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VARIETAL RESPONSE OF BITTER GOURD GERMPLASM AGAINST *MYROTHECIUM RORIDUM* TODE EX. FR. CAUSING MYROTHECIUM LEAF SPOT DISEASE AND ITS CHEMOSYNTHETIC MANAGEMENT

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ABSTRACT

The fungus *Myrothecium roridum* is the chief hazard to crops including cucurbits inhabit in soil as saprophyte, on diseased plant debris and also as facultative parasite on vegetables, fruits and ornamental plants. Keeping in view the damage caused by *M. roridum* to bitter gourd, screening of nine bitter gourd varieties (BG34F1, Manika 7004, CBT 36, BSS 616, KHBG 037, Leena 7005, Raja, Tipu and CO2NO3) was done in Plant Pathology Research Institute, Ayub Agricultural Research Institute Faisalabad under field conditions. All the varieties showed moderately susceptible to highly susceptible reaction ranging between 25-75% infections on leaves. Significant disease severity was recorded in the genotype Tipu while the lowest disease severity was recorded in the genotype BG34F1. Maximum numbers of flowers were recorded in the variety Tipu (24.33) followed by Raja (17.33) and CO2NO3 (15.33). Maximum number of fruits were recorded in the variety Tipu (40.00) followed by BSS 616 (11.00) and CO2NO3 (10.33). M. roridum from the diseased samples was isolated and identified morphologically as white cylindrical conidia with rounded ends under the microscope. In vitro management of the fungus M. roridum was done with fungicides having novel mode of action including Antracol[®] (Propineb), Cabrio Top[®] (Pyraclostrobin + Metriam Complex), Nativo[®] (Tebuconazole + Trifloxystrobin), Topsin M[®] (Thiophenate Methyl) and Score[®] (Difenoconazole) at concentrations of 50,100,150,200,250 µg/mL to inhibit the mycelial growth. Among all the fungicides tested, Score significantly reduced the mycelial growth of *M. roridum* with a value of 49% decrease over control followed by Nativo and Topsin M with a value of 48% and 47% respectively. Antracol was found to be the least effective in reducing the mycelial growth of M. *roridm* with a value of 22% decrease over control.

Keywords: Bitter gourd, Myrothecium leaf spot, varietal response, Difenoconazole, (Tebuconazole+Trifloxystrobin)

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is native to the tropical regions of India and China and currently grown in Asia, South America and East Africa (Yang and Walters, 1992). It is extensively cultivated as a summer vegetable throughout Pakistan on an area of 6107 ha with an annual production of 56949 tons (MNFSR, 2015). The fruit of bitter gourd has a unique bitter taste and considered as a rich source of minerals and vitamins (Desai and Musmade, 1998). In recent years, the crop productivity is going down due

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to various diseases. Several diseases are known to cause yield losses but leaf spot caused by (*Cercospora* spp. and *Myrothecium roridum*), powdery mildew (*Sphaerotheca fuliginea*), Fusarium wilt (*Fusarium oxysporum* f. sp. *niveum*) and downy mildew (*Pseudoperonospora cubensis*) are worth mentioning (Khan and Kamal, 1962; 1968; Maholay, 1986; Ali *et al.*, 1988). Among all fungal diseases, bitter gourd leaf spot caused by *Myrothecium roridum* Tode ex Fr has caused severe losses in various bitter gourd producing regions of the world including Pakistan (Costa *et al.*, 2006). The pathogen is a common soilinhabiting fungus with a relatively wide host range that includes agronomic crops including cotton, tomato, cacao, coffee, potato, soybean, cucurbits, as well as various ornamental plants (Bharath *et al.*, 2006).

Diseases caused by *M. roridum* are generally thought to be associated most frequently with warmer environments during wet conditions (Fitton and Holliday, 1970). The fungus was first reported to be pathogenic to muskmelons (*Cucumis melo*) in Texas (McLean and Sleeth, 1961). In Pakistan, *M. roridum* has been reported to be associated with bottle gourd, sponge gourd, bitter gourd and red gourd (Shaukat *et al.*, 1988; Wahid *et al.*, 1991; Shakir and Mirza, 1992). On bitter gourd leaves, symptoms appeared as tiny circular to irregular water soaked spots turning from yellowish to purplish brown and finally become black in color. As these spots enlarge in size, they merged, covering whole leaf which finally dried out (Shaukat *et al.*, 1988).

Apparently wide range of susceptibility among bitter gourd cultivars in the field has been observed which suggests a moderately elevated level of resistance to Myrothecium leaf spot in some cultivars. Absence of resistance/tolerance against diseases of bitter gourd varieties is supposed to be one of the main reasons for their low yield in Pakistan. Keeping in view the severe damage to bitter guard crop by *M. roridum*, present study was designed to screen out some resistance source of bitter gourd germplasm and *in vitro* evaluation of fungicides for effective management of *M. roridum*.

MATERIALS AND METHODS

Screening of different bitter gourd germplasm against M. roridum: Screening of nine different varieties/lines (BG. 34F1, Manika 7004, CBT 36, BSS 616, KHBG 037, Leena 7005, Raja, Tipu, CO2NO3) of bitter gourd was done using a 0-5 disease rating scale, where 0(no symptoms on leaves; highly resistant), 1(<5% infection on leaves; resistant), 2(5-25% infection on leaves; moderately resistant), 3(25-50% infection on leaves; moderately susceptible), 4(51-75% infection on leaves; susceptible), 5(>75% infection on leaves; highly susceptible) (Vir and Grewel, 1974), in the research area of Plant Pathology Agricultural Research Institute Section, Ayub Faisalabad. Artificial spray of inoculum was done by grinding the infected leaves of bitter gourd. The experiment was laid out in augmented design by maintaining P×P distance of 1 foot and bed size as 6×6 feet. Data on total number of flowers and fruits were recorded periodically. Disease severity was calculated by using the following formula given by Rauf *et al.* (2007).

Disease severity (%) = $\frac{\text{Sum of all disease rating}}{\text{No. of disease plants}} \times 100/5$

Isolation, purification and identification of M. roridum: Isolations were made from symptomatic leaves of bitter gourd collected from research area of Plant Pathology Section, Ayub Agricultural Research Institute Faisalabad. The diseased samples were brought to Plant Disease Diagnostic Laboratory, Department of Plant Pathology, University of Agriculture Faisalabad for isolation, purification and identification of *M. roridum* associated with diseased samples. Isolation was made following the method of Alam et al., (2017). The samples were surface sterilized in 0.1% sodium hypochlorite (NaOCl) for 30 s, rinsed three times with sterile distilled water, dried on blotter paper and then placed on potato dextrose agar (PDA) medium in petri plates under laminar flow cabinet. The plates were incubated at ambient temperature of 25°C. After 3-5 days, mycelia from colonies that emerged from the plated leaf tissues were hyphal tipped and transferred to PDA plates and identification was done based on morphological characteristics of the fungus with the help of available literature (Sutton, 1980).

In vitro efficacy of various fungicides against *M.* roridum: In vitro efficacy of various fungicides including Topsin M, Antracol, Score, Cabrio Top and Nativo was tested against *M. roridum* by using poisoned food technique described by Borum and Sinclair (1968). All fungicides were tested at 50, 100, 150, 200 and 250 μ g/mL concentrations. After solidification of PDA, the plates were inoculated by placing 5mm discs of 7 days old culture of *M. roridum*. The experiment was carried out by making three replications of each treatment; the treatment without fungicide served as control. The inoculated plates were incubated at 25°C and data on the radial colony diameter was recorded after 5 days of incubation.

STATISTICAL ANALYSIS

The collected data was analyzed using SAS/STAT statistical software (SAS Institute, 1990). Means were separated using Fisher's protected least significant difference (LSD procedure). Data for disease management was subjected to statistical analysis under completely randomized design with M.STAT software for the significance and non-significance of fungicides (Russel and Eisensmith, 1983).

RESULTS

Percent diseases severity: All the varieties of bitter gourd showed moderately susceptible to highly susceptible reaction against Myrothecium leaf spot (*M. roridum*) by following disease rating scale of Vir and Grewel, 1974. The data on diseases severity was recorded after 8,9,10 and 11th weeks of crop sowing and presented in Table 1. Disease severity varied with the plant life cycle phase. After 11th week, maximum mean disease severity was recorded in the

genotype Tipu (41.97%) which was designated as the most susceptible genotype followed by BSS 616 (38.95%) and KHBG 037 (37.16%) while the least mean disease severity (27.5%) was recorded in the genotype BG.34F1. Moreover, when the data of disease severity was subjected to regression analysis (Figure 1), the results revealed that there was a positive correlation between disease progress and weeks in all genotypes except BG34F1 and Leena 7005 which showed negative correlation. The data collected at the 11th week of crop after germination showed the highest values of disease severity.

Table 1. Disease severity of *Myrothecium roridum* on different lines/ varieties of bitter gourd after 8,9,10 and 11th weeks post inoculation.

Varioty		Moon			
variety	8	9	10	11	Mean
BG34F1	25.00±0.58i	25.00±0.87i	30.00±0.90gh	30.00±0.84gh	27.50±0.83g
Manika 7004	29.16±0.44h	30.00±0.84gh	40.00±0.84d	42.85±0.61c	35.50±1.84d
CBT- 36	30.00±0.58gh	33.30±0.47f	33.30±0.53f	37.50±0.55e	33.53±0.83e
BSS 616	33.30±0.57f	37.50±0.64e	40.00±0.95d	45.00±0.95b	38.95±1.32b
KHBG 037	33.30±0.42f	35.00±0.10f	37.50±0.59e	42.85±0.61c	37.16±1.11c
Leena 7005	25.00±0.56i	31.25±0.13g	35.00±0.20f	40.00±0.58d	32.81±1.66e
Raja	25.00±1.15i	26.00±0.87i	28.57±0.24h	34.37±0.42f	28.48±1.14f
CO2NO3	25.00±0.98i	29.16±0.49h	37.50±0.55e	39.28±0.19de	32.74±1.79e
Tipu	33.33±0.41f	35.00±0.20f	46.42±1.05b	53.12±1.06a	41.97±2.49a
Mean	28.79±0.74d	31.36±0.80c	36.48±1.04b	40.55±1.24a	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).



Figure 1. Regression analysis of disease severity on different genotypes (V1 = BG34F1, V2 = Manika 7004, V3 = CBT-36, V4 = BSS 616, V5 = KHBG 037= Leena 7005, V7 = Raja, V8 = CO2NO3 and V9 = Tipu) of bitter gourd over weeks.

Total number of flowers: The data was recorded after 8, 9 and 10th week of crop germination. The results indicated that number of flowers was significantly high in all the varieties of bitter gourd. Maximum numbers of flowers were recorded in the variety Tipu (24.33) followed by Raja (17.33) and CO2NO3 (15.33) while the total number of flowers was significantly low (7.00) in Table 2. Total number of flowers will dod by different units.

the variety BG34F1. Maximum 25 flowers were recorded of Tipu cultivar at the 10th week of the bitter gourd crop and minimum 7 flowers were seen on the bitter gourd plant in the 8th week on the KHBG 037 variety. There was no significant difference in the total number of flowers in the different weeks in the variety Leena 7005 (Table 2).

Variatu		Moon			
vallety –	8	9	10	Medll	
BG34F1	8.00±0.58ij	5.00±0.58k	8.00±1.53ij	7.00±0.71e	
MANIKA 7004	7.00±0.58jk	10.00±0.58ghi	17.00±1.73d	11.33±1.58d	
CBT- 36	11.00±0.58fgh	8.00±0.58ij	13.00±1.73ef	10.67±0.91d	
BSS 616	12.00±0.58fg	9.00±1.15hij	12.00±0.58fg	11.00±0.65d	
KHBG 037	7.00±1.00jk	10.00±0.58ghi	16.00±1.73d	11.00±1.45d	
LEENA 7005	13.00±0.58ef	10.00±0.58ghi	13.00±0.58ef	12.00±0.58d	
RAJA	17.00±0.58d	15.00±0.58de	20.00±1.73c	17.33±0.91b	
CO2 NO3	16.00±1.00d	13.00±0.58ef	17.00±1.15d	15.33±0.76c	
TIPU	20.00±0.58c	28.00±0.58a	25.00±0.58b	24.33±1.20a	
Mean	12.33±0.88b	12.00±1.24b	15.67±0.99a		

Table 2. Total number of flowers yielded by different varieties of bitter gourd after ten weeks.

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Total number of fruits: The results regarding total number of fruits indicated that number of fruits on the bitter gourd plants were significantly high in all the bitter gourd varieties. Maximum total number of fruits of bitter gourd was recorded in the variety Tipu (40.00) followed by BSS 616 (11.00) and CO2NO3 (10.33). While the total

number of fruits were significantly low (4.67) in the variety CBT-36. Maximum 40 fruits were recorded at the Tipu genotype in the 10th week of the bitter gourd crop and minimum 5 flowers were seen on the bitter gourd plant in the 8th week on the two varieties CBT-36 and Leena 7005 (Table 3).

Table 3. Total number of fruits yielded by different varieties of bitter gourd after ten weeks.

Variety		Mean		
vallety	8	9	10	
BG34F1	3.00±0.58lm	6.00±1.00jk	9.00±0.58hi	6.00±0.94d
MANIKA 7004	4.00±1.15kl	7.00±0.58ij	14.00±1.15de	8.33±1.56c
CBT- 36	1.00±0.00m	4.00±1.15kl	9.00±1.73hi	4.67±1.31d
BSS 616	9.00±1.15hi	11.00±1.15fgh	13.00±1.15def	11.00±0.82b
KHBG 037	2.00±0.58lm	6.00±0.58jk	10.00±0.58gh	6.00±1.19d
LEENA 7005	2.00±0.58lm	4.00±0.58kl	9.00±1.15hi	5.00±1.12d
RAJA	6.00±0.58jk	11.00±1.15fgh	12.00±1.15efg	9.67±1.05bc
CO2 NO3	6.00±0.58jk	10.00±0.58gh	15.00±0.58d	10.33±1.33b
TIPU	31.00±1.15c	43.00±1.15b	46.00±1.15a	40.00±2.36a
Mean	7.11±1.73c	11.33±2.27b	15.22±2.20a	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Cultural and morphological characters of *M. roridum:* The colonies were white, floccose, and somewhat raised in the center. Conidia were cylindrical-rod-shaped with rounded ends and measured 4 to 6.5×1.4 to $2.6 \mu m$. Conidia formed dark green to black masses on sessile sporodochia in concentric zones (Alam *et al.*, 2017).

Efficacy of various fungicides on mycelial growth of *Myrothecium roridum:* All the fungicides inhibited the

growth of the pathogen invariably. The effect of fungicides, their concentration and interaction of fungicides and concentration were found statistically highly significant on the mycelial growth of *M. roridum*. It was recorded that Nativo, Topsin M and Score were statistically at par with each other at the concentration of 250 μ g/ml in reducing mycelial growth of *M. roridum*. On the other hand, Antracol was the least effective against *M*.

roridum among all tested fungicides. Maximum mycelial colony of test fungus was recorded in the control which was 2.80 cm. All fungicidal treatments showed reduced

growth of *M. roridum* as compared to corresponding control petri plates that were kept free of fungicides treatments (Table 4).

Table 4. Effect of various concentrations of fungicides against mycelial growth of *Myrothecium roridum* after seven days of incubation at 25±2°C

Fungicide -	Conc. (µg/ml)				Moon	Percent decrease	
	50	100	150	200	250	Mean	over control
Antracol	2.13±0.09cd	1.73±0.22fgh	2.53±0.03ab	2.57±0.09ab	1.97±0.09def	2.19±0.10b	21.78%
Cabriotop	2.37±0.12bc	1.77±0.09fgh	2.07±0.09de	1.97±0.15def	1.83±0.18efg	2.00±0.07c	28.57%
Nativo	1.50 ± 0.15 h-k	1.60±0.12ghi	1.50±0.12h-k	1.17±0.12lhk	1.53±0.09hij	1.46±0.06d	47.85%
Topsin M	1.53±0.15hij	1.53±0.12hij	1.50 ± 0.06 h-k	1.60±0.06ghi	1.30±0.06jkl	1.49±0.05d	46.78%
Score	1.23±0.07kl	1.37±0.03i-l	1.53±0.03hij	1.53±0.09hij	1.43±0.09jkl	1.42±0.04d	49.28%
Control	2.80±0.00a	2.80±0.00a	2.80±0.00a	2.80±0.00a	2.80±0.00a	2.80±0.00a	
Mean	1.93±0.14a	1.80±0.12a	1.99±0.13a	1.94±0.14a	1.81±0.13a		

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

DISCUSSION

Myrothecium roridum is a soil borne fungus which continues its life cycle as a saprophyte in dead and decaying plant tissues (Souza-Motta et al., 2003; Castlebury et al., 2004; Costa et al., 2006; Domsch et al., 2007; Quezado et al., 2010). Despite the saprophytic character, M. roridum can cause diseases, mainly in the aerial parts of number of plant hosts, including vegetable crops, fruit plants and ornamentals (Murakami and Shirata, 2005; Domsch et al., 2007). M. roridum has been reported as a pathogen of more than two hundred plant species belonging to distinct botanical families (Quezado et al., 2010). Bitter gourd leaf spot caused by Myrothecium roridum Tode is a serious threat to bitter gourd production in the Punjab province of Pakistan because it has caused considerable losses to farmer community. The pathogen has also been documented as a serious and destructive pathogen causing fruit rot of bitter gourd in India (Sharma and Bhargara, 1978) and leaf spots on watermelon in Korea and Pakistan (Chase, 1983; Kim et al., 2003; Alam et al., 2017). The recent plethora of first reports of *M. roridum* causing disease on plants aforementioned not observed to be susceptible which suggests that either regional change in weather resulting in local growing conditions more conducive to *M. roridum* infection has occurred or an evolution of *M.* roridum sub-species or races has occurred that facilitates a broader host range (Kim et al., 2003; Worapong *et al.*, 2009).

In present studies, all the bitter gourd varieties showed diseased symptoms on leaves invariably. It was observed that all 9 local germplasm screened, showed susceptible to highly susceptible reaction ranging between 25-75% infections on leaves. The disease

severity increased with passage of time. The highest disease severity was recorded in variety "Tipu" with a mean value of disease severity (41.97%) followed by BSS 616 (38.95%), KHBG 037 (37.16%), Manika 7004 (35.50%), CBT- 36 (33.53%), Leena 7005 (32.81%) CO2NO3 (32.74%), Raja (28.48%), BG34F1 (27.50%). Our results are in line with already reported results of screening trials. Five commercial cultivars against Myrothecium leaf spot were tested to find out some resistant cultivars against M. roridum (Norman, 2003). The results indicated that all the evaluated commercial cultivars showed susceptibility against M. roridum. Sixtyseven indigenous varieties were screened against Myrothecium leaf spot under field conditions (Maji et al., 2009). The findings revealed that 29 varieties out of 67 were susceptible against M. roridum.

In present study, genotype "Tipu" yielded maximum number of flowers and fruits among the bitter gourd varieties evaluated. Flower and fruit production had a high variation and thus may be used as representative variables for varietal selection in genomic improvement programs (Ibitoye *et al.*, 2009). The genotype RAJA had the second maximum number of flowers, but it did not result in the maximum production of fruit. This is possibly due to the fact that various cultivars are more sensible to abiotic factors in the propagative phase, such as radiation and temperature. Hot temperatures are responsible for reduction in flowers, low viability, and lead to a lower fruit formation (Pek and Helyes, 2004; Gusmao *et al.*, 2006).

Effectiveness of different fungicides against *M. roridum* revealed that synthetic fungicides are the most important means to manage diseases (Singh and Sharma, 2007). They are used alone or in combinations

(Ismail and Zhang, 2004). Different fungicides showed different mode of action. Topsin M reported to affect wide range of fungi and inhibits fungal tubule function (mitosis structure). Nativo is known as a broadspectrum fungicide, curative with long-lasting protectant activity. It enhanced plant health from the strongest strobilurin in vegetable crops. Score is supposed to be taken up by the plant and acts on the fungal pathogen during penetration and haustoria formation. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes. Cabrio Top offers extended residual protection and functions as a preventive fungicide (stopping infection and/or reinfection by prohibiting the germination of fungal spores). Among all the tested fungicides in present studies, Nativo, Topsin M and Score showed statistically meaningful results against M. roridum at the concentration of 250 µg/ml while Antracol was least effective against *M. roridum*. Similar findings have been reported in numerous studies. Foliar sprays of fungicides like Topsin M gave maximum efficiency of disease control for *M. roridum* (87.48%) on bitter gourd (Sultana and Ghaffar, 2009). Trimiltox forte, Dithane M-45 and Pencozeb were effective to inhibit the mycelial growth of M. roridum (Ali et al., 1988). Considerable reduction in mycelia growth was observed when the plates were treated with fungicides like iprodione or captan (Chase, 1990). The efficacy of the three fungicides (Trimiltox forte, Topsin M and Pencozeb) was compared to control M. roridum on the seeds of bitter guard and found significant results (Ali et al., 1988; Bharath et al., 2006).

CONCLUSION

Genotype BG.34F1 showed less disease severity among all the bitter gourd germplasm under study. It could be further used in breeding program to acquire resistance against Myrothecium leaf spot disease. Score showed promising activity by reducing the disease 49% over control. So this fungicide is a best option for reducing the losses to bitter gourd growers and could be helpful to increase their income.

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