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PATHOGENESIS AND CONTROL OF *MYROTHECIUM* SPP., THE CAUSE OF LEAF SPOT ON BITTER GOURD (*MOMORDICA CHARANTIA* LINN.)

NASREEN SULTANA AND A. GHAFFAR

Crop Diseases Research Institute, PARC,
Karachi University Campus, Karachi-75270, Pakistan and
Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Seed and soil inoculation by *M. roridum* and *M. verrucaria* reduced seed germination and caused seed rot, damping off, root rot and spots on aerial parts of bitter gourd. Isolates of *M. roridum* were found more pathogenic than isolates of *M. verrucaria.* This is the first report of seedling and root infection of *M. roridum* and *M. verrucaria* and also the first report of *M. verrucaria* leaf spot disease in bitter gourd. Isolates of *M. roridum* were found more pathogenic than isolates of *M. verrucaria*. Foliar sprays of fungicides and oil cakes extract significantly reduced the incidence and were found superior to other treatments in controlling disease. Maximum percent efficiency of disease control was observed where Topsin-M was sprayed on *M. roridum* (87.48%) and *M. verrucaria* (95.79%) inoculated leaves of bitter gourd.

Introduction

Myrothecium sp., *M. roridum* Tode ex Fr. and *M. verrucaria* (Alb. Schew) Ditm ex Fr., are soil borne as well as seed borne pathogens and attack several plant species. *Myrothecium* sp., generally cause round dark-brown leaf spot in cucurbits, which on later stage coalesces to form blighted areas on the leaves (Belisario *et al*., 1999). *M. roridum* causing leaf spot / blight has been reported as a common pathogen of cucurbits (Ali *et al*., 1987). *M. roridum* has been isolated from seeds of bottle-gourd (Shakir & Mirza, 1992), Indian gourd, red gourd, sponge gourd (Wahid, 1985; Wahid *et al*., 1991; Shakir *et al*., 1995), pumpkin (Manthachitra, 1971) and melon (Lima *et al*., 1997). *M. verrucaria* has showed a high incidence on seeds of watermelon (Bharath *et al*., 2006). Belisario *et al*., (1999) have also reported the occurrence of *M. verrucaria* on muskmelon seed *In vitro* conditions. *Myrothecium roridum* and *M. verrucaria* were isolated frequently from seeds of bitter gourd and found associated with rotted and un-germinated seeds during seed health testing. Isolates of *M. roridum* and *M. verrucaria* obtained from rotted seeds of bitter gourd were used to test their pathogenic effect on germination, seedling mortality, root infection and leaf spot disease of bitter gourd and effect of foliar spray by different fungicides and oil cake extracts for the control of *Myrothecium* leaf spot of bitter gourd was elucidated.

Materials and Methods

Two isolates each of *Myrothecium roridum* and *M. verrucaria* of bitter gourd were used to test their pathogenicity by seed, root and leaf inoculation techniques. Seeds were inoculated by rolling on 10 days old sporulated colonies raised on PDA plates. Spore suspension of *M. roridum* and *M. verucarria* were prepared from two weeks old PDA plate culture. Conidia were removed from plates using sterilized distilled water and spatula and adjusted at 10^8 conidia per ml by means of haemocytometer. Roots of 2

weeks old seedling were dipped in freshly prepared spore suspension (10^8spores/ml). The inoculated seeds and seedling were transferred to pot containing autoclaved soil. There were 10 seeds per pot with 5 replications. Similarly for leaf inoculation 1 month old seedlings raised in pot containing sterilized soil were sprayed with spore suspension (10^8 spores / ml) by means of hand sprayer. Plants sprayed with distilled water served as control. The plants were covered with polythene bags for 24 h after inoculation. Observations were made on seed germination, seedling mortality and root infection. After 40 days the plants were uprooted, plant sizes were recorded in cms. Symptomatic plant parts viz., seeds, stem, leaves and roots after surface disinfection with 2% $NaOCL_2$ were plated on PDA plates.

Fungicides viz., Carbendazim, Topsin-M, Aliette, Benlate and Ridomil were used @ 0.3% w/w. Oil cakes i.e., mustard, neem and castor cake @ 200 gm in a litre of water were soaked for overnight. The mixture was filtered through muslin cloth and filtrates were directly sprayed. Bitter gourd seeds were sown in pots @ 10 seeds per pot and one month old seedling were sprayed on leaves by aforesaid treatments after 24 h inoculation by *Myrothecium roridum* and *M. verrucaria*. The disease index was calculated by adopting disease-rating scale 0-5 (Vir & Grewel, 1974) where 0= no symptoms on leaves, 1= <5% infection on leaves, 2=5-25% infection on leaves, 3=25-75% infection on leaves, 4= >75% infection on leaves. Disease index and percent efficiency of disease control were calculated by the following formulae:

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

$$\text{Percent efficiency of disease control} = \frac{\text{Disease index in control - Disease index in treatments}}{\text{Disease index in control}} \times 100$$

Result and Discussion

Seed inoculation with isolates of *M. roridum* and *M. verrucaria* gave variable results among species but not significantly different in isolates of species (Fig. 1). Seed rot, damping off, root rot and infection on aerial parts were significantly increased in isolates of *M. roridum*. Lesion on cotyledons, cotyledonary and first form leaves were initially yellow and later became brown and finally turned black. The whole seedling collapsed as the aerial portion dried. Similarly root rot and aerial parts infection were significantly increased in seedlings where roots were inoculated by isolates of *M. roridum*. Reduction in seed germination by *M. roridum* have been reported in sponge gourd (Shakir *et al.*, 1995) and 100% in *Striga hermonthic* (Czerwenka-Wenkstetten *et al.*, 1997). Both species of *Myrothecium* viz., *M. roridum* and *M. verrucaria* caused seed rot, damping off, root rot and infection on aerial parts. *M. roridum* was found more pathogenic than *M. verrucaria* as also reported by Cunfer *et al.*, (1969) in red clover. *M. roridum* attacked the emerging seedlings of mungbean and killed most of the transplanted seedlings (Ram Nath *et al.*, 1970). The fungus was also reported to kill seedlings of watermelon and produced necrosis on cotyledonary leaves (Bharath *et al.*, 2006). Although Wensely (1956) implicated *M. verrucaria* in transplanted problems of peaches, the soil borne fungi generally attacked above ground plant organs. Symptomatic plant parts viz., seeds, stem, leaves and roots after surface disinfection with 2% $NaOCl_2$ were plated on PDA plates. After 7-10 days of inoculation the pathogens re-isolated from the diseased plants showed well-developed dark green sporodochia. This is first report of seedling and root rot infection of *M. roridum* and *M. verrucaria* on bitter gourd.

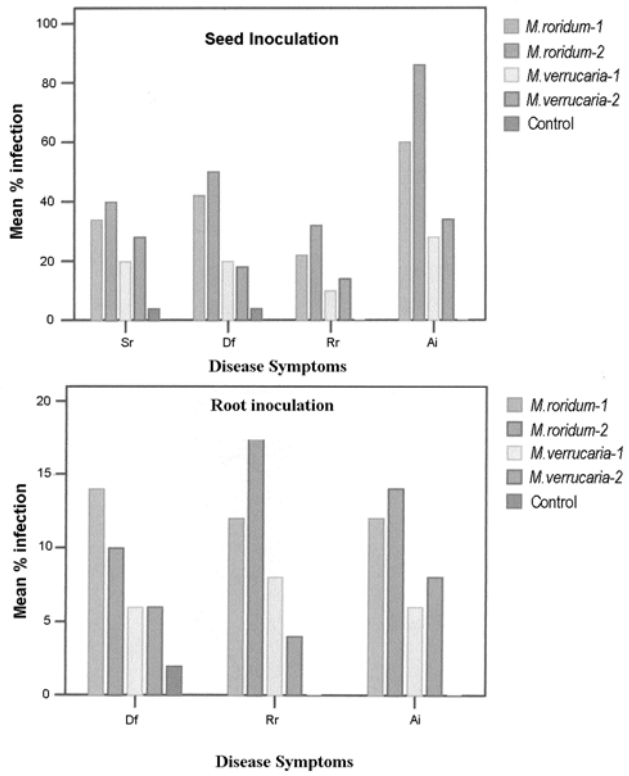

Fig. 1. Effect of isolates of *Myrothecium roridum* and *M. verrucaria* on disease development on bitter gourd by seed and root inoculations.
Sr= Seed rot, Df= Damping off, Rr= Root rot, Ai= Aerial infection

Infection index of leaves on the basis of 0-4 scales were significantly increased on leaves inoculated with isolates of *M. roridum* as compared to isolates of *M. verrucaria* (Table 1). Symptoms developed on leaves after 3 days of inoculation were yellowing of leaves; chlorosis and browning of leaf margins were the most prominent symptoms and were evident after 6-8 days of inoculation. In few cases concentric layer of darker brown rings developed on leaves. As the chlorotic and necrotic areas extended, the leaves curl and die. *Myrothecium* species generally caused brown, dark brown leaf spot in cucurbits. Leaves on later stages coalesce to form blighted area on the leaf (Belisario *et al.*, 1999). Both isolates of *M. roridum* were found more pathogenic than isolates of *M. verrucaria*. *M. roridum* has been reported to cause leaf spot in bitter gourd (Shaukat *et al.*1988). This is first report of *M. verrucaria* leaf spot disease in bitter gourd. Yang & Jong (1995) have described the host range of *M. verrucaria* isolated from leafy spruge. *M. roridum* and *M. verrucaria* have been reported to be foliar pathogen of red clover (*Trifolium pretense*) and favoured by prolonged hot and humid weather (Cunfer *et al.*, 1969). *M. roridum* has been recognized as a seriously damaging leaf spot and crown rot pathogen of foliage plants (Chase, 1983) and also reported as fruit rot pathogen of bitter gourd in India (Sharma & Bhargara, 1978).

Table 1. Effect on foliar leaf spots development on the bitter gourd plant inoculated with conidia of isolates of *Myrothecium roridum* and *M. verrucaria*.

Treatments	Disease index
M. roridum isolate- I	50.8
M. roridum isolate-II	41.8
M. verrucaria isolate-I	24.1
M. verrucaria isolate-II	12.5
Control	0.0

Table 2. Effect of foliar spray of different fungicides and oil cake extracts on *Myrothecium* leaf spot of bitter gourd.

Treatments	Disease index		% Efficiency of disease control	
	M. roridum	***M. verrucaria***	***M. roridum***	***M. verrucaria***
Aliette (3%)	19.2e	4.9b	71.7e	88.16e
Benlate (3%)	10.4b	2.2a	84.6h	94.60f
Carbendazim (3%)	16.4d	4.7b	75.8f	88.50e
Mancozeb (3%)	12.7c	2.3a	81.3g	94.26f
Topsin-M (3%)	8.5a	1.7a	87.4i	95.79f
Mustard cake (20%)	31.2f	10.2c	54.2d	75.42d
Neem cake (20%)	36.7g	14.2d	46.2c	65.75c
Castor cake (20%)	39.4h	28.1e	42.2b	33.01b
Control (untreated)	68.1i	41.6f	0.0a	.00a

Mean followed by the same letter within a column are not significantly different (p=0.05) according to Duncan's multiple range test.

All treatments invariably reduced *M. roridum* and *M. verrucaria* leaf spot incidence over control (Table 2). Foliar sprays of fungicides and oil cakes extract significantly reduced the incidence and were found superior to other treatments in controlling disease. Maximum percent efficiency of disease control was for *M. roridum* (87.48%) and *M. verrucaria* (95.79%) where Topsin-M was sprayed on inoculated leaves of bitter gourd. This was followed by Benlate, Mancozeb, Carbendazim and Aliette. The treatments comprising of oil cakes extracts significantly reduced disease index. Percent efficiency of disease control was 54.27% for *M. roridum* and 75.42% for *M. verrucaria* where mustard cake extract were sprayed on inoculated leaves of bitter gourd followed by neem and castor cake extract. Ali *et al*., (1988) have reported that three fungicides viz., Trimiltox forte, DithaneM-45 (Mancozeb) and Pencozeb were found equally good and significantly controlled the *Myrothecium* leaf spot of bitter gourd.

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