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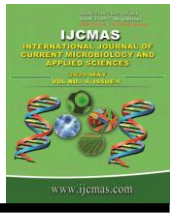

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Efficacy of Plant Extracts and *Trichoderma viride* against Leaf Spot of Maize caused by *Curvularia lunata*

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A B S T R A C T

An experiment was conducted to evaluate the effect of five plant extracts, one bio agents and one fungicide in *in vitro* and *in vivo* againstleaf spot of maize caused by *Curvularia lunata*. Mancozeb @ 0.25% was effective in the inhibition of mycelial growth (69.35%) of *C.lunata*. Among the bio-agents, *Trichoderma viride* was found effective in the inhibition of mycelial growth (50.77%). Among the plant extracts, neem leaf extract @ 10% was found effective in the inhibition of mycelial growth (42.84%) followed by eucalyptus leaf extract @ 10% (40.66%), bael leaf extract @ 10% (37.45%), tulsi leaf extract (34.82) and onion bulb extract (31.80). The plant extracts, potential bio agent and fungicide found effective in *in vitro* were tested against the curvularia leaf spot of maize under field conditions during *kharif* 2017-2018. Among all the treatments, mancozeb @ 0.25% was found effective in the disease reduction (35.85%), followed by *Trichoderma viride* @ 2% (49.12%). Amoung the plant extracts, neem @ 10% was found effective in disease reduction (45.70%) followed by eucalyptus @ 10% with (45.94%), bael leaf exract @ 10% (47.39%), tulsi leaf extract (48.64%), and onion bulb extract (49.55%).

Introduction

Maize (*Zea mays* L.), belonging to the family Gramineae is one of the important cereal crops of the world. The maize kernel, like that of other cereal grains, includes pericarp (6%), endosperm (82%) and germ (12%). The main structural component of the endosperm is starch, a complex carbohydrate that constitutes on an average 71 per cent of the grain and is a source of concentrated energy. Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. The maize grain accounts for about 15 to 56 per cent of the total daily calories in diets of people in about 25 developing countries, particularly in Africa and Latin America, where animal protein is scarce and expensive and consequently, unavailable to a vast sector of

the population (Prasanna *et al.*, 2001). Maize is currently produced on nearly 100 million hectares in 125 developing countries and is among the three most widely in grown crops in 75 of those countries (Anonymous, 2012).These include seedling blights, stalk rots, foliar diseases, downy mildews and ear rots . Among the fungal diseases *Curvularia lunata (Cochliobols lunatus)* was recorded on maize by *Curvularia* leaf spot is potentially an important foliar disease in areas where the temperatures drop at night while the humidity is high. The disease is known to affect maize from seedling stage till harvest. Loss in grain yield will be more if it occurs at flowering, silking and grain filling stages. *Curvularia* is a hyphomycete (mold) fungus which is a facultative pathogen of many plant species and of the soil. Conidia develop at the tips and sides of the spores and have a smooth texture. *C. lunata* is differentiated from other *Curvularia* species by its 3 septa and 4 cells, with the first and last cell usually of a paler shade of brown than those in the middle. Conidia range from 9-15 μm in diameter and have a curved appearance (Macri and Dilenna, 1974). Importance of maize, it is being plagued by an array of diseases which include the leaf spot of maize, caused by *Curvularia lunata*. This disease is a very important seed and soil borne prevalent in the hot, humid maize areas. The disease produces or chlorotic spot with a light colored halo lesions are about 0.5 cm per spot when fully developed and this cause significant damage to maize up to 60 per cent due to great loss of photosynthetic region of the crop(Akinbode, 2010).

Materials and Methods

Isolation of the pathogen

The pathogen was isolated from the disease infected plants and it was identified as the *Curvularia* leaf spot of maize infected leaves were collected. The infected leaves were cut into small pieces (0.5 cm^2) surface sterilized with mercuric chloride (0.1%) for 15-30 seconds, rinsed with three changes of sterile distilled water to remove the disinfectant and blotted dry. The sterilized pieces were plated (4 pieces/ dish) on potato dextrose agar (PDA) medium in petri dishes under aseptic conditions and incubated at 25 ^{0}C for 2 weeks. For obtaining sufficient quantity of inoculums, pure cultures were obtained by subculturing. For this purpose, small bits of the fungus was taken at the tip of a sterilized needle and transferred aseptically to the centre of fresh PDA medium in petri dishes. The dishes will be incubated for 2 weeks at 25^{0}Cin the dark.

Preparation of bio-agents spray

Amount of bio-agents formulation calculated and weighed according to following formula for required concentration and then mixed in required amount of water. Freshly prepared suspension used for spray.

$$A = \frac{\text{required concentration (\%)} \times \text{required}}{\text{Active ingredient (\%)}}$$

Preparation of fungicidal spray solution

The fungicidal spray solution of desired concentration as per treatment freshly prepared every time at the site of experimentation just before the start of spraying operations. The quantity of spray materials required for average of crop gradually increased as the crop advanced in age.

The spray solution of desired concentration prepared by adoption of the following formula.

$$A = \frac{T \times P}{a.i.}$$

Where,

A = Quantity of a formulated pesticide required.
T = Total spray fluid required.
P = Percentage strength required.
a. i. = Given percentage strength of a formulated pesticide.

The spraying undertaken immediately after the appearance of the disease. Five plants in each plot used as representative plants to score for disease severity and data converted into per cent disease index (PDI).Disease severity will be estimated by using 1-5 disease rating scale of Payak and Sharma (1983) as detailed here:

Grade	Type
1.0	Very slight to slight infection.
2.0	Light infection,
3.0	Moderate infection,
4.0	Heavy infection,
5.0	Very heavy infection

Per cent Disease Index (PDI) calculated by using formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical disease ratings}}{\text{No. of plants observed} \times \text{Max.disease rating}} \times 100$$

Poisoned food technique

Five millimetre diameter disc of *Curvularia lunata* kept at the centre of each Petri plate containing the plant extracts of required concentration dissolved in PDA. Three replications maintained. The plates were incubated at 27±1^{o}C 96 hours and colony diameter recorded. Per cent inhibition of mycelial growth calculated by using the formula given by Vincent (1947).

Evaluation of bio-agents *In-vitro*

Antagonistic microorganisms like, *Trichoderma viride* evaluated for their antagonist properties against *Curvularia lunata* by dual culture technique. Twenty millilitre of PDA poured into sterile petriplates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the petriplate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3 to 4 cm gap. For this actively growing cultures were used. One control maintained where in only test fungus grown. The treatments were replicated three times. The plates incubated for four days at 27±1 ^{o}C. After incubation, the colony diameter of *Curvularia lunata* recorded. Per cent inhibition calculated the using the formula given by Vincent (1947).

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

Where:

C = Colony diameter in control

T = Colony diameter in treatment,

Results and Discussion

Efficacy of plant extracts and *Trichoderma viride* against *Curvularia* leaf spot of maize *in- vitro*

The data reported in table 4.2 and depicted in figure 4.1 showed the response of plant extracts and bio-agents on redial inhibition (%).

Leaf extract of Neem (10%), Tulsi (10%), Onion bulb (10%), Eucalyptus (10%) and Bael leaf extract (10%) were tested against *Curvularia lunata.* All the botanicals tested were significantly effective in inhibion growth (%) of pathogen over control

(Untreated).

Among different plant extracts tested Neem (57.15%) @ 10% showed maximum inhibition of *Curvularia lunata* followed by Eucalyptus (59.33), Bael (62.54%) and Tulsi (65.17%) least effectiveness was found in Onion bulb (68.19%). One fungal bio-agents, *T. viride* were evaluated against *Curvularia lunata* in dualculture technique by usingpotato dextrose agar (PDA) as basal medium.

The observations revealed that the maximum reduction in colony growth of *Curvularia lunata* was recorded in*T. viride* (49.22%). The results revealed that the *T. viride* exhibited fungi static activity and significantly inhibited redial growth of *Curvularia lunata* and *T. viride* were found less effective in inhibited redial growth of *Curvularia lunata* .

Evaluation of plant extracts and bio-agents against *Curvularia lunata in-vivo* condition

The data presented in table 4.3 and depicted in figure 4.2 showed the response of plant extracts and bio-agents on PDI at 45, 60 and 75 DAS.

At 45 DAS, the minimum average plant disease intensity (%) of *Curvularia lunata* was observed in T_1-Neem (30.02%) followed by T_2-Eucalyptus (30.16%), T_3-Bael (30.36%), T_4-Tulsi (30.45%), T_6- *T. Viride* (31.13%), T_5-Onion bulb (31.45%) as compared to the treated control T_7-Mancozeb (26.47%) and untreated T_0- Control (32.55%). All the treatments were found statistically significant over T_0-Control (Untreated) and among the treatments (T_5, T_3, T_4, T_2 andT_1) and (T_2 and T_1) were found non- significant to each other.

Table.1 Redial growth (mm) of *Curvularia lunata* as affected by different treatments

Treatment		**Redial growth (mm) of *Curvularia lunata***			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
T_1	Neem leaf extract		10.58	13.78	17.81
T_2	Eucalyptus leaf extract	6.38	11.35	14.31	18.49
T_3	Bael leaf extract	6.49	12.43	15.20	19.49
T_4	Tulsi leaf extract	6.74	13.18	16.42	20.31
T_5	Onion bulb extract	6.92	13.33	17.62	21.25
T_6	*Trichoderma viride*	6.28	9.42	11.24	15.34
T_7	Mancozeb (Treated control)	5.28	6.42	7.76	9.55
T_0	Control (Untreated)	7.17	13.70	22.21	31.16
Mean		6.45	11.36	14.82	19.18
F- test		**S**	**S**	**S**	**S**
S. Ed. (±)		0.078	0.105	0.159	0.173
C. D. (P = 0.05)		0.234	0.314	0.977	0.984
C.V.		2.094	1.589	1.859	0.554

Table.2 Per cent plant disease intensity is leaf spot at 45, 60 and 75 DAS as affected by treatments

Treatments		Concentration (%)	Disease intensity (%)			
			45 DAS	60 DAS	75 DAS	Mean
T_1	Neem leaf extract	10	30.02	35.57	45.70	**37.11**
T_2	Eucalyptus leaf extract	10	30.16	36.60	45.94	**37.37**
T_3	Bael leaf extract	10	30.36	37.46	47.39	**38.15**
T_4	Tulsi leaf extract	10	30.45	39.39	48.64	**39.16**
T_5	Onion bulb extract	10	31.45	39.28	49.55	**40.16**
T_6	*Trichoderma viride*	10 g/l	31.13	38.7	49.12	**39.65**
T_7	Mancozeb (Treated control)	0.25	26.47	30.07	35.85	**30.80**
T_0	Control (Untreated)	-	32.55	40.04	59.81	**44.13**
Overal Mean			30.32	37.13	**47.76**	
F- test			**S**	**S**	**S**	
S. Ed. (±)			0.42	0.36	**0.69**	
C. D. (P = 0.05)			**1.27**	**1.10**	**2.10**	

Values are average of three replicate

At 60 DAS, the minimum average plant disease intensity (%) of *Curvularia lunata* was observed in T_1-Neem (35.57%) followed by T_2-Eucalyptus (36.60%), Bael T_3-(37.46%), T_4-Tulsi leaf extract (38.49%), T_5-Onion bulb (38.70%), T_6-*T. viride* (39.28%) as compared to the treated control T_7-Mancozeb (30.07%) and untreated T_0-Control (40.04%). All the treatments were found statistically significant over T_0-control (Untreated) and among the treatments (T_6 and T_4), (T_4 and T_3), (T_3 and T_4), (T_4 and T_2), (T_4, T_2 and T_1) and (T_1 and T_0) were found non-significant to each other.

After 75 DAS, the minimum average plant disease intensity (%) of *Curvularia lunata* was observed in by T_1-Neem (45.7%) followed byT_2-Eucalyptus (45.94%), T_3-Bael (47.39%), T_4-Tulsi (48.64%), T_5-Onion bulb(49.12%), T_6-*T. viride* (49.55%)as compared to the treated control T_7-Mancozeb (35.85%) and untreated T_0- Control (59.81%). All the treatments were found statistically significant over T_0-control (Untreated) and among the treatments (T_5, T_3 and T_4), (T_4, T_2 and T_2) and (T_2 and T_1)were found non-significant to each other.

In conclusion, neemleaf extract @ 10% recorded the minimum disease incidence (%), maximum yield (q/ha) and highest cost to benefit ratio, where as *Trichoderma viride* recorded the highest mycelial inhibition (%). The results of present experiment are limited to one crop season under Prayagraj agro climate conditions as such more trials should be carried out in future to validate the findings.

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