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Evaluation of Antifungal Efficacy of Bioagents and Botanicals Against *Curvularia lunata* Causal Agent of Maize Leaf Spot

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Abstract Maize is an important cereal crop of India. Recently the crop is severely affecting with curvularia leaf spot of maize caused by *Curvularia lunata* (Wakker) Boedijn and it is endemic in the maize growing areas of Northern Karnataka. An attempt was made to manage the disease by holistic approach with five bioagents and seven botanicals. *In vitro* evaluation of five bioagents were tested by dual culture technique which showed that *Trichoderma viride* registered significantly maximum mycelial inhibition (84.44%) followed by *Trichoderma harzianum* (69.44%) and *Bacillus subtilis* (68.33%). Whereas among the seven plant extracts tested, maximum inhibition of 75.19% was recorded in Nimbecidine 1500 ppm followed by *Emblica officianalis* (61.48%) and neem seed kernel extract (61.11%), both are on par with each other at 5% concentration. While at 10% concentration, maximum inhibition of mycelial growth was recorded in Nimbecidine 1500 ppm (84.04%) which was significantly superior to all other treatments.

Keywords Curvularia leaf spot, *Curvularia lunata*, *In vitro*, Bioagents, Botanicals.

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Introduction

Maize (*Zea mays* L.) is an important cereal crop belonging to the family Poaceae. Maize not only an impotant human nutrient but also a basic element of animal feed and raw material for manufacture of many industrial products. It is also being recently used as biofuel and also for extraction of starch. Maize is versatile crop grown over a range of agro climatic conditions. It is cultivated in tropics, subtropics and temperate regions under irrigated and rainfed conditions. Maize ranks third in the world after wheat and rice in area and production. However, as far as productivity is considered it ranks first. In India, about 50 to 60% of maize production is consumed directly as food, 30 to 35% goes for poultry, piggery and fish meal, 10 to 12% in wet milling industry e.g., starch and oil and about 3% in dry milling for traditional requirements like Dalia and Sattu and other food industry such as corn bread and corn chips, brewery one percent and as seed, one per cent [1]. In last one decade it has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirements.

Maize is subjected to as many as 112 diseases on global basis. In India there is record of 35 diseases. Curvularia leaf spot of maize was earlier considered to be of minor importance. It has now increased all over the maize growing areas in India [2]. Symptoms of the disease start as minute, chlorotic,

pinhead sized translucent spots on the leaf surface. Subsequently the spots increases in size and necrosis start from the center. In Karnataka the disease was reported for the first time in 2008 [3]. Natural plant extracts and bioagents are new means and nonselective pesticides for control of plant diseases. Many plant products have been reported to be antimicrobial against plant pathogenic fungi. Earlier the disease was considered to be minor importance. It has now increased all over the maize growing areas. Hitherto, no systematic work has been carried out on this disease. Keeping these points in view the present investigation of *in vitro* evaluation of bioagents and botanicals studies were carried out. *T. viride* had a marked significant inhibitory effect on the growth of *C. lunata* [4]. Further studies reported that plant extracts were highly effectively in inhibiting the growth of *Helminthosporium maydis* causal agent of maydis leaf blight of maize [5].

Materials and Methods

In vitro evaluation of bioagents

Five bioagents viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichodema harzianum*, *Trichoderma koningii* and *Trichoderma viride* were tested under *in vitro* condition by following dual culture technique. Twenty ml of PDA was poured into sterile petriplates and allowed them to solidify. From the previously grown young cultures of both fungal bioagents and host pathogens, 5 mm of the test fungus and respective bioagents were transferred aseptically to petriplates simultaneously by leaving sufficient space in between two discs. In case of bacteria, mycelial discs of the pathogen was kept at opposite ends and bacteria streaked at the center. Five replications were maintained for each treatment. The petriplates were incubated at 28±2°C till the growth of the colony touches the periphery in the control plate. Colony diameter of both the test pathogen and bioagents were measured and per cent inhibition was calculated by using the following formula and data was analyzed statistically.

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

Where, I = Per cent inhibition of mycelium, C = Growth of mycelium in control, T = Growth of mycelium in treatment.

In vitro evaluation of botanicals

Fresh leaves or fruits or kernals of seven test plant were collected and washed with tap water followed by distilled water. Then 100 g of fresh sample is crushed in a surface sterilized pestle and morter by adding 100 ml of sterile distilled water (1 : 1 w/v). The extracts were filtered through two layers of muslin cloth. Filtrate thus obtained was used as a stocked solution and commercial formulation of neem oil (Nimbecidine 1500 ppm) directly used at tested concentrations. To study the antifungal mechanism of plant extracts the poisoned food technique was followed. Five and ten ml of stock solutions were mixed with 95 and 90 ml of sterilized molten PDA media respectively so as to get 5 and 10% concentration. The medium was thoroughly shaken. Twenty ml of medium was poured into each of the 90 mm sterilized petriplates. Each plate was seeded with 5 mm mycelial discs taken from the periphery of eight day old fungal culture and were incubated at 28±2°C till the growth

Details of the botanicals used for the study.

Sl. No.	Botanical name	Common name	Family	Parts used	Concentration (%)	
1	*Adahathoda vasica* A. Juss	Malabar nut	Acanthaceae	Leaves	5	10
2	Nimbecidine 1500 ppm	Neem	Meliaceae	Oil	5	10
3	*Azadirachta indica* Nees	Neem	Meliaceae	Seed	5	10
4	*Emblica officinalis* L.	Amla	Phyllanthaceae	Fruits	5	10
5	*Lawsonia inermis* L.	Henna/Mehandi	Lythraceae	Leaves	5	10
6	*Murraya koenigii* L.	Curry leaf	Rutaceae	Leaves	5	10
7	*Ocimum sanctum* L.	Tulasi	Lamiaceae	Leaves	5	10

Table 1. *In vitro* evaluation of bioagents against *Curvularia lunata*. *Arcsine transformed values.

Antagonist	Inhibition of mycelial growth (%)
Bacillus subtilis	68.33 (55.74)*
Pseudomonas fluorescens	62.78 (52.38)
Trichoderma harzianum	69.44 (56.44)
Trichoderma koningii	65.83 (54.21)
Trichoderma viride	84.44 (66.75)
SEm±	0.31
CD at 1%	1.10

of colony touches the periphery in control plate. The disc is placed upside down in the center of the petriplates, so that the mycelium is in direct contact with the medium poisoned with the requisite plant extract at required concentration. In which three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs were inoculated into the center of PDA plates without plant extracts. Mean colony diameter in each treatment was recorded by taking the diameter of the colony in two directions. Radial growth over the control was measured and per cent inhibition of mycelial growth over control was calculated by using the following formula.

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

Where, I = Per cent inhibition of mycelium, C=Growth of mycelium in control, T=Growth of mycelium in treatment.

Results and Discussion

In vitro evaluation of bioagents

Table 1 indicated that antagonistic fungi significantly suppressed the growth of *Curvularia lunata*. *Trichoderma viride* registered significantly maximum mycelial inhibition (84.44%) followed by *Trichoderma harzianum* (69.44%) and *Bacillus subtilis* (68.33%). Least mycelial inhibition was observed with bacterial antagonistic organisms such as *Pseudomonas fluorescens* with 62.78%. *Trichoderma viride* besides maximum inhibition of fungus it also over grew on the pathogen. These results are in accordance with earlier literature who have carried out studies on *C. lunata* causal agent of grain discoloration in rice [6], who reported that *B. subtilis* and *T. viride* and *T. harzianum* were found effective in inhibiting the radial growth of the fungus *Curvularia lunata*. Similarly another author [4] reported that *T. viride* had a marked significant inhibitory effect on the growth of *C. lunata. Trichoderma* is a fast growing fungus it reached the pathogen within 3–4 days and overgrew on the pathogen in 8—10 days. Further reports showed that *T. harzianum* strain Th-13 showed maximum inhibition of mycelial growth of *C. lunata* [7].

Table 2. *In vitro* evaluation of botanicals against *Curvularia lunata*. *Arcsine transformed values.

Phytoextracts	Per cent inhibition of mycelial growth Concentration (%) 5	10	Mean
Adahathoda vasica	2.96 (9.87)*	21.48 (27.59)	12.22 (18.73)
Nimbecidine 1500 ppm	75.19 (60.10)	84.04 (66.46)	79.61 (63.28)
Azadirachta indica (NSKE)	61.11 (51.40)	62.96 (52.52)	62.03 (51.96)
Emblica officianalis	61.48 (51.62)	65.19 (53.82)	63.33 (52.72)
Lawsonia inermis	26.67 (31.08)	32.96 (35.02)	29.81 (33.05)
Murraya koenigii	23.70 (29.12)	53.33 (46.89)	38.51 (38.00)
Ocimum sanctum	17.04 (24.37)	28.15 (32.03)	22.59 (28.20)
Mean	38.30 (36.79)	49.73 (44.90)	
Phytoextracts	P	C	P×C
SEm±	0.61	0.53	0.71
CD at 1%	2.11	2.10	2.35

In vitro evaluation of botanicals

Results from the Table 2 revealed that among two concentrations tested maximum inhibition was recorded at 10% concentration with 49.73% and least was observed at 5% concentration with 38.30% inhibition. Among the different treatments tested maximum inhibition of 79.61% was recorded in

Nimbecidine 1500 ppm which was significantly superior to all other treatments followed by *Emblica officianalis* (63.33%) and neem seed kernel extract (62.03%), both are on par with each other. Least mycelial inhibition was observed with *Adahathoda vasica* (12.22%).

In the interaction between phytoextract × concentration maximum mycelial inhibition (84.04%) at 10% concentration followed by same phytoextract with was observed with Nimbecidine 1500 ppm with 75.19% inhibition at 5% concentration and least was observed with *Adahathoda vasica* (2.96%) at 5% concentration. It is opined that inhibitory action of Nimbecidine 1500 ppm at both 5 and 10% concentration may be due to triterpenoid azadirchtin which retards the growth and activation of the pathogen . These results are in support with earlier work Kumar et al. [5] who reported that plant extracts were highly effective in inhibiting the growth of *Helminthosporium maydis*. Neem and tulsi extracts were found to be effective as growth inhibitors. Inhibitory action of Nimbecidine 1500 ppm may be due to triterpenoid azadirachtin which retards the growth and activation of the pathogen.

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