The Andhra Agric. J 71 (4): 406-409, 2024
doi :10.61657/aaj.2024.152

In vitro evaluation of fungicides against *Macrophomina phaseolina*, the incitant of blackgram dry root rot

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ABSTRACT

Blackgram *Macrophomina phaseoolina* has become big problem to the farmers for the effective measures chemical control is been chosen and *invitro* efficacy against the pathogens has been tested at two different concentrations results at 500 ppm are complete inhibition of mycelial growth was recorded with Carbendazim, propiconazole, carbendazim+ mancozeb, flusilazole+ carbendazim, tebuconazole+ sulphur, azoxystrobin+ difenoconazole, captan+ hexaconazole, penfluzen+ trifloxystrobin and tebuconazole+ trifloxystrobin (each 0.00 cm).

Key words: *Blackgram, Dry root rof, Gungicided and In vitro evaluation*

Pulses, including black gram, are vital in agriculture due to their ability to fix atmospheric nitrogen, which enhances soil fertility and supports soil health. This process contributes to sustainable farming practices and helps reduce greenhouse gas emissions. Black gram, in particular, stands out as a crucial pulse crop, offering a rich source of dietary proteins and fibers.

In India, pulses are highly valued for their protein content, which ranges from 17% to 25%, significantly higher than the 6% to 10% found in cereals. Pulses are also low in fat and rich in essential nutrients like fiber, potassium, magnesium, zinc, and iron. Including pulses in the diet is beneficial for overall health, as they are linked to a lower risk of obesity, diabetes, heart disease, and cancer. Health organizations worldwide recommend incorporating pulses into daily diets for their numerous health benefits.

It belongs to the family Fabaceae with 2n=22 and is believed to have originated in India (Chatterjee and Bhattacharya, 1986). Blackgram has many common names, viz., urd, urd bean, urad, mash, black maple and klai (Chatterjee and Randhawa, 1952), is mentioned in ancient texts such as Kautilya's *Arthashastra*. The name "black gram" derives from the Latin word *legere*, meaning "to gather," reflecting the traditional method of hand-harvesting its seeds. In India, it is commonly referred to as "urad dal" and is highly valued for its protein-rich content. Pulses like black gram are essential to Indian agriculture due to their significant role in food security.

In Andhra Pradesh, black gram holds particular significance as it integrates well into the rice-pulse cropping system, serving as a relay crop under low input management, especially in the Krishna-Godavari and north coastal zones as a *rabi* rice fallow pulse. It is cultivated over 1.25 million acres, yielding approximately 329,000 tonnes, with a productivity of 263 kg per acre (Vyavasaya Panchagam, 2018). Of the several problems hindering the realization of good yields in blackgram, soil borne diseases in *rabi* rice fallows are increasing day by day due to continuous cultivation of the crop in the same soils solely relying on chemical fertilizers leading to depletion of the soil organic carbon content having adverse effect on the beneficial soil microbial fauna.

Among the fungus, the root rot/stem rot/ charcoal rot disease is caused by *M. phaseolina* (Tassi.) Goid which is a major disease, distributed worldwide occurring from seedling stage to the maturity stage (Meena, 2018). Various chemical applications are currently employed to control the *M. phaseolina* disease. Several workers have attempted to control *Macrophomina phaseolina* by use of different systemic fungicides and contact fungicides .The main objective of this study was to evaluate the different fungicides at different concentrations for

***In vitro* evaluation of fungicides**

testing their efficacy against mycelial growth inhibition of *Macrophomina phaseolina* under *in vitro* condition.

MATERIAL AND METHODS

Isolation and Pathogenicity of the Pathogen

The disease causing fungus was isolated by using tissue segment method. The fungus *Macrophomina phaseolina* was frequently isolated from the root rot affected blackgram plant. The cultures were purified by hyphal tip method (Dasgupta, 1988).

Mass Multiplication of Pathogen

For pathogenicity test *M.phaseolina* was mass multiplied on sorghum grains. About 50 g of sorghum grains were taken in 250 ml capacity conical flask and soaked overnight. The flasks were then sterilized at 15 psi pressure for 15 minutes (121 °C) over two successive days. After sterilization the sorghum seeds in flask were inoculated with 5mm mycelial discs taken from 7 days old actively growing pure culture of *M.phaseolina* and flasks were incubated for 15 days at 28°C ± 2°C. Flasks were shaken regularly for proper mycelial growth of the pathogen.To prove pathogenicity test, twenty day old sorghum grain culture of *M.phaseolina* was inoculated in the top two inches soil of 15kg soil pot at 50grams and watered sufficiently. The test pathogen was re-isolated from the infected plant parts to prove Koch's postulates.

Different concentrations of systemic and non-systemic fungicides were tested for the growth inhibition of *M. phaseolina* by using poisoned food technique (Sinclair and Dhingra 1985) . The required quantity of each chemical was incorporated aseptically in 100 ml of PDA in 250 ml flasks to make various concentrations fungicides. The medium was shaken well to give uniform dispersal of the chemical and then 20 ml of medium was poured aseptically to each plate with three replications. After solidification, the plates were inoculated with mycelial discs of 4 mm diameter of five days old culture. The mycelium disc which was placed in the center of the plates, in an inverted position to make a direct contact with the poisoned medium which was incubated at 28 ± 1 ^{0}C for seven days. The linear growth of the fungal colonies was measured from two different angels in millimeter (mm) and the average values were calculated. The per cent inhibition of growth of the fungus in each treatment was calculated by using the following formula described by Vincent (1947).

$$I = \frac{C - T}{C} X100$$

Where, I= Per cent inhibition
C= Colony diameter in control (mm)
T= Colony diameter in respective treatment (mm)

RESULTS AND DISCUSSION

In vitro Evaluation of Fungicides Against *Macrophomina phaseolina*

All the treatments showed significant difference along with control. Observations at two days after inoculation revealed that the radial mycelial growth ranged from 0.00-3.04 cm in different fungicide treatments when compared to control plates (4.12 cm). However, complete inhibition of mycelial growth was recorded with Carbendazim, propiconazole, carbendazim+ mancozeb, flusilazole+ carbendazim, tebuconazole+ sulphur, azoxystrobin+ difenoconazole, captan+ hexaconazole, penfluzen+ trifloxystrobin and tebuconazole+ trifloxystrobin (each 0.00 cm). This followed by metalaxyl+ mancozeb (1.04 cm), mancozeb (1.53 cm), hexaconazole (1.84 cm), and metalaxyl+ chlorothalonil(1.95cm), propineb (2.26cm) and mycobutanil (3.04).

When compared to control plates (5.68 cm) at 4 DAI, the radial mycelial growth in different treatments ranged from 0.00 cm to 4.42 cm. At 5 days after inoculation (DAI), not all fungicides demonstrated the same level of effectiveness as observed at 3 DAI, carbendazim, propiconazole, carbendazim+ mancozeb, flusilazole+ carbendazim, penfluzen+ trifloxystrobin and tebuconazole+ trifloxystrobin showed 0.00 cm mycelial growth at 4DAI (Table 1).

The final observation was recorded at 6DAI, when the pathogen was grown to cover the complete control plate (9 cm) revealed that complete (100%) mycelial inhibition was recorded with the six fungicides viz., Carbendazim, propiconazole, carbendazim+ mancozeb, flusilazole+ carbendazim, penflufen+trifloxystrobin, tebuconazole+ trifloxystrobin showed 100% inhibition followed by tebuconazole+ sulphur(82%), captan+ hexaconazole (80%), azoxystrobin+ difenoconazole (79%), metalaxyl+ mancozeb (73%), hexaconazole(63%), metalaxyl+ chlorothalonil (56%), mycobutanil (50%), mancozeb(48%) and propineb (40%).

Table 1. Invitro evaluation of fungicides against M. Phaseolina

S.No.	Treatment (500ppm)	Diameter of the colony (cm)			Inhibition Per centage
		2DAI	4DAI	6DAI	
1	Carbendazim	0.00(1.00)^{g}	0.00(1.00)^{i}	0.00 (1.00)^{i}	100%
2	Mancozeb	1.53(1.425)^{e}	3.34(1.95)^{d}	4.61 (2.26)^{c}	48%
3	Propineb	2.26(1.661)^{c}	4.42(2.21)^{b}	5.35(2.40)^{b}	40%
4	Propiconazole	0.00(1.00)^{g}	0.00(1.00)^{i}	0.00(1.00)^{i}	100%
5	Carbendazim+ mancozeb	0.00(1.00)^{g}	0.00(1.00)^{i}	0.00(1.00)^{i}	100%
6	Flusilazole+ carbendazim	0.00(1.00)^{g}	0.00(1.00)^{i}	0.00(1.00)^{i}	100%
7	Tebuconazole+ sulphur	0.00(1.00)^{g}	1.04(1.24)^{h}	1.55(1.41)^{h}	82%
8	Azoxystrobin+ difenoconazole	0.00(1.00)^{g}	1.19(1.30)^{h}	1.81 (1.51)^{g}	79%
9	Captan+ hexaconazole	0.00(1.00)^{g}	1.21(1.30)^{h}	1.79 (1.51)^{g}	80%
10	Penfluzen+ trifloxystrobin	0.00(1.00)^{g}	0.00(1.00)^{i}	0.00(1.00)^{i}	100%
11	Hexaconazole	1.84(1.53)^{d}	2.63(1.76)^{f}	3.33 (1.94)^{e}	63%
12	Metalaxyl+ mancozeb	1.04(1.24)^{f}	1.78(1.51)^{g}	2.4 (1.70)^{f}	73%
13	Tebuconazole+ trifloxystrobin	0.0(1.00)^{g}	0.00(1.00)^{i}	0.00(1.00)^{i}	100%
14	Mycobutanil	3.04(1.88)^{b}	3.76(2.06)^{c}	4.44 (2.21)^{c}	50%
15	Metalaxyl+ chlorothalonil	1.95(1.56)^{d}	2.98(1.86)^{e}	3.90 (2.09)^{d}	56%
16	Control	4.12 (2.14)^{a}	5.68(2.48)^{a}	9.00 (3.08)^{a}	
	P value	1.637819e-47***	2.969268e-^{47***}	1.925654e-^{49***}	
	SEm(±)	0.0081	0.0089	0.0091	
	C.D. ($P \leq 0.05$)	0.0233	0.0257	0.0261	
	CV (%)	1.043	1.041	0.957	

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Received on 14.10.2024 and Accepted on 05.11.2024