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Inhibitory Effects of some Fungicides against *Macrophomina phaseolina* Causing Charcoal Rot

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ABSTRACT

No fungicides have been registered to control *Macrophomina phaseolina* in Pakistan. Therefore, in the present study, nine fungicides belonging to different groups were evaluated for their *in vitro* and *in vivo* effectiveness against *M. phaseolina*. Highly significant inhibitory effects of fungicides were recorded on the growth of *M. phaseolina*. All the fungicides caused significant inhibition of the fungus over control. Maximum individual inhibition of growth of the fungus was recorded with Benomyl (83.89%) followed by Carbendazim (79.11%) at a concentration of 150 ppm. Copper oxychloride at a concentration of 50 ppm gave the minimum inhibition (12.50%). Concentrations also had significant inhibitory effects on the growth of the fungus. All the fungicides caused maximum inhibition of the growth of the fungus at a concentration of 150 ppm. With a decrease in the concentration, the inhibition in the growth also decreased. The inhibition of growth was found to be directly proportional to the concentration. Fungicides also affected significantly the plant survival of green gram and black gram over control. Maximum plant survival was observed where the seeds were treated with Benomyl followed by Carbendazim. However, Copper + Mancozeb and Copper oxychloride treated seeds gave the minimum germination and survival of plants. Doses also had a significant effect on the germination and plant survival. Maximum germination and survival were recorded where the seeds were treated with a concentration of 150 ppm and minimum was recorded in case of 50 ppm concentration. With a decrease in the concentration, the germination and survival decreased significantly showing a direct relationship between concentrations and plant survival. Benomyl at 150 ppm concentration showed the highest rate of plant survival of 76.67% whereas Carbendazim and Propineb with same concentration exhibited 66.67 and 63.33% plant survival respectively. Copper oxychloride proved to be the least effective.

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Authors' Contribution
UI designed the study, executed experimental work, analyzed the data and prepared the manuscript. TM helped in designing the study and supervised the experimental work.

Key words
Charcoal rot, Chemical control, Fungicides, Growth inhibition, Plant survival

INTRODUCTION

Pulses being important food stuff are used in different forms in almost all the tropical and subtropical countries and constitute an essential part of human diet. Pulses are rich in proteins (20-26%), vitamins (A, B, C and niacin) and minerals (potassium, phosphorus and calcium) vital for human body (Malik, 1994). They are regarded as economically important in cropping systems to substitute expensive nitrogenous fertilizers by fixing atmospheric nitrogen through nitrogen fixing rhizobacteria which result in an increase in the fertility of soils.

Green gram (*Vigna radiata* L.) Wilczek. and black gram (*Vigna mungo* L.) Hepper are two important summer pulse crops of Pakistan, cultivated on an area of 245.9 and 32.5 thousand hectares with a total production of 177.7 and 17.3 thousand tons respectively (Anonymous, 2011) under a wide range of agro ecological zones. The average yields of these pulses in Pakistan are very low as compared to their potential yields obtained in many other countries of the world. Legions of biotic and abiotic factors are responsible for this low yield. Among biotic factors, diseases are the most destructive (Ashfaq *et al*., 2017; Aslam *et al*., 2017a, b, 2019a, b; Fateh *et al*., 2017; Javed *et al*., 2017a, b: Kayani *et al*., 2017; Khan *et al*., 2017; Mukhtar *et al*., 2017a, b, 2018; Kayani and Mukhtar, 2018; Mukhtar, 2018; Tariq-Khan *et al*., 2017). Up to 44 percent yield losses to pulse crops have been reported due to diseases, depending upon the crop variety (Bashir and Malik, 1988). Green gram and black gram are invaded by about 26 diseases in the world (Charles, 1978); charcoal rot incited by *Macrophomina phaseolina* (Tassi) Goid, is of prime significance in reducing crop yield especially in arid regions of the world (Hoes, 1985; Iqbal and Mukhtar, 2014; Iqbal *et al*., 2014). The pathogen is distributed all over the world in diverse climatic conditions from arid to

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tropical regions. It has a broad host range (Cottingham, 1981; Abawi and Pastor-Corrales, 1990) infecting more than 500 hosts including legumes and cereals (Dhingra and Chagas, 1981; Sinclair, 1982).

M. phaseolina is a soil and seed borne pathogenic fungus and produces cushion shaped black sclerotia (Wheeler, 1975) and disease severity is correlated with viable sclerotia present in the soil. All the growth stages of plants are infected by charcoal rot. The disease manifests in the form of dark lesions on the epicotyls and hypocotyls resulting in seedling death due to occlusion of xylem vessels. The pathogen causes red to brown lesions on roots and stems, produces dark mycelia and black microsclerotia. Severe infections cause defoliation and wilting (Abawi and Pastor-Corrales, 1990) resulting in 100% yield losses (Bashir and Malik, 1988). Due to lack of resistant cultivars in the country, non availability of commercialized biological control agents and the associated problems in their use, management of charcoal rot is mainly relied on synthetic fungicides. In Pakistan no fungicides have been registered for the management of charcoal rot of green gram and black gram. Mostly, the fungicides labeled to control other soil borne pathogens are currently used to manage this disease which do not give satisfactory control. For these reasons, in the present study, nine fungicides belonging to different groups were evaluated for their *in vitro* and *in vivo* effectiveness against *M. phaseolina* with the objective to identify some new fungicides to update disease management strategies.

MATERIALS AND METHODS

Isolation, purification and identification of M. phaseolina

The fungus used in the study was isolated from stem bark tissues of black gram bearing fungal sclerotia and characteristic charcoal rot symptoms. The samples were cut into small pieces (5-10 mm long), surface sterilized with 1% sodium hypochlorite for 2 minutes and then rinsed thrice in sterilized distilled water. The pieces were placed on Chloroneb Mercury Rose Bengal Agar (CMRA) medium (Meyer *et al*., 1973) in Petri dishes and incubated in dark at 25 ± 1°C for 7 days. A small portion of the actively growing colony of *M. phaseolina* was taken from the periphery of 90 mm diameter Petri dish and spread onto Petri dishes containing glucose agar medium (glucose, 20 g; agar, 20 g and water, 1000 ml) and incubated in dark at 25 ± 1°C for 7 days. A small portion of the colony having sclerotia was taken into a drop of sterilized water and agitated with a sterilized needle to separate the sclerotia from the mycelia. Sclerotia were then transferred to 90 mm diameter Petri dishes containing CMRA medium. Colonies appearing from single sclerotium were again transferred to CMRA medium in 90 mm Petri plates, incubated as mentioned above and identified on the basis of standard key (Barnett and Hunter, 1972).

Multiplication of M. phaseolina *for pot assay*

Sorghum seeds were water soaked overnight, air dried under room temperature and placed in conical flasks. The mouth of each flask was plugged with cotton wool, wrapped in aluminum foil and autoclaved at 15 psi (121°C) for 20 minutes. After cooling, the seeds in flasks were inoculated with 4 mm mycelial plugs from a 7-day old culture of *M. phaseolina* and incubated at 25 ± 1°C for 15 days. The flasks were shaken at alternate days for uniform colonization of the grains. The inoculum thus produced was used in pot experiments.

Evaluation of fungicides for their effectiveness against M. phaseolina

Nine fungicides (Table I) were tested for their effectiveness against *M. phaseolina in vitro* and in pot culture assay.

Table I.- Fungicides evaluated for their effectiveness against charcoal rot fungus.

Sr. No.	Fungicide	Chemical name	Formulation	Manufacturer
1	Antracol	Propineb	70 WP	Bayer (Pvt) Ltd.
2	Trimiltox Forte	Copper+ Mancozeb	41 WP	Syngenta
3	Dithane M-45	Mancozeb	80 WP	Rohm & Hass Ltd
4	Derosal	Carbendazim	50 WP	Bayer (Pvt) Ltd.
5	Captan	Captan	50 WP	ICI (Pvt) Ltd.
6	Benlate	Benomyl	50 WP	Du Pont
7	Ridomil Gold	Matalaxyl+ Mancozeb	68 WP	Syngenta
8	Cobox	Copper oxychloride	50 WP	Pak Agro
9	Daconil	Chlorothalonil	75 WP	Syngenta

In vitro evaluation of fungicides

The effectiveness of nine fungicides was tested by using poisoned food technique (Nene and Thapliyal, 1982). Requisite quantity of active ingredient of each fungicide was mixed in autoclaved PDA to obtain the required concentrations of 50, 100 and 150 ppm. Poisoned medium was then poured into each sterilized 90-mm-dia. sterilized petriplate, allowed to solidify and proceeded as described above.

Pot culture assay

For testing the affectivity of fungicides against *M. phaseolina* (MP-7) in pots, surface sterilized seeds each of green gram (NM-92) and black gram (Mash-98) were treated with 1, 2 and 3 g of a.i of each fungicide as slurry method. Control seeds were treated with sterile distilled water. Ten seeds in five replications were sown in sterile pots containing a mixture of soil and sand at the rate of 1: 1 (v: v) amended with the rice seeds colonized with *M. phaseolina* @ 2g/kg soil. The pots were kept in growth rooms at 30 °C. Data on percentage germination/plant survival was recorded after 20 days.

Statistical analysis

The experiments were conducted twice. Percent reduction in mycelial growth and increase in seedling emergence were calculated over controls prior to statistical analysis (Hussain *et al*., 2016; Kayani *et al*., 2018). All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's Protected Least Significant Difference Test at ($P \leq 0.05$). No significant interaction was observed between the data of both the experiments, so the two sets of data were combined for analysis. Standard errors of differences of means were calculated in Microsoft Excel 2007.

RESULTS

Radial growth of M. phaseolina

Highly significant inhibitory effects of fungicides ($F = 380.04$; $df = 8, 18$; $P<0.001$) and their concentrations ($F = 594.69$; $df = 2, 36$; $P<0.001$) were recorded on the growth of *M. phaseolina*. The interaction between fungicides and their concentrations was also found to be highly significant ($F = 13.09$; $df = 16, 36$; $P<0.001$).

All the fungicides caused significant inhibition of the fungus over control. Maximum individual inhibition of growth of the fungus was recorded with Benomyl (83.89%) followed by Carbendazim (79.11%) at a concentration of 150 ppm. Copper oxychloride at a concentration of 50 ppm gave the minimum inhibition (12.50%). The individual inhibitions caused by the fungicides are given in the Table II. Concentrations also had significant inhibitory effects on the growth of the fungus. All the fungicides caused maximum inhibition of the growth of the fungus at a concentration of 150 ppm. With a decrease in the concentration, the inhibition in the growth also decreased. The inhibition of growth was found to be directly proportional to the concentration.

Table II: *In vitro* radial growth inhibition of *M. Phaseolina* by fungicides .

Fungicide	% growth inhibition at			
	50 ppm	100 ppm	150 ppm	Average
Propineb	41.11 ± 1.61	50.33 ± 1.88	61.44 ± 1.36	50.96
Copper+ Mancozeb	8.33 ± 1.26	26.11 ± 1.80	53.56 ± 1.17	29.33
Mancozeb	21.44 ± 0.60	30.89 ± 1.01	56.11 ± 1.15	36.15
Carbendazim	63.33 ± 1.61	73.00 ± 1.30	79.11 ± 1.30	71.81
Captan	19.22 ± 2.17	31.67 ± 1.04	41.44 ± 1.17	30.78
Benomyl	66.89 ± 1.45	78.67 ± 1.59	83.89 ± 1.15	76.48
Meatalaxyl+ Mancozeb	42.56 ± 1.01	51.44 ± 1.45	57.78 ± 0.87	50.59
Copper oxychloride	12.56 ± 0.93	23.89 ± 0.00	34.22 ± 1.01	23.56
Chlorothalonil	25.89 ± 1.30	34.44 ± 1.44	53.33 ± 2.75	37.89

Values are the means of the five replicate samples. Figures following ± are standard errors

Plant survival of green gram and black gram

Fungicides also affected significantly the plant survival of green gram ($F = 13.76$; $df = 8, 18$; $P<0.001$) and black gram ($F = 27.62$; $df = 8, 18$; $P<0.001$) over control. Maximum plant survival was observed where the seeds were treated with Benomyl followed by Carbendazim. However, Copper + Mancozeb and Copper oxychloride treated seeds gave the minimum germination and survival of plants. Doses also had a significant effect on the germination and plant survival. Maximum germination and survival was recorded where the seeds were treated with a concentration of 150 ppm and minimum was recorded in case of 50 ppm concentration. With a decrease in the concentration, the germination and survival decreased significantly showing a direct relationship between concentrations and plant survival. Benomyl at 150 ppm concentration showed the highest rate of plant survival of 76.67% whereas Carbendazim and Propineb with same concentration exhibited 66.67 and 63.33% plant survival respectively. Other fungicides also showed enhanced effect at 150 ppm as compared to other concentrations but having minimum plant survival rate (40-56%). Copper oxychloride remained behind in its effectiveness in terms of disease incidence and plant survival. The individual germinations and survivals of greem gram and black gram have been given in Tables III and IV, respectively.

Table III.- Effect of fungicides on plant survival of green gram against charcoal rot (*M. phaseolina*).

Fungicide	Plant survival at			
	50 ppm	100 ppm	150 ppm	Average
Propineb	43.33 ± 0.33	50.00 ± 0.58	63.33 ± 0.33	52.22
Copper+ Mancozeb	26.67 ± 0.33	33.33 ± 0.33	43.33 ± 0.33	34.44
Mancozeb	30.00 ± 0.00	36.67 ± 0.33	46.67 ± 0.33	37.78
Carbendazim	46.67 ± 0.33	53.33 ± 0.33	66.67 ± 0.33	55.56
Captan	30.00 ± 0.58	36.67 ± 0.33	46.67 ± 0.33	37.78
Benomyl	50.00 ± 0.00	56.67 ± 0.33	76.67 ± 0.33	61.11
Meatalaxyl+ Mancozeb	43.33 ± 0.33	50.00 ± 0.00	56.67 ± 0.33	50.00
Copper oxychloride	26.67 ± 0.33	33.33 ± 0.33	46.67 ± 0.33	35.56
Chlorothalonil	40.00 ± 0.00	46.67 ± 0.33	56.67 ± 0.33	47.78

Values are the means of the five replicate samples. Figures following ± are standard errors

DISCUSSION

Charcoal rot caused by *M. phaseolina* is one of the most important diseases of pulses incurring heavy yield losses. No fungicides have been registered in Pakistan to combat this disease. Therefore, in the present studies, nine fungicides registered for other soil borne pathogens were tested for their effectiveness against the fungus. Of all the fungicides, Benomy and Carbendazim proved to be the most effective. Benomyl has successfully controlled many diseases of different crops as leaf spot in sugar beet (Kalaoglanidis *et al*., 2003), rice blast (Kamerwar, 1976), scab and powdery mildew of apples, cucurbits and strawberries (Scot *et al*., 1979). Marley and Genga (2004) found that benomyl reduced the mycelial growth of *Stenocarpella maydis in vitro*. It also inhibited the growth of *Fusarium oxysporum* (El-Tobshy *et al*., 1981). Mamza *et al*. (2010) reported that benomyl along with thiram and tricyclazole suppressed growth of *F. pallideroseum* isolated from castor. Khan and Khan (2006) found that both benomyl and carbendazim inhibited 100% mycelial growth of *M. phaseolina*. Carbendazim also inhibited the growth and sclerotial production of *M. phaseolina* (Suryawashi *et al*., 2008). Similarly, seed dressing with fungicides enhanced seedling emergence and reduced mortality rate in legumes (Muthomi *et al*., 2007).

Table IV.- Effect of fungicides on plant survival of black gram against charcoal rot (*M. phaseolina*).

Fungicides	Plant survival at			
	50 ppm	100 ppm	150 ppm	Average
Propineb	40.33 ± 0.00	50.00 ± 0.58	63.33 ± 0.33	51.22
Copper+Man-cozeb	20.00 ± 0.00	33.33 ± 0.33	43.33 ± 0.33	32.22
Mancozeb	23.33 ± 0.33	36.67 ± 0.33	46.67 ± 0.33	35.56
Carbendazim	43.33 ± 0.33	53.33 ± 0.33	66.67 ± 0.33	54.44
Captan	26.67 ± 0.33	36.67 ± 0.33	46.67 ± 0.33	36.67
Benomyl	46.67 ± 0.33	56.67 ± 0.33	76.67 ± 0.33	60.00
Meatalax-yl+Mancozeb	36.67 ± 0.33	50.00 ± 0.00	56.67 ± 0.33	47.78
Copper oxychlo-ride	20.00 ± 0.00	33.33 ± 0.33	46.67 ± 0.33	33.33
Chlorothalonil	33.33 ± 0.33	46.67 ± 0.33	56.67 ± 0.33	45.56

Values are the means of the five replicate samples. Figures following ± are standard errors

A number of mechanisms are involved in the suppression and inhibition of pathogens by fungicides. It was found from the present investigation that fungicides significantly caused reduction in growth of *M. phaseolina* and enhanced germination of green gram and black gram. Fungicides act by binding with b-tubulin polymers of pathogens which play a key role in nuclear division and result in inhibition of polymerizing activity of microtubules. These also cause hindrance in different regulatory cellular activities including mitosis, meiosis and cell shape maintenance etc. (Nene and Thapliyal, 1993). Similarly, Carbendazim inactivates tubulin function of pathogen necessary for their maintenance and growth (Butlers *et al*., 1995).

CONCLUSION

It is concluded from the present studies that fungicides registered for other soil borne pathogens can be effectively used against the charcoal rot causing fungus *Macrophomina phaseolina*. As Benomyl, Carbendazim, Propineb and Meatalaxyl+Mancozeb proved to be the effective against the fungus and hence recommended for the control of charcoal rot in Pakistan.

Statement of conflict of interest

Authors have declares that there is no conflict.

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