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IN VITRO FUNGICIDAL ACTIVITY OF AQUEOUS EXTRACTS OF CROP AND WASTELAND WEEDS AGAINST *Myrothecium roridum* TODE

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ABSTRACT

Application of aqueous weed extracts is an environment friendly approach to manage destructive plant pathogens and is an emerging tool in biological control of pathogens. In the present study, aqueous extracts of nine weeds Chenopodium album L., Parthenium hysterophorus L., Trianthema portulacastrum L., Malvestrum coromandelianum (L.) Garcke, Coronopus didymus (L.) Sm., Sphaeranthus indicus L., Digera muricata (L.) Mart., Solanum nigrum L. and Nicotiana plumbaginifolia Viv. were applied against Myrothecium roridum Tode strain Mr 10 (accession no. 1155) by food poison technique. Aqueous extracts of weeds were prepared by macerating 20g of fresh leaves in 20 mL of sterilized distilled water (100% w/v stock). The extracts were double filtered through muslin cloth and Whatman filter paper no. 1 and added in PDA medium under asceptic conditions before pouring. The extract of N. plumbaginifolia exhibited growth inhibition of 88%, P. hysterophorus (71%) and S. nigrum, C. didymus, S. indicus and T. portulacastrum L. restrained the colony growth up to 66, 65, 64 and 60%, respectively. Digera muricata was least effective with 11% of colony growth.

Key words: Antifungal potential, aqueous extract, crop and waste land weeds, *Myrothecium roridum*.

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INTRODUCTION

Myrothecium roridum is a seed- and soil-borne fungus with a wide host range of vascular plants. It has been isolated frequently

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from seeds of bitter gourd (*Momordica charantia* L.) and found associated with rotted and un-germinated seeds. It has become a problematic pathogen affecting the yield and quality of bitter gourd crop in Punjab, Pakistan (Sultana and Ghaffar, 2007). Appearances of dark brown leaf spots with concentric rings of olive green to black colored sporodochia are signs of the presence of myrothecium leaf spot disease. At later stage, these spots coalesce to form blighted areas on the leaves (Belisario *et al*., 1999). Very little information is available on myrothecium leaf spot disease and associated promoting factors of climate and need experimental elaboration.

Score (Difenoconazole DMI group), and zinc and copper based fungicides like Captan and Maneb are generally recommended to control *M. roridum* but their efficacy can be affected by climatic conditions and growth stage of the plants (Sultana and Ghaffar, 2009; McMillan, 2010). Application of commercial synthetic fungicides may also have negative effect on produce quality and grower environment. There is a need to investigate efficient, effective and economical ways for environmental friendly management strategies against plant pathogens. Study of allelopathic potential of plants in managing several pathogens may lead to cost effective and environmental friendly approach and provides an excellent alternative to synthetic chemical applications (Vyvyan, 2002). Scientists are working on the aqueous and organic solvent extracts of flowering plants like *Azadirachta indica, Eucalyptus* spp., *Syzygium cumuni, Curcuma longa, C. didymus, C. album,* and *Aloe vera* to control wide range of fungal plant pathogens (Davicino *et al*., 2007; Dellavalle *et al*., 2011; Javaid and Iqbal, 2014).

The archeological references reveal that the concept of application of phyto pesticides is centuries old in the Indian subcontinent and Africa. This phenomenon is supported due to presence of essential compounds which can further be exploited for managing plant pathogens (Srivastava and Lawton, 1998; Root, 1973). The inhibitory effect of *S. indicus* has been reported against *Alternaria solani, Fusarium oxysporum* and *Penicillium pinophilum* (Dubey *et al*., 2000; Galani *et al*., 2010). *Parthenium hysterophorus* was reported to have antifungal potential against soil borne pathogens (Bajwa *et al*., 2001). Antifungal potential of *D. muricata* and *C. didymus* under *in vitro* and *in vivo* conditions was found to reduce the incidence of *Alternaria alternata* and *Sclerotium rolfsii* against vegetable and cereals (Shafique *et al*., 2006; Sharma and Vijayvergia, 2013). *Myrothecium roridum* is an emerging threat for bitter gourd crop in Pakistan. Few synthetic fungicides have been evaluated against *M. roridum* but there is scarcity of information on exploring antifungal activity of weeds. Host susceptibility and broader

host range of *M. roridum* demands for exploring new strategies for integrated management of the disease. Therefore attempt is made to explore the antifungal potential of aqueous extracts of endemic crop and wasteland weeds.

MATERIALS AND METHODS

Fungal culture

Myrothecium roridum strain Mr 10 (FCBP accession no. 1155) isolated from bitter gourd leaves in Seed and Post Harvest Pathology Lab, Institute of Agricultural Sciences, University of the Punjab, Lahore was maintained on potato dextrose agar medium (potatoes, 200g; dextrose, 20g; agar, 16g and distilled water; 1L) at 25°C.

Collection of weed plants

Tender plants of *N. plumbaginifolia, C. album*, *P. hysterophorus, T. portulacastrum, M. coromendelianum, C. didymus, S. indicus, D. muricata* and *S. nigrum* were collected from the crop and wasteland fields of Lahore and its suburbs.

Preparation of aqueous extracts

Plants were thoroughly washed under running tap water to remove dust and other contaminants and surface dried on the blotter paper. Aqueous weed extracts were prepared by macerating 20g of fresh leaves in 20 mL of distilled water (100% w/v stock) and double filtered through muslin cloth and filter paper (Javaid *et al*., 2010). Stock extracts were stored at 4 °C and used within 2-3 days.

Food poison assay

The experiment was laid out in CRD with five replicates and five 90 mm Petri plates in each replicate. Each of the tested weed extract was added @ 10% in 2% PDA medium before pouring under sterilized conditions. Control PDA plates were not amended with weed extracts. A disc of 3mm diameter from the actively growing colony margins of 10 days old *M. roridum* culture was transferred to the PDA plates amended with the weed extracts. The plates were incubated at 28±2 °C and colony growth was measured after 4, 7, 10 and 14 day interval. Inhibition percentage was measured at day 14 by the following formula given below.

$$\text{Radial growth inhibition \%} = \frac{\text{Growth in control - growth in weed amended medium}}{\text{Growth in control}} \times 100$$

Morphological response was assessed on colony macroscopic characters i.e., colony texture, colony margins, colony form, colony elevation and physiological response on microscopic character i.e., spore production were recorded. Data were subjected to analysis of variance (ANOVA) followed by Tukey's HSD test using computer software SPSS version 15.0.

RESULTS AND DISCUSSION

Radial mycelia growth of *M. roridum* was observed against tested weed extracts at 4, 7, 10 and 14 incubation day (Fig. 1). There was a significant increase in radial growth with increase in incubation period. The highest radial growth (i.e. 87 mm) was observed in control treatment during all incubation periods (Fig. 2). Among the tested weed extracts, *N. plumbaginifolia, P. hysterophorus, S. nigrum, C. didymus* and *S. Indicus* did not exhibit any radial growth at day 4. This suppression was maintained in *N. plumbaginifolia* up to day 7. Weed extracts of *C. album*,*T. portulacastrum, M. coromendelianum, D. muricata* were proved least effective as they showed increasing pattern in radial growth at 4, 7, 10 and 14 day of incubation. At 14 day incubation period, *N. plumbaginifolia, P. hysterophorus, S. nigrum, C. didymus, S. indicus, C. album*, *T. portulacastrum, M. coromendelianum* and *D. muricata* exhibited 11, 26, 30, 31, 32, 36, 42, 72 and 81 mm radial growth respectively (Fig. 2). Among tested aqueous extracts, the highest antifungal potential was found in *N. plumbaginifolia* extract that inhibited the colony radial growth up to 88% followed by *P. hysterophorus* that reduced the growth up to 71% over control (Fig. 3). *S. nigrum*, *C. didymus, S. indicus* and *T. portulacastrum* L restrained the colony growth up to 66%, 65%, 64% and 60% respectively. *C. album* slows down the colony growth up to 54%. *D. muricata* was least effective with 11% of colony growth.

Variation in physiological response was recorded on the basis of macroscopic characters like colony color, texture, margins, spore production and elevation (Table-1). *M. roridum* produce circular, flat colonies with floccose texture and filiform margins on PDA at 25°C. A large number of conidia produced after 3-4 days on colony surface while mycelium continues growing from margins. *Nicotiana plumbaginifolia* and *P. hysterophorus* extracts revealed to inhibit the colony radial growth whereas *N. plumbaginifolia* did not exhibit any spore production till last reading. This might due toof presence of potent antifungal compounds in aqueous extracts that provide inhibition in the fungal growth. *S. indicus* and *M. coromendelianum* produce submerged colonies as compared to the control treatment. *C. album* and *M. coromendelianum* extracts produce irregular shapes colonies with lobate margins.

Inhibition potential of the weed plants is due to the presence of several chemical constituents like phenols, alkaloids, terpenoids, coumarins and tannins. Some of them already have been discovered and known for their antifungal potential but many needed to be explored. Singh *et al.* (2010) observed antibacterial activity of aqueous and methanol extracts of *N. plumbaginifolia* on five human pathogenic bacteria Viz *Bacillus cereus, Bacillus fusiformis, Salmonella*

typhimurium Staphylococcus aureus and *Pseudomonas aeruginosa*. Phytochemical evaluation of leaves of *N. plumbaginifolia* revealed the presence of alkaloids, saponin, tannin, flavonoides, cardiac glycosides, phenolic compounds, steroids, terpenoides and carbohydrates (Singh *et al.,* 2010). Stukkens *et al.* (2005) reported terpenoids compounds in *N. plumbaginifolia* are responsible for its antifungal properties. *P. hysterophorus* is known to have chemical constituents like parthenin, p-coumaric acid, ferrulic acid, vanillic acid and caffeic acid that act as antifungal compounds (Kanchan and Jayachandra, 1980; Das and Das 1995). The plant parts and their extracts suppressed *Penicillium* spp., inhibited germination of spores of *Drechslera rostrata, Fusarium oxysporum, Alternaria alternata, Corynespora cassiicola, Aspergillus fumigatus, A. niger, A. sulphureus and Microsporum gypseum* (Luke, 1976; Kumar *et al.,* 1979; Shrivastava *et al.,* 1984; Sharma and Gupta, 2012). Plant extract inhibited mycelial growth and sporulation in pathogen *Aspergillus flavus* (Lokesha *et al.,* 1986). *Digera muricata* is reported to contain antifungal compounds and showed significant reduction in growth of *Fusarium oxysporum* and *Aspergillus niger* but in the present study it exhibited least inhibition against *M. roridum* (Kohli *et al*., 1998; Sharma and Vijayvergia, 2013). This may be attributed resistance reaction of the test fungus to its compounds but detailed investigations are needed.

Further, *in vivo* investigations of highly effective concentration of aqueous extracts and exploitation of their organic fractions are in progress.

CONCLUSION

It is concluded that aqueous extracts of *N. plumbaginifolia* and *P. hysterophorus* possess significant antifungal activity against *M. roridum* under *in vitro* conditions.

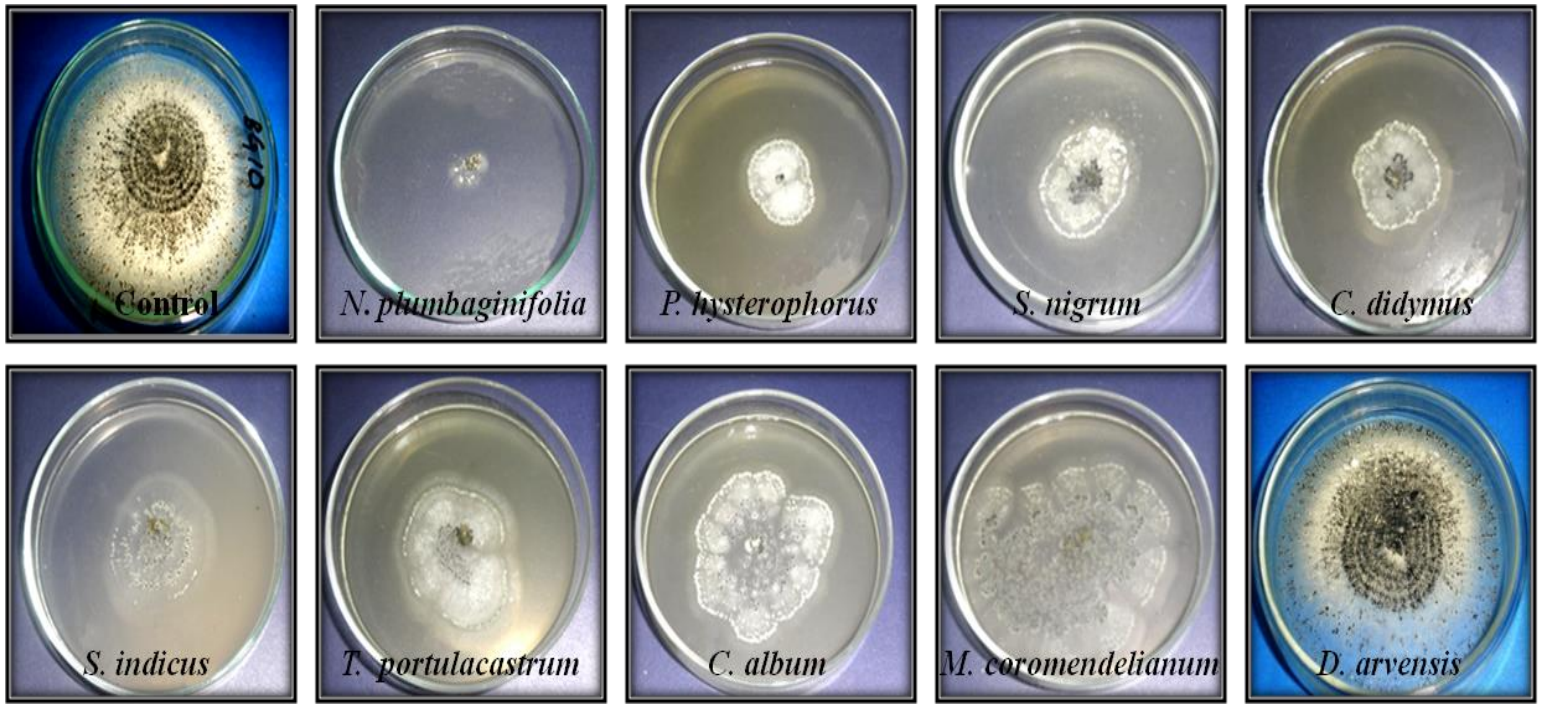

Figure 1. Effect of different aqueous weed extracts on colony growth of *M. roridum*.

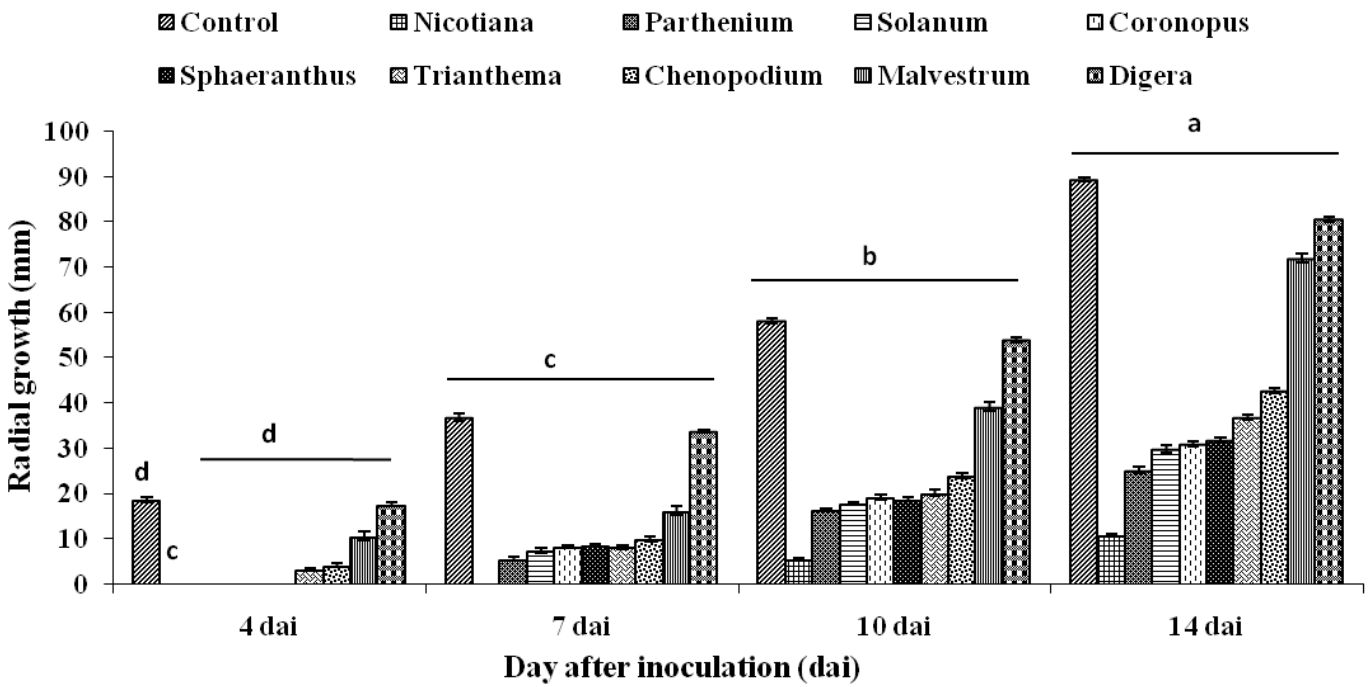

Figure 2. Effect of different aqueous weed extracts on colony growth of *M. roridum*. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD method.

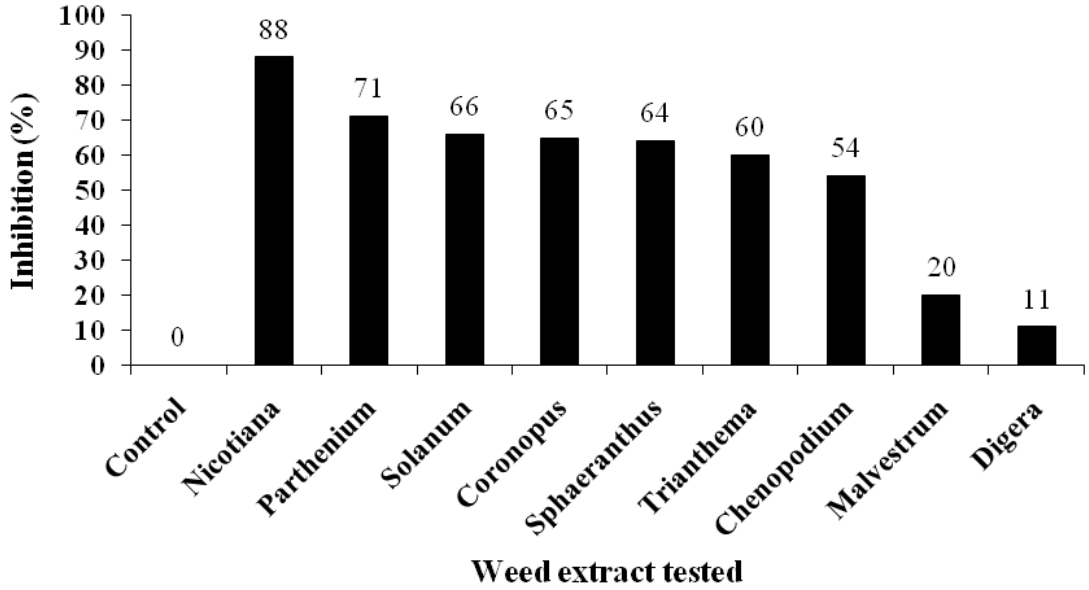

Figure 3. Evaluation of colony growth inhibition of *M. roridum* against aqueous weed extract

Table-1. Assessment of macroscopic colony characters of *M. roridum* under the stress of weed aqueous extracts

Weed extract	Colony form	Colony elevation	Colony texture	Colony margins	Spore production
Control	Circular	Flat	Floccose	Filiform	+
N. plumbaginifolia	Irregular	Umbonate	Filamentous	Filiform	-
P. hysterophorus	Filamentous	Raised	Floccose	Filiform	-
S. nigrum	Filamentous	Raised	Floccose	Filiform	+
C. didymus	Filamentous	Flat	Floccose	Undulate	+
S. indicus	Irregular	Submerged	Sparsely filamentous	Entire	+
T. portulacastrum	Filamentous	Crateriform	Sparsely floccose	Filiform	+
C. album	Filamentous	Crateriform	Floccose	Undulate	+
M. coromendelianum	Irregular	Submerged	Sparsely filamentous	Lobate	+
D. muricata	Circular	Flat	Floccose	Filiform	+

+: Produce spores, - : No spore production

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