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Response of mungbeen germplasm against collar rot disease, caused by *Phytophthora Megasperma* and its chemical management

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Abstract

Mungbean (*Vigna radiata*) is grown mainly for its edible seeds. Collar rot disease caused by *Phytophthora megasperma* is a serious limiting factor for its cultivation. The current research was planned to evaluate the response of eighty mungbean germplasm against collar rot disease. These germplasm were sown in sick plot in augmented design with 10 cm plant to plant and 30 cm row to row distance at research area of Plant Pathology Research Institute (PPRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Check varietywas sown after every five entries. Data was recorded on plant mortality basis according to disease rating scale. No germplasm was found immune. Eight germplasm exhibited highly resistant while 15 germplasm showed resistance response against the disease. Nineteen germplasm were moderately resistant. Twenty-seven germplasm were found susceptible and eleven were highly susceptible with more than 50% plant mortality.Then efficacy of different fungicides was evaluated against *P. megaspermain vitro*. Culture of *P. megasperma* was isolated from infected mungbean samples on PARP media [Pimaricin, Ampicillin, Rifampicin, Pentachloronitrobenzene (PCNB)]. For evaluation of fungicides Potato Dextrose Agar (PDA) amended with different fungicides at different concentrations (10, 50, 100 and 200 ppm) was used. Experiment was conducted in completely randomized design with fifteen replications. Data was recorded on mycelial growth (mm) of fungus. Ridomil Gold (Mancozeb + Metalaxyl-M) significantly inhibited the growth at all the concentrations. After this Revus (mandipropamid), Acrobat (Dimethomorph) and Amistar Top (azoxystrobin + difenoconazole) inhibited the growth as compared to control respectively. Score (difenconazole) was significantly least effective as it even at 200 ppm exhibited 50.8 (mm) mucelial growth of *P. megasperma*. The current research is useful to find out resistance source of mungbean germplasm against collar rot disease and its chemical management.

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Introduction

The grain legumes are grown for important and cheap food supply in the world and also improve fertility of soil by fixing atmospheric nitrogen (Haq, 1999). Legumes are excellent source of plant protein which provides major dietary source of nutritional values, rich source of vitamins and minerals (Taylor *et al.,* 2005). Pakistan produces mungbean (*Vigna radia* L. Wilczek) crop as valuable food and considered an alternate source of meat and proteins.Its seeds are very much edible, delicious and appetizing in comparison with other groups of legume crops. It is high in fat 0.6%, ash 0.05% and protein 24.7 % (Potter and Hotchkiss, 1997). It is ranked 2nd next to chickpea (*Cicer arietinum*) cultivation and production wise in Pakistan. Cultivation of mungbean in Pakistan is on 127.4 thousand ha area, which produced 98.7 thousand tones (Anonymous, 2015). *Phytophthora* is a cosmopolitan genus of Oomycete and is devastating pathogen of many crops, vegetables, ornamentals and fruit plants (Erwin and Ribeiro, 1996).Collar rot of mungbean, caused by *Phytophthora megasperma* is potentially important disease of mungbean in irrigated areas of Pakistan. The disease caused heavy plant mortality at seedling and vegetative stages, resulting in poor plant growth and lower yield.This is water mold fungus that can be active in conditions of free moisture or of flooding. *Phytophthora* collar rot has become a serious problem. The disease is primarily a problem on sitesthat are poorly drained or are irrigated by flooding. High soilmoisture is essential for the survival and movement of, and infection by, the *Phytophthora* fungus. Management of this disease is difficult because soil treatments are ineffective in most of the cases. These survive in a dormant state or as resistant structures (oospores) capable of surviving in the environment until they meet their next susceptible host.

Characteristic symptoms of the disease arewater-soaked slightly sunkenlesions on collar portion of stems. Lesions girdle the stem andthe foliage dries up. Previously *P. megasperma* caused heavy plant mortality in *Vigna mungo*, resulting in poor plant stand and lower yield (Khan *et al*., 2006).

Phytophthora is also a production constraint in pigeonpea (Mishra and Shukla, 1987; Chauhan *et al.,* 2002). Fontma *et al.* (1996) estimated crop losses by *Phytophthora* pathogen raised to 12-67% in early growing season and later on 14-52 %. Vishwakarma *et al.* (2002) reported 40-50 % plant mortality in tomato. *Phytophthora* disease depends on relationship among the environmental conditions and severity of disease.The disease yield losses extended to 1.7-3.2 thousand dollars (Matthew *et al.,* 2006).

Plant resistance is one of the most attractive approaches in suppressing the diseases. It is not only compatible with management techniques but also eco-friendly. The protection of crop is very much dependent on artificial chemical control through the chemical pesticides. These chemical pesticides have simple, straight and fast process to kill the pathogen and helped to solve the problem. The current research was planned to find the source of resistance in different mungbean germplasm against *P. megasperma* and its *in-Vitro* management through different fungicides. The results of these experiments will provide information useful to breeders searching for germplasm to breed for resistance to *P. megasperma*.

Materials and methods

Evaluation of mungbean germplasm against P. megasperma

Seeds of 80 mungbean germplasm were collected from Pulse Research Institute (PRI), Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan and were sown in sick field of Plant Pathology section, AARI Faisalabad. Trial was laid out in augmented design on plain field with single line of each germplasm and after every five lines, susceptible check variety was sown. Plants were spaced 10 cm apart in the row and 30-cm between rows. Data was recorded on percent plant mortality basis by using Mayee and Datar (1986) scale where 0%=immune, ≤ 1%= highly resistant, 2-10% = resistant, 11-20%= moderately resistant, 21-50%= susceptible and > = highly susceptible. The following formula of plant mortality was used.

$$\text{Mortality \%} = \frac{\text{No. of plants recorded as dead}}{\text{Total plants}} \times 100$$

Preparation of fungus inoculum

Young mungbean plants showing infection at collar portion were collected from the experimental area and processed for isolation of casual fungus in the lower fungi laboratory of Plant Pathology Research Institute, AARI, Faisalabad, Pakistan. A selective medium known as PARP (Pimaricin Ampicillin Rifampicin Pentachloronitrobenzene, PCNB) was used for isolation of fungus (Stevenson *et al*., 2000). The isolated fungus was purified and multiplied which was later on identified as *Phytophthora megasperma* (Waterhouse, 1970).

In-vitro evaluation of fungicides against P. megasperma

Poisoned food technique as suggested by Nene and Thapliyal (1982) was used for evaluating the effect of fungicides on the growth of *P. megasperma*. Five different fungicides viz., Acrobat (Dimethomorph), Amistar Top (azoxystrobin + difenoconazole), Revus (mandipropamid), Ridomil Gold (Mancozeb + Metalaxyl-M) and Score (difenconazole) were included in the trial. Potato dextrose agar medium (PDA) was prepared and then amended with test fungicides at 10, 50, 100 and 200 ppm levels by following Nene and Thapliyal (1982).

The amended medium was then poured in sterilized Petri dish (90 mm dia.). A 5-7 mm disc of test fungus cut from the margins of seven days old cultures were placed centrally in each of the Petri dish and were incubated at 20± 2^0C. The radial growth of fungi in each Petri dish was measured after seven days and compared with their respective control.

Statistical analysis

Data were subjected to ANOVA and differences among the means were partitioned at *P*=0.05 according to least significant difference (LSD) test (MSTAT version 3.1).

Results

The results of response of mungbean germplasm revealed that none of the germplasm was found immune to *P. megasperma*. However, five groups among 80 germplasm were observed i.e., highly resistant, resistant, moderately resistant, susceptible and highly susceptible. Response of eight germplasm *viz*., 15033, 15067, 15094,15102, 15106, 15100, 16039, 16051 was highly resistant to disease.

Table 1. Reaction of mungbeangermplasm to collar rot disease according to Mayee and Datar (1986) scale.

Disease %	Reaction*	Varieties/line	No. of varieties/line
0%	Immune	None	-
1 or less	Highly resistant	15033,15067, 15094,15102, 15106, 15100, 16039, 16051	08
1-10 %	Resistant	12005, 12006, 12008, 12009, 14001, 14005, 15040, 15043, 15053, 15072, 15088, 15093, 15095, 15141, 16010	15
11-20 %	Moderately resistant	12001, 12002, 12004, 13001, 13004, 14002, 15005, 15006, 15030, 15045, 15057, 15073, 15077, 15089, 15097, 16005, 16015, 16017, 16036	19
21-50 %	Susceptible	12007, 13001, 13005, 13008, 13009, 14003, 14004, 14006, 15001, 15002, 15003, 15004, 15018, 15034, 15052, 15054, 15057, 15071, 15085, 15087, 15098, 15107,16013, 16026, 16094, 16106, AZRI-06	27
51 % or more	Highly susceptible	13002, 13037, 14007, 15007, 15009, 15055, 15086, 15099, 16037, 16038, 16102	11
Total			80

*Reaction based on percent plant mortality

In second group, 15 germplasm *viz.*, 12005, 12006, 12008, 12009, 14001, 14005, 15040, 15043, 15053, 15072, 15088, 15093, 15095, 15141, 16010 were found to be resistant where below 10 % mortality was observed. In third group, 19 germplasm *viz.*, 12001, 12002, 12004, 13001, 13004, 14002, 15005, 15006, 15030, 15045, 15057, 15073, 15077, 15089, 15097, 16005, 16015, 16017, 16036, showed 11-20 % plant mortality and grouped under moderately resistant. In fourth group, 27germplasm *viz.*, 12007, 13001, 13005, 13008, 13009, 14003, 14004, 14006, 15001, 15002, 15003, 15004, 15018, 15034, 15052, 15054, 15057, 15071, 15085, 15087, 15098, 15107,16013, 16026, 16094, 16106, AZRI-06 exhibited susceptible response against the *P.megasperma*.

Fifth group, showed more than 51 % plant mortality. Eleven mungbean germplasm *viz.*, 13002, 13037, 14007, 15007, 15009, 15055, 15086, 15099, 16037, 16038, 16102 were categorized in highly susceptible group.

The results of *in-vitro* evaluation of different fungicides varied significantly (*P=0.05*) in terms of mycelia growth of *P. megasperma*. It is clear from the data the fungicides at different concentrations significantly reduced the growth of *P. megasperma*. Among fungicides, Ridomil Gold and Revus were significantly effective as compared to other test fungicides (Table 2).

Table 2. Mycelial growth (mm) of *Phytophthora megasperma* on PDA amended with different fungicides at different concentrations after seven days of incubation.

Treatment	Mycelial growth (mm)				Mean MG (mm)	
	10 ppm	50 ppm	100ppm	200ppm	MG (mm)	GI %
Acrobat	71.2 E[1]	45.8 J	35.2 L	20.2 O	43.1	51.9
Revus	50.8 I	31.0 M	25.6 N	15.6 P	30.7	65.7
Amistar Top	81.4 C	67.6 F	54.0 H	40.6 K	60.9	32.1
Score	87.8 B	74.2 D	62.4 G	50.8 I	68.8	23.3
Ridomil Gold	0.00 Q	0.00 Q	0.00 Q	0.00 Q	0	100.0
Control	90.0 A	89.8 A	89.4 A	89.4 A	89.6	
		LSD= 1.21				

[1]Means within a column sharing the same letter are not significantly different from each other at *P* = 0.05 according to Least Significant Difference Test.

MG= Mycelial growth; GI= Growth inhibition.

Ridomil Gold completely inhibited the growth of *P. megasperma* at all the concentrations, 10, 50, 100 and 200 ppm, while Amistar top and Score were least effective because these two fungicides exhibited 40.6 and 50.8 mm mycelia growth respectively even at 200 ppm. Maximum growth inhibition % was determined in Ridomil Gold (100 %) and Revus (65.7 %) as compared to their respective control and minimum was in Amistar top and Score, 32.1 and 23.3 respectively. Intermediate mycelial growth and growth inhibition % was recorded in media amended with Acrobat fungicide (Table 2). It is evident from the results as concentration of the fungicides increased from 10 to 200 ppm the mycelia growth decreased. This similar trend was noticed in all the treatments.

Discussion

Host-pathogen response of collar rot disease of mungbean germplasm was variable during current research. This difference in response to *P. megasperma* among mungbean germplasm might be due to their genotypic response (Dorrance *et al.*, 2003; Grau *et al.*, 2004).The use of resistant cultivars is considered to be the best way of controlling the disease.

Khan *et al.* (2003) screened sixty seven varieties/lines against collar rot pathogen. None of the germplasm was found immune to pathogen. Similarly, Aslam *et al.* (2007) evaluated 40 germplasm of mash (*Vigna mungo*) against *P. megasperma* and found that none of the varieties/lines were immune to disease.

Evaluation of wild species of Pigeonpea, indicated that a few accessions of *Cajanus platycarpus* (ICWP 61, ICWP 66 and ICWP 67) have shown high levels of resistance against prevailing isolates of *P. drechsleri* f. sp. *Cajani* (Masood *et.al.* 2005). Chaudhary and Dhar (2008) evaluated 739 germplasm, breeding lines and selections of short, medium and long duration Pigeonpea in a sick field for five years revealed 20 selections as moderately resistant, while 51germplasm, breeding lines and selections were tolerant and remaining 668 lines were susceptible and none of the Pigeonpea line was resistant. Pande and Sharma (2009) evaluated a large number of wild species of *Cajanus*, newly developed Pigeonpea lines and hybrids, under natural infection conditions found that most of them succumb to *P. drechsleri* f. sp. *cajan I* isolate with 40 % plant mortality.

Most of lines identified as resistant by various researchers were later found susceptible to *P. drechsleri*f. sp. *cajani*under natural epidemic conditions in Deccan Plateau (Sharma *et al.* 2006).Frequent evolution of new pathotypes and co-existence of more than one pathotype at a location has become difficult in developing resistant genotypes against *Phytophthora* blight.

The use of fungicides has become inevitable in the management of plant diseases particularly in absence of availability of resistant varieties.There are nearly 150 different fungicidal compounds, formulated and sold proprietary products, are used in world agriculture to protect plants against fungal diseases (Brent and Hollomon, 2007).Our results are in conformity with Khan *et al.*, 2003; Aslam *et al.*, 2007). They described that Ridomil MZ-72 and Sandofan were significantly effective out of tested fungicides.

These two fungicides completely checked the growth of *Phytophthora* even at 10 ppm, whereas Captan was effective at 200 ppm only. However,Aliette and Dithane M-45 were less effective. Subramanyam (2009) reported that metalaxyl MZ at 250 ppm recorded 100 percent inhibition of radial growth of *P. capsici*.Peshney*et al.* (1990) reported that Metalaxyl, Copper oxychloride and Zirum have been effective for inhibition of mycelial growth and Carbendazim, Thiophanate methyl, Biloxazole, Metalaxyl and Fosetyl-Al reduced sporangia formation of *P. nicotianae*var. *parasitica*under in-vitro conditions.

Conclusion

Compatible host-pathogen response indicated that our mungbean germplasm lack resistance gene to pathogen. Hence, there is a need to transfer resistance gene to our mungbean breeding material to avoid the damage from collar rot pathogen. Ridomil Gold and Revus proved significantly good results against collar rot pathogen and should be recommended to growers for plant protection.

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