

Original Research Article

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## ***In vitro* Evaluation of Antagonistic Potential of Native *Trichoderma* spp., Botanicals and Fungicides against *Curvularia spicifera* causing *Curvularia* Leaf Spot of Tomato in Manipur**

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### **A B S T R A C T**

Tomato (*Solanum lycopersicum* Mill.) is one of the most remunerative and widely grown vegetables in the world. 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<sup>-1</sup> during 2016-17. The major constraints in production of tomato are biotic and abiotic stress. Among the biotic stress *Curvularia* leaf spot caused by *Curvularia spicifera* 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 *Curvularia spicifera* causing *Curvularia* leaf spot 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 55% and 50% respectively. Botanicals used in this study significantly inhibited the growth of the fungus, among which garlic (*Allium sativum*) gave the best results by showing 73.33% of inhibition at 10% concentration followed by garlic 5% and ginger 10% showed 55.55 to 53.86% inhibition, among fungicides Propiconazole 13.9% + Difenconazole 13% gave the best results by showing of 100% inhibition at 0.1% concentrations.

#### **Keywords**

*Curvularia spicifera*,  
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### **Introduction**

Tomato (*Solanum lycopersicum* Mill.) is one of the most remunerative and widely grown vegetables in the world. It is an annual crop belonging to the family Solanaceae and native of "South America" (Sabina 1819). Tomato holds second rank next to potato in world acreage although it is first among processing

crops. Tomato is grown for its edible fruits, which can be consumed either fresh or in the form of various processed products such as paste, powder, ketchup, sauce, soup and canned whole fruits. Tomato is also known for high medicinal value and nutritional values. The pulp and juice are digestible, promoter of gastric secretion and blood purifier.

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<sup>-1</sup> 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).

Mandokhot and Basu Chaudhary (1972) evaluated ten fungicides belonging to different groups of chemicals and assayed for their effectiveness against leafspot disease of maize caused by *Curvularia clavata*. Among the fungicides evaluated, difolaton 80 WP was found the most effective.

Archana (2008) tested the bioagents and phytoextracts against *Curvularia penniseti* in pearl millet. The maximum inhibition was observed with *Trichoderma viride* (53%) followed by *Trichoderma harzianum* (47%). Among the phytoextracts, *Emblica officinalis* and *Azadirachta indica* were found effective.

Sumangala *et al.*, (2008) evaluated various fungicides, botanicals and bioagents against *C. lunata* causal agent of grain discoloration in rice. Among the four systemic fungicides, maximum inhibition of mycelial growth was obtained from Difenconazole (98.8%) and Propiconazole (98.10%) at 0.1 per cent concentration. Among the nonsystemic fungicides, Mancozeb (98.8%) was found the most effective. Among the seven botanicals

tested, garlic bulb extract (96.44%) at 10 per cent concentration was found effective. Among the bioagents *Bacillus subtilis* (97.77%), *T. viride* (96.44%) and *T. harzianum* (93.5%) were found effective in inhibiting the radial growth of the fungus.

## Materials and Methods

### Isolation of fungus

*Curvularia* leaf spot 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 hypochlorite. Later the pieces 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°C in BOD incubator and were observed periodically for the fungal growth. Purified cultures of the fungus were obtained by hyphal tip culture methods. Identification was done according to the key of (Leslie and Summerell, 2006)

### ***In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Curvularia spicifera* causing *Curvularia* leaf spot 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

### **Bell's scale with slight modification**

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

Class II: The antagonist overgrew at least 2/3<sup>rd</sup> 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 of contact

Class V: The pathogen overgrew the mycoparasite

Class VI: The pathogen and antagonist 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).

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

Where,

C = radial growth of fungus in control

T = radial growth of fungus in treatment

### **Effect of Botanicals against growth of *Curvularia spicifera* causing *Curvularia* leaf spot 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

### **Effect of fungicides and a fungicidal combination against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato**

Fungicides and a fungicidal combination *viz.*, Propiconazole 25%, Difenconazole 25% and Propiconazole 13.9% + Difenconazole 13% 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

## Results and Discussion

### **In vitro** evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato

All the *Trichoderma* spp exhibited different antagonistic potential against the *Curvularia spicifera*. Among three *Trichoderma*spp tested *Trichoderma viride* showed highest colony growth (3.77 cm) and inhibition percentage (52.06%) followed by *Trichoderma harzianum* (3.67 cm and 53.33%), *Trichoderma asperellum* (3.57 cm and 54.06%), mix (*Trichoderma harzianum* + *Trichoderma asperellum*) (3.47 cm and

55.87%) and mancozeb (1.87cm colony growth and 76.19% respectively).

All the bio control agents significantly inhibit the growth of the test fungus. According to Bell’s scale the antagonistic nature of all the three *Trichoderma* spp. were classified under Class III in which the antagonist colonized on half of the growth of the pathogen surface (50% over growth) (Table 1).

### **Effect of Botanicals against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato**

In *Curvularia spicifera* best result showed by Garlic (10%) colony growth 2.1 cm and 73.33% inhibition, followed by Garlic(5%) showed (3.5 cm and 55.55%) and Ginger (10%) showed (3.63 cm and 53.86%), Ginger (5%) and Turmeric (10%) showed same as (3.8and 51.74%) remaining plant extracts that’s as Garlic (2.5%),Turmeric (2.5%) and (5%) showed more are less same colony growth that’s is 4.1 to 4.25 cm and inhibition is 46.00 to 47.65%. lowest results is showed by Ginger (2.5%) colony growth is 4.43cm and inhibition 43.73% (Table 2).

**Table.1** *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato

Treatment No.	Treatment details	Dose (%)	<i>Curvularia spicifera</i>	
			Colony growth(cm)*	Inhibition % over control*
T1	<i>Trichoderma asperellum</i> (25)	10	3.57	54.60
T2	<i>Trichoderma harzianum</i> (69)	10	3.67	53.33
T3	<i>Trichoderma viride</i>	10	3.77	52.06
T4	Mix (T1+T2)	10	3.47	55.87
T5	Mancozeb	0.3	1.87	76.19
SE(d)				0.04
CD(0.05)				0.12

\*Mean of three replications

**Table.2** Effect of botanicals against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato

Botanicals (Parts used)	Concentration (%)	<i>Curvularia spicifera</i>	
		Colony growth(cm)*	Inhibition % over control*
Garlic (Cloves)	2.5	4.1	47.93
	5.0	3.5	55.55
	10	2.1	73.33
Ginger (Rhizome)	2.5	4.43	43.70
	5.0	3.8	51.74
	10	3.63	53.86
Turmeric (Rhizome)	2.5	4.2	46.66
	5.0	4.13	47.51
	10	3.8	51.74
SE(d)±			0.04
CD(0.05)			0.14

\*Mean of three replications

**Table.3** Effect of fungicides and a fungicidal combination against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato

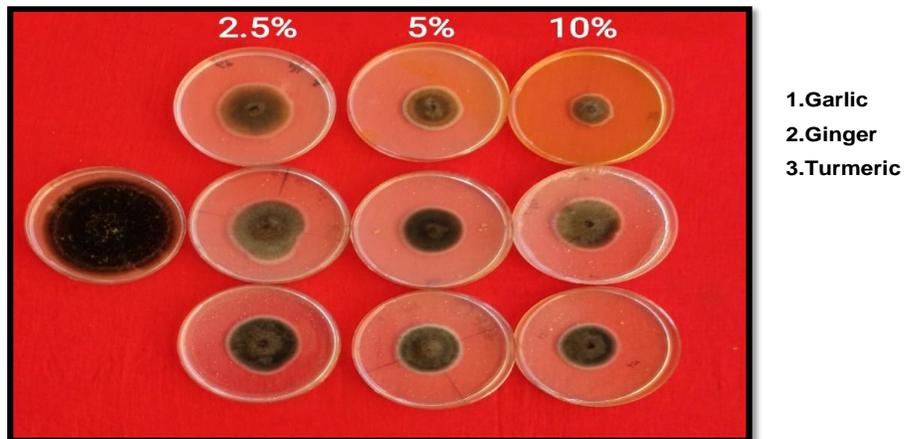
Fungicide	Concentration	<i>Curvularia spicifera</i>	
		Colony growth	Inhibition over control
Propiconazole25%EC	0.1	0.63	91.95
	0.05	1.2	84.76
	0.025	3.8	51.74
Difenoconazole25% EC	0.1	0.4	94.92
	0.05	4.8	39.04
	0.025	5.8	26.34
Propiconazole13.9% + Difenoconazole13% EC	0.1	0	100
	0.05	0.13	98.30
	0.025	0.14	98.17
SE(d)±			0.04
CD(0.05)			0.14

\*Mean of three replications

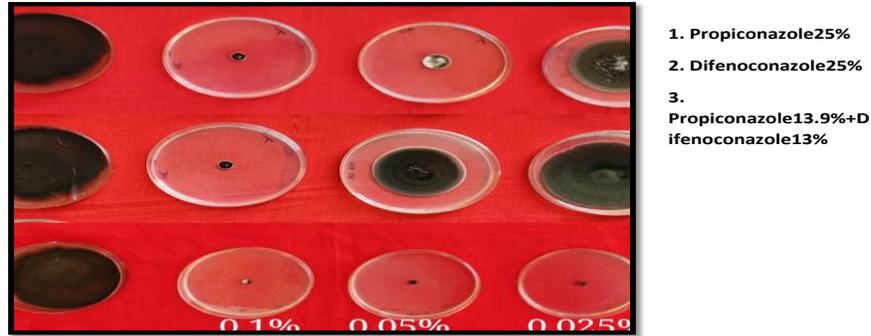
**Plate.1** *In vitro* evaluation of antagonistic effect of native *Trichoderma* spp. against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of tomato



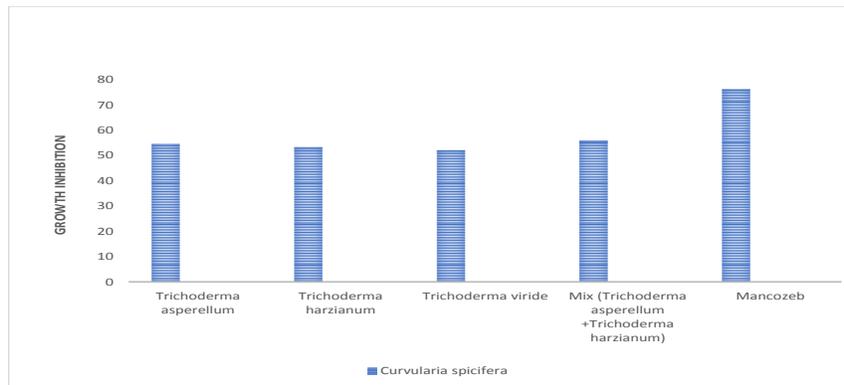
**Plate.2** Effect of Botanicals against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of tomato



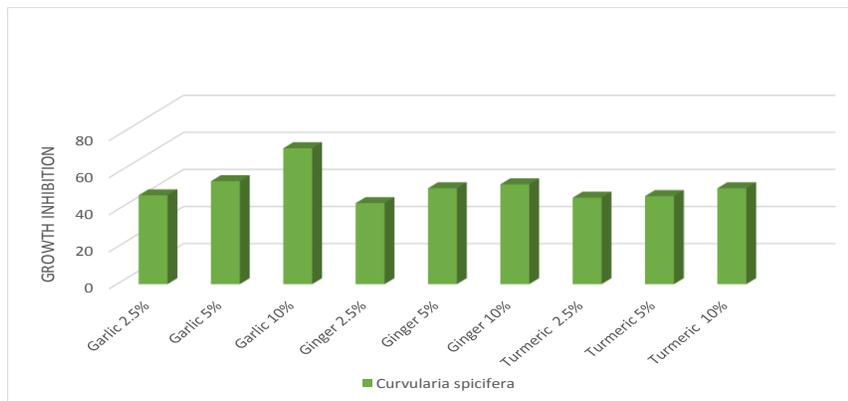
**Plate.3** Effect of fungicides and a fungicidal combination against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato



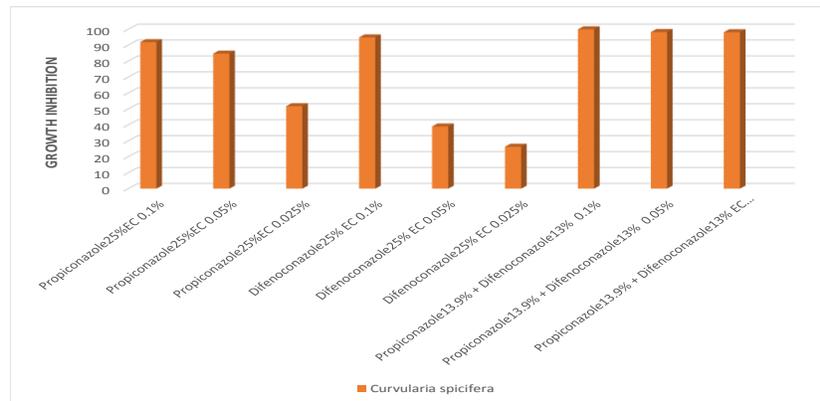
**Graph.1** In vitro evaluation of Antagonistic effect of native *Trichoderma* spp. against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of tomato



**Graph.2** Effect of botanicals against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato



**Graph.3** Effect of fungicides and a fungicidal combination against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato



**Effect of fungicides and a fungicidal combination against growth of *Curvularia spicifera* causing *Curvularia* leaf spot of Tomato**

*Curvularia spicifera* at 0.1% Propiconazole 13.9% + Difenconazole 13% was found to be the best with no colony growth and 100% growth inhibition followed by Propiconazole 13.9% + Difenconazole 13% (0.05% and 0.025%) showed nearly same (0.1 cm and 98.5% respectively), Difenconazole 25% (0.1%) and Propiconazole 25% (0.1%) shows more are less same (0.4 to 0.6 cm colony growth 91% to 94 % inhibition respectively), Propiconazole 25% (0.05 and 0.025%) showed (1.2 cm & 3.8% colony growth and 84%-51.74% inhibition respectively), and lowest result shown by Difenconazole 25% (0.025%) with 5.8 cm and 26.34% respectively (Table 3).

In conclusion it is evident that all the *Trichoderma* spp. used in this investigation exhibited antagonism in suppressing the mycelial growth of *Curvularia spicifera*.

These findings showed that for management of *Curvularia spicifera*, *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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