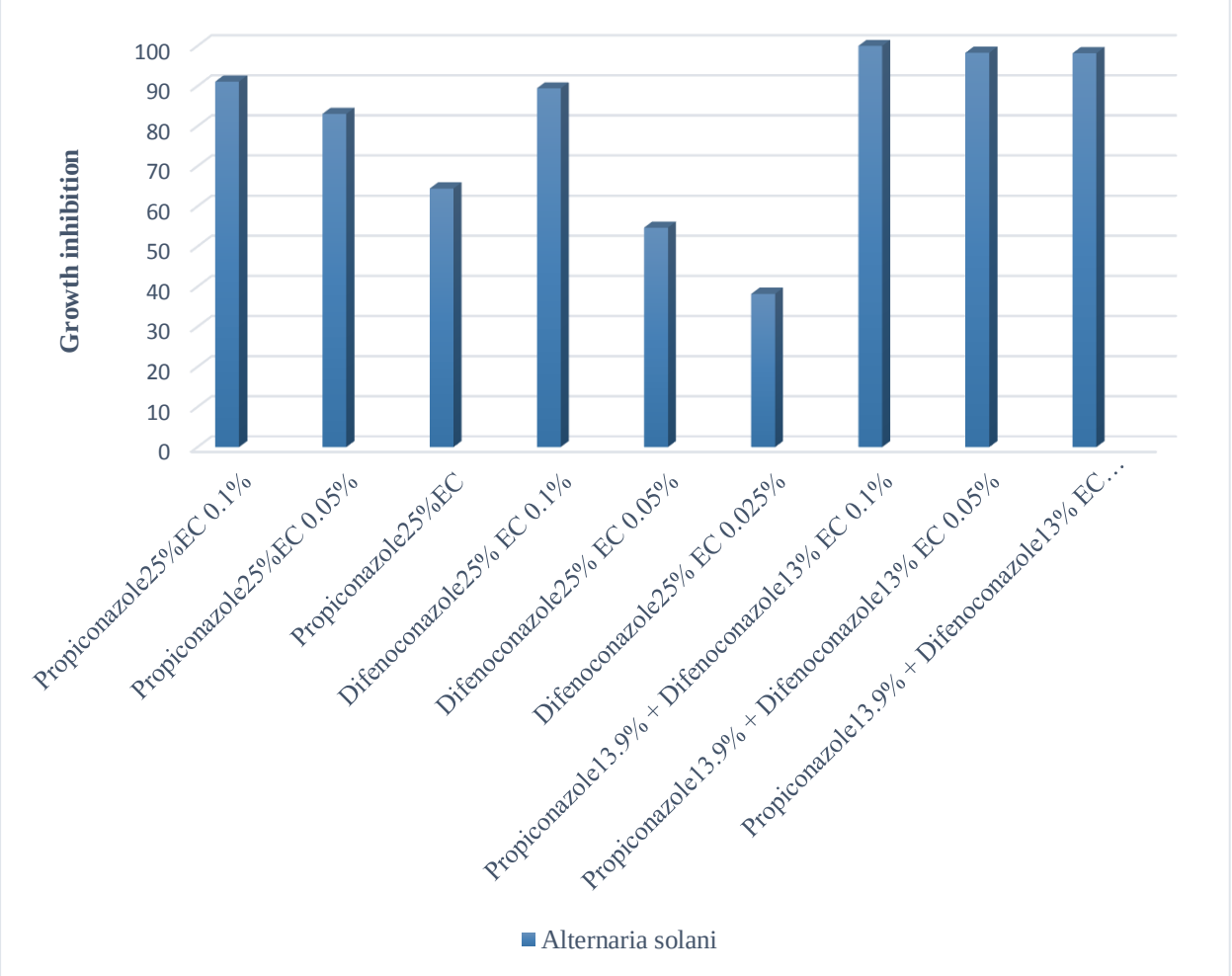
Graph 3. Effect of fungicides and a fungicidal combination against growth of *Alternaria solani* causing Early blight of Tomato



4. Conclusion

It is evident that all the Trichoderma spp. used in this investigation exhibited antagonism in suppressing the mycelial growth of *Alternaria solani*. These findings showed that for management of *Alternaria solani*, Trichoderma spp. can be used as bio control agent. All the fungicides tested effectively inhibit the growth of pathogen. Among all the plant extracts garlic and Ginger showed the best result, all the bio control agents also significantly inhibit the growth of pathogen.

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