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In vitro efficacy of botanicals and biocontrol agents against Fusarium leaf blight of tomato

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Abstract
Fusarium blight is a potentially destructive disease in tomato occurring on the oldest leaves as small, brownish to black lesions which are due to foliar blight disease and it was caused by *Fusarium oxysporum*. The disease causes huge yield as well as post-harvest losses. *Fusarium oxysporum* exhibited dull to fluffy white mycelium with a slight faint reddish tinge at the middle on PDA. Eight plant extracts and four biocontrol agents were evaluated following poison food technique and dual culture. Garlic+Neem+Datura and only garlic extract induced 100% growth reduction at 10% and 20% concentrations for both the pathogens followed by Neem oil up to 69% for *Fusarium oxysporum*. Neem leaf extract recorded least growth inhibition of 5.81% and 5.9% in 10% and 20% concentration respectively. Among three fungal bio-control agents *Trichoderma viride* recorded maximum growth inhibition against *F. oxysporum* (81.27%). *Pseudomonas fluorescens* inhibited growth 80.70% against *F. oxysporum.*

Keywords: Karanj, *Fusarium oxysporum, Pseudomonas fluorescens, Trichoderma viride*

Introduction
The tomato is a warm season crop and world's largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables [11]. A general climate of Odisha is warm and humid with mild winter and a hot summer which is very much conducive for rapid growth of pathogenic micro-organisms. Like other crops this crop is also subjected to several diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors [2]. The crop suffers from a number of foliar diseases such as early blight, also called Alternaria leaf blight [3] (*Alternaria solani*), late blight (*Phytophthora infestans*), Septoria leaf spot (*Septoria lycopersici*), Gray mold (*Botrytis cinerea*) and leaf mold (*Fulvia fulva*), Fusarium blight incited by *Fusarium oxysporum* f.sp. *lycopersici*. Among all fungal diseases Fusarium blight is more common in Orissa. The first symptoms appear when fruit begins to mature. Lower leaves turn yellow. This is followed by leaf and stem wilting. The fusarium blight is soil borne and can persist for many years in soil even if no host plants are grown. Present experiment for evaluate the botanicals and bioagents for eco safe management of Fusarium blight in tomato crop.

Materials and Methods
In vitro evaluation of Plant extracts for management of causal pathogen
The present investigation was carried out to evaluate different plant species for the possible presence of fungi toxicant properties by poisoned food technique. The list of botanicals used in the study is presented in Table 1. Hundred grams of fresh leaf material was taken and cut into small pieces, 20 ml of 5 percent acetone was added and the samples were ground thoroughly. Different plant extracts of varying concentrations i.e. 10.0 and 20.0 percent were tested in three replications. *In vitro* evaluations of leaf extracts was done against *Fusarium oxysporum* using Potato dextrose agar medium. The leaf extracts were mixed to the medium by proper stirring and poured to Petri plates and allowed for solidification. Seven mm disc from twelve days actively growing culture was transferred aseptically using a cork borer to the Petri plates containing leaf extracts. The PDA plates without any plant extracts served as control. The plates were incubated at 27±1 °C for 10 days and the colony diameter was recorded. Percent inhibition was worked out according to the equation of [13].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition of the mycelium C = Growth of the mycelium in control
T = Growth of the mycelium in treatment.

Table 1: List of plant extracts used

SI No.	Common name	Botanical name	Plant parts used
1	Garlic+Neem+Datura	Combination	Clove + Leaf
2	Datura	*Datura stramonium*	Leaf
3	Garlic	*Allium sativum*	Clove
4	Mint	*Menthe piperita*	Leaf
5	Onion	*Allium cepa*	Bulb
6	Turmeric	*Curcuma longa*	Rhizome
7	Azadirachtin oil	*Azadirachta indica*	Neem seed
8	Neem	*Azadirachta indica*	Leaf
9	Karanj	*Pongamia pinnata*	Leaf

***In vitro* evaluation of Neem oil for management of test pathogen**

The experiment was carried out by taking 1% oil concentration for management of mycelia growth. The appropriate concentration of oil after emulsifying with Tween 20 was mixed with sterilized potato dextrose agar media and thoroughly mixed with the media. Twenty ml of media was poured into each Petri dish and allowed for solidification.

***In vitro* evaluation of bio control agents**

The efficacy of biocontrol agents were tested against the casual organism by dual culture technique. Biocontrol agents like *Trichoderma viride, Trichoderma hamatum, and Trichoderma harzianum, Pseudomonas fluorescens* (Table-2) were tested against the fungus. The fungal antagonist was grown in potato dextrose agar media and bacterial antagonist in nutrient agar media to get a fresh active culture for the experiment

Table 2: Biocontrol agents

SI No.	Biocontrol agents	Place of Collection
1	*Trichoderma viride*	Department of Agriculture Entomology, College of Agriculture, OUAT, Bhubaneswar.
2	*Trichoderma hamatum*	Department of Plant pathology, College of Agriculture, OUAT, Bhubaneswar.
3	*Trichoderma harzianum*	Central Horticultrual Experiment Station, Bhubaneswar.
4	*Pseudomonas fluorescens*	AICRP on ground nut, Bhubaneswar.

Dual culture technique

About 20ml of potato dextrose media for fungus and nutrient agar media for bacteria was poured into petri dishes and allowed to cool down. The fungal mycelial disc (5 mm) was transferred to one end of the plate and fungal antagonist culture disc placed opposite to it leaving 5-6 mm distance from the periphery of the plates. In case of bacterial antagonist, the bacterium was streaked at one side of the plate and fungal culture disc at the other side of the plate. Each treatment was replicated thrice. The inoculated plates were taken. The data analyzed statistically. The efficacy of biocontrol agents were expressed as percentage inhibition of mycelia growth over control and calculated as [13].

$$I = \frac{C - T}{C} \times 100$$

I = Percent inhibition, C= Radial growth in control, T = Radial growth in treatment

Statistical Analysis

The experiments were done under controlled laboratory conditions, and the data were analyzed following completely randomized design (CRD).

Results and Discussion

Fusarium oxysporum

All the tested plant extracts also exhibited significant growth reduction against *Fusarium oxysporum* in comparison to control and among them self in both the concentrations. Garlic+Neem+Datura and only garlic extract induced 100% growth reduction in both the concentration followed by Neem oil up to 69%. There was no significant difference among Neem leaf extract, Karanj and control plates for the inhibition of the growth of test pathogen in 10% concentration. Neem leaf extract recorded least growth inhibition of 5.81% and 5.9% in 10% and 20% concentration respectively. Mint leaf extract (14.41% & 14.85%) and Onion bulb extract (16.79% & 16.85%) also recorded negligible growth reduction of test pathogen in both 10% and 20% concentration [1, 4, 5&7]. (Table-3 Plate-1 & 2).

Fungal and bacterial bioagents were also tested in dual culture method for the inhibition of the growth of foliar pathogen i.e. *Fusarium oxysporum.* Radial growth (mm) was recorded when growth in the control plate reached 85 mm and the data present in Table 4. Among three fungal bio agents tested are *Trichoderma viride* recorded maximum growth inhibition (81.27) followed by *Trichoderma harzianum* (74.70%) against *Fusarium oxysporum*,

Pseudomonas fluorescence inhibited 80.70% growth of *Alternaria solani*. *Trichoderma viride* recorded 81.27% growth inhibition against *Fusarium oxysporum* and at par with *Pseudomonas fluorescence* (80.70%). *Trichoderma hamatum* was found to be less effective only reducing approximately 70% growth of the both the pathogen [10]. (Table-4 Plate-3).

Table 3: Efficacy of different plant extracts in inhibiting the radial growth of Fusarium oxysporum

Treatments	10%		20%	
	Mean radial growth(mm)	Percent inhibition	Mean radial growth(mm)	Percent inhibition
Combination	0(0.71)	100	0(0.71)	100
Datura leaf extract	36.93(6.11)	52.31	28.60(5.39)	60.37
Garlic bulb extract	0(0.71)	100	0(0.71)	100
Mint leaf extract	66.27(8.17)	14.41	61.45(7.87)	14.85
Onion bulb extract	64.43(8.06)	16.79	60.01(7.77)	16.85
Turmeric corm extract	43.27(6.61)	44.12	36.20(6.05)	49.84
Azadiractin oil	25.43(5.09)	67.16	22.27(4.77)	69.14
Neem leaf extract	72.93(8.57)	5.81	67.91(8.27)	5.90
Karanj leaf extract	71.93(8.51)	7.1	43.50(6.64)	39.73
T_{10}(Control)	77.43(8.83)	-	72.17(8.52)	-
SEm(±)	0.09		0.07	
CD (5%)	0.3		0.2	

*Figures in the parentheses indicate $\sqrt{(x+0.5)}$ transform values

Table 4: Efficacy of fungal and bacterial Bio-control agents against radial growth of foliar pathogen of tomato

Treatments	*Fusarium oxysporum*	
	Mean radial growth(mm)	Percent inhibition
Trichoderma viride	16.16	81.27
Trichoderma hamatum	26.3	69.52
Trichoderma harzianum	21.83	74.70
Pseudomonas fluorescens	16.66	80.70
T_5(Control)	86.3	-

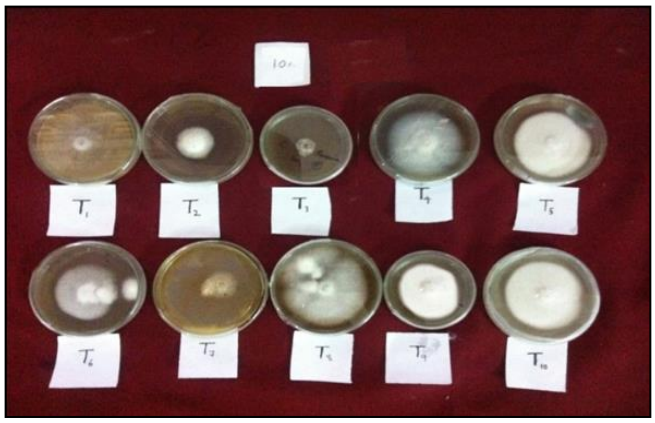

Plate 1: (10%conc) Efficacy of various plant extracts against radial growth of *Fusarium oxysporum*

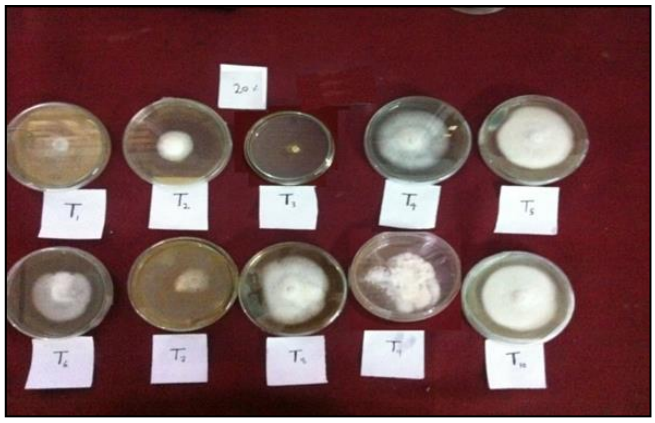

Plate 2: (20%conc) Efficacy of various plant extracts against radial growth of *Fusarium oxysporum*

T_1-Garlic+Neem+Datura(combination), T_2-Datura leaf extract, T_3Garlic bulb extract, T_4-Mint leaf extract, T_5-Onion bulb extract,T_6-Turmeric corm extract, T_7-Azadiractin oil T_8-Neem leaf extract, T_9-Karanj leaf extract, T_{10}-Control.

Plate 3: Efficacy of various bioagents against growth of *Fusarium oxysporum*

T_1- *Trichoderma viride*, T_2- *Trichoderma hamatum*, T_3- *Trichoderma harzianum*, T_4- *Pseudomonas fluorescens*, T_5- Control

Conclusion

This investigation reveals that among all bio agents (garlic, Neem and Datura) combination and only garlic bulb extract, *Trichoderma viride*, *Pseudomonas fluorescens* are effective for managing the test pathogen under lab conditions. Present study helpful for further investigation *in vivo* management of fungal blight.

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