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RISK OF *MYROTHECIUM RORIDUM* LEAF SPOT IN LOCAL CUCURBITACEOUS CROPS OF PAKISTAN

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ABSTRACT
Myrothecium roridum is a disastrous pathogen of grain, fruit and vegetable crops including cucurbits. It has been reported to parasitize a number of crops in Pakistan. To find out the cucurbitaceous host range of the fungus, pathogenicity was done on eight cucurbit species in controlled conditions. The results reveal that *M. charantia, C. melo, C. sativus L.* and *C. lanatus* are least resistant hosts of this disease with D.I. of 49.84, 48.21, 45.25 and 44.86% respectively. On the other hand three vegetables species viz. *L. aegyptaca* (17.61%), *L. siceraria* (22.72%) and *C. pepo* (23.57%) showed least resistance. One species i.e. *P. fistulosus* (38.13%) showed moderately susceptible response. All the results were evaluated by using M.STAT software under completely randomized design.

KEYWORDS: *Myrothecium roridum*, cucurbits, host range, water melon, Bitter gourd, sponge gourd, muskmelon, cucumber.

INTRODUCTION
Apart from agronomic crops, vegetables are a significant source of income for our small land holders as well as for tunnel farmers. There are more than 36 vegetables grown commercially in Pakistan including cucurbits as major food crops of the country (Malik *et al*., 2010). According to Agricultural Statistics of Pakistan (2015) area under vegetable cultivation and total production was 265.7 thousand ha and 3132.7 thousand tons respectively. However, heavy crop losses (20-30% in horticulture crops and 10-15% in agronomic crops) due to disease attacks are hampering the profitability of the national agricultural industry (Chandel and Kamal, 1995). The real yield potential of these vegetables is far from being probed out and production in the country is not up to the mark when compared with world, vegetable production.

A number of viruses, bacteria, fungi and other annoyances attack these plants at various phases of their development (Tapwal *et al.*, 2011) thus reduce the yield and lead to massive losses for human beings (Singh *et al.*, 2014). Among them pathogenic fungi are ubiquitous in environment and are more common (Dellavalle *et al.*, 2011). They cause upto 12% preharvest losses of crops in developing countries (Lee *et al.*, 2001). Cucurbits are vulnerable to numerous fungal diseases like downy mildew, anthracnose, scab or gummosis, gummy stem blight, charcoal rot, damping off, powdery mildew, Alternaria leaf spot and Myrothecium leaf spot or blight.

Myrothecium species are the chief hazards to crops as well as to cucurbits. The genus *Myrothecium* is acknowledged to consist of several species, mostly saprophytic (Quezado, 2010) and survive in this environment on dead and decaying plant tissue (Souza-Motta *et al.*, 2003; Costa *et al.*, 2006; Domsch *et al.*, 2007). The concept and classification of species within this genus is not clear and still needs revision. Among these species only *M. roridum* Tode is considered as an expressive plant pathogen. However *M. verrucaria* Ditmar is also regarded as a weak phytopathogen (Ahrazem *et al.*, 2000).

This pathogen causes about 30% yield losses of soybean in India (Talukdar and Dantre, 2014). Symptomology of infection suggests that it has dark-brown rings or bulky uneven spots on most of host leaves. However zonal development of the abrasions is also noticed. Small sclerotium like marks can also be observed on the façade of the abrasions under humid environment (Kim *et al.*, 2003). High relative humidity for a long period helps *M. roridum* to perpetuate and the disease may occur in epidemic form (Khan *et al.*, 2015).

It has been found associated with cucurbit species grown in Pakistan (Sheikh, 1990) like bottle gourd (Shakir and Mirza, 1992), bitter gourd (Ali *et al.*, 1988), Indian gourd (Wahid *et al.*, 1988), red gourd (Wahid *et al.*, 1991) and sponge gourd (Shakir *et al.*, 1995). Specifically it has become an emerging threat for bitter gourd production in the country. Sultana and Ghaffar (2009) also isolated *M. roridum* and *M. verrucaria* from bitter gourd seeds belonging to different regions of the country. Specifically it has become an emerging threat for bitter gourd crop in Pakistan. A research was planned to find out which of 8 cucurbits grown in Pakistan on commercial scale can be victimized by this hostile pathogen.

MATERIALS AND METHODS

Raising of Cucurbit plants

One locally cultivated variety of each of 8 vegetables was obtained from Vegetable Research Institute, AARI Faisalabad. All these plants were grown in plastic pots containing sterilized soil and pots were placed in screen house of Plant Pathology Research Field at University of Agriculture, Faisalabad.

Table 1: List of Cucurbit species and varieties used for screening against *M. roridum*.

Cucurbit Vegebles	Botanical Names	Variety/ Cultvar
Cucumber	*Cucumis sativus L.*	Local Sialkot
Tinda gourd	*Praecitrullus fistulosus*	Tinda Dil Pazeer
Muskmelon	*Cucumis melo*	T-96
Sponge gourd	*Luffa aegyptaca*	Faisalabad round
Water melon	*Citrullus lanatus*	Sugar baby
Bitter gourd	*Momordica charantia*	Black king
Pumpkin	*Cucurbita pepo*	Vegetable marrow
Long gourd	*Lagenaria siceraria*	Ghiya tori Local

Preparation of Media

Plating was done on Potato Dextrose Agar (PDA) following the composition described by MacFaddin (1985). All the ingredients were mixed in 1000 ml of distilled water. This mixture was then placed of Magnetic stirrer at 200 rpm for 30 minutes to mix up thoroughly. Digital autoclave was used for the sterilization of media and glassware at 121 ^{0}C and 15 Psi for 15 minutes. Glassware was wrapped in newspaper before autoclaving. After cooling down the media was poured into 9 cm diameter plates at the rate of 20 ml per plate then allowed to solidify and placed in a refrigerator for further use.

Isolation and Purification

Infected bitter gourd leaf tissues were obtained from vegetable area of Ayub Agricultural Research Institute, Faisalabad. Infected portion of leaves with healthy part were cut in 2-3 mm diameter and sterilized with 70% ethanol for one minute (Ali *et al.*, 1988). Then washed with mercuric chloride solution and then dipped into distilled water for 2 minutes to remove residues. Plating, isolation and identification of *M. roridum* was done in laminar flow hood at Plant Nematology Laboratory of Department of Plant Pathology University of Agriculture, Faisalabad.

Pathogenicity of *M. roridum* in cucurbits

Pathogenicity was done at two true leaf seedling stage by spray inoculation of *M. roridum* conidial suspension at the rate of 10^6 conidia per ml of distilled water. Heamocytometer was used for the counting of conidia. Pots were placed in relative humidity chamber and covered with polythene bags for 24 hours to ensure maximum humidity (Sultana and Ghaffar, 2009) required for penetration of fungus. Completely randomized design with three replications was applied. Separate control (3 replications) was kept for each species. Plants were examined on daily basis and data for disease incidence were recorded after 7 days by using 0 to 4 rating scale.

Table 2: Disease rating scale used for disease severity of *M. roridum* on cucurbit leaves.

Ratings	Symptoms	Result
0	No symptoms	Immune
1	1-25% leaf area infected	Moderately resistant
2	26-50% infection	Moderately Susceptible
3	51-75% infected tissue	Susceptible
4	76-100% leaf area infected	Highly susceptible

(Fish *et al.*, 2012)

Formula used for disease severity:

$$\text{Disease index} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves assessed}} \times \frac{100}{4}$$

(Sultana and Ghaffar, 2009)

RESULTS AND DISCUSSIONS

In this study 8 locally grown cucurbit crops in Pakistan, were tested for the pathogenicity response to an ascomycete fungus *M. roridum.* All the vegetables under experiment showed susceptible response. This fungus

shows ubiquitous nature as it has previously shown some 200 hosts in the world and a number of its hosts present in the country have been manipulated. In the present experiments maximum disease incidence was shown by variety/ cultivar of each of *M. charantia* (Black King), *C. melo* (T-96), *C. sativus* L (Local Sialkot) and *C. lanatus* (Sugar baby) as 49.84, 48.21, 45.25 and 44.86% respectively. Other three vegetable species viz. *L. aegyptaca*, *L. siceraria* and *C. pepo* showed least susceptibility as 17.61, 22.72 and 23.57% respectively while one species i.e. *P. fistulosus* was moderately susceptible to the attack of fungus with 38.13% disease incidence.

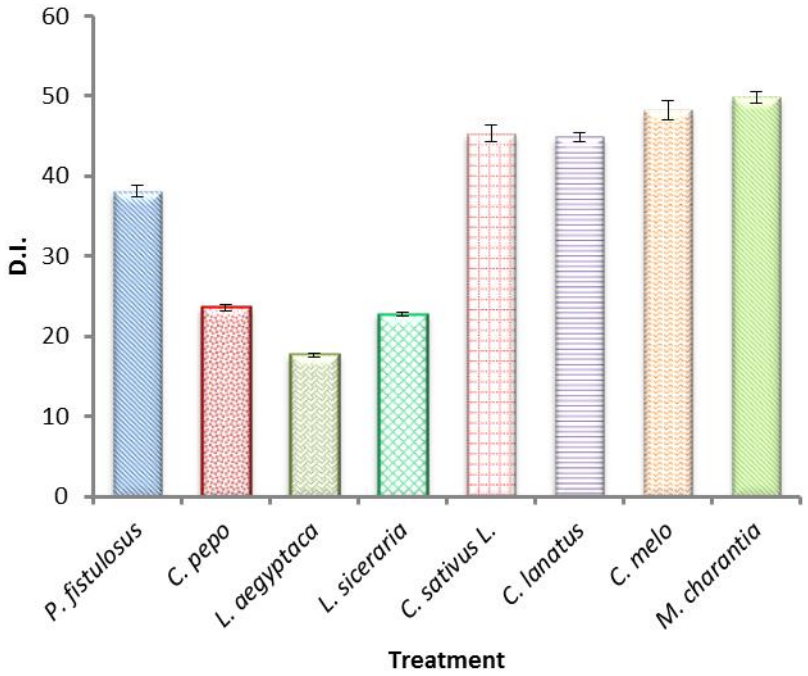

Fig. 1: Susceptibility response of Cucurbits for M. roridum.

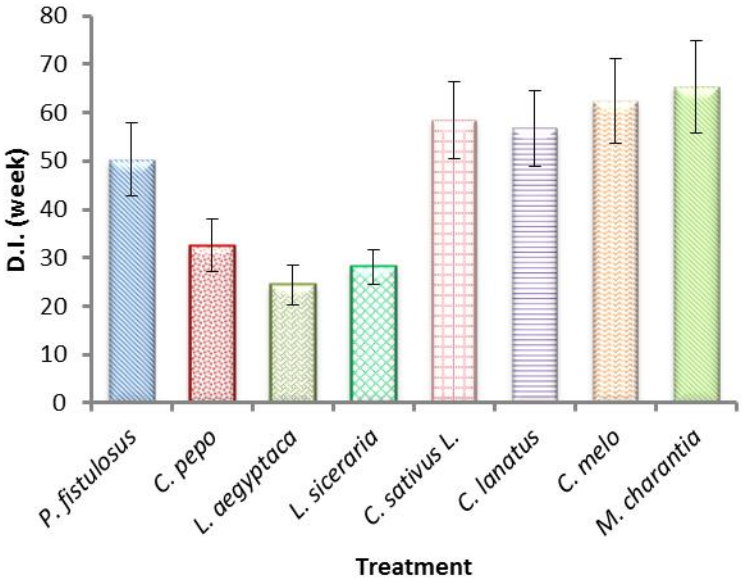

Fig. 2: Susceptibility response of Cucurbits for M. roridum in relation to weeks of infection.

All the statistical trials were performed by using statistical software SAS/STAT (SAS Institute, 1990). Means were separated using Fisher's protected least significant difference (LSD procedure). Data for *in-vitro* evaluation of chemicals was subjected to statistical analysis under complete randomized design with

M.STAT software for determining the significance and non-significance of chemicals (Russel and Eisensmith, 1983).

Table: Means of disease severity of *M. roridum* on different cucurbit species.

Treatment	Mean ± SE
P. fistulosus	38.13 ± 0.72 C
C. pepo	23.58 ± 0.37 D
L. aegyptaca	17.61 ± 0.28 E
L. siceraria	22.72 ± 0.22 D
C. sativus L.	45.25 ± 1.04 B
C. lanatus	44.86 ± 0.53 B
C. melo	48.21 ± 1.18 A
M. charantia	49.84 ± 0.68 A

Means sharing similar letters are statistically non-significant (P>0.05)

Table: Analysis of Variance Table for D.I. shown by different cucurbits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	7	3532.76	504.680	335.0**
Error	16	24.04	1.502	
Total	23	3556.80		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table: Analysis of Variance Table for D.I. in relation to number of weeks of infection.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Week	2	2227.14	1113.57	74.95**
Treatment	7	5667.31	809.62	54.49**
Error	14	208.00	14.86	
Total	23	8102.45		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table: Means disease severity of *M. roridum* in relation to number of weeks of infection.

Week	Mean ± SE
W1	35.94 ± 4.59 C
W2	46.49 ± 5.65 B
W3	59.49 ± 7.21 A

Means sharing similar letters are statistically non-significant (P>0.05).

Leath and Kendall (1983) observed rot of roots in red clover and alfalfa fields. The fungus *M. roridum* was first reported to be pathogenic to muskmelons (C*ucumis melo*) by Mclean and Sleeth (1961). The fungus then showed its ubiquitous behavior when it was isolated from tomato (*Solanum lycopersicum*) plant belonging to another family, solanaceae by Khan and kamal (1968) in Pakistan. This fungus contains relatively wide host range including horticultural and field crops such as cotton, potato, tomato, coffee, soybean, cocoa and cucurbits as well as numerous ornamental plants (Bruton *et al*., 1996; Chase, 1983; Ponappa, 1970). Ali *et al*. (1988) found infected leaves of bitter gourd with small yellow to purplish brown and black, circular to irregular spots at Vegetable Research Area of Ayub Agricultural Research Institute, Faisalabad.

Quezado *et al*. (2010) also found out some unfamiliar hosts of the Myrothecium fungi in Brazil. In their study they isolated *M. roridum* and *M. verrucaria* from three vegetable plants (sweet pepper, tomato and cucumber), four ornamental species (*Spathiphyllum wallisii, Solidago canadensis, Anthurium andeanum* and *Diffenbachia amoena*) and a weed belonging to solanaceae family (*Nicandra physaloides*) and confirmed pathogenicity.

Gaikwad (1988) studied the host range of *M. roridum*. He observed moth (*Phaseolus acontifolius* Jacq), green gram (*Phaseolus mungo L.*), cowpea (*Vigna radiate Endl*), pigeon pea (C*ajanus cajan L.* Millsp), pea (*Pisum sativam L.*), okra (*Abelmoschus esculantum L.* Moench), soybean (*Glycine max merr,*), peanut (*Arachis hypogaea L.*), potato (S*olanum tuberosum L.*), cotton (*Gossyipum hirsutum L.*), wheat (*Triticum aestivum L.*), pearl millet guar (*cyamopsis tetragonoloba),* tomato (*Lycopersicum esculantum* Mill*)* and maize (*Zea mays L.*) as most effective host for the pathogen. Meyer *et al.* (2006) studied the leaf spots disease caused by *M. roridum* (Tode ex Fr.) on cotton field in Maranhao state of Brazil, triggering yield loss of above 60%. The disease symptoms were lesions with concentric, necrotic rings, with striking structures (sporodochia) described irregularly.

CONCLUSION

Bitter gourd, muskmelon, cucumber and water melon are more likely to be infected by disease with D.I. of 49.84, 48.21, 45.25 and 44.86% respectively while three other vegetables viz. sponge gourd, long gourd and pumpkin showed least resistance with 17.61, 22.72 and 23.57% disease incidence respectively. Only Tinda gourd (38.13%) showed moderate susceptibility. The overall result reveals that all the 8 cucurbits were hosts of *M. roridum*.

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