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Bio-efficacy of phytoextracts and oil cakes on *Macrophomina phaseolina* (Tassi) causing stem rot disease of jute, *Corchorus spp*.

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Abstract: In the present study efforts were made to explore the efficacy of various plant extracts and oil cakes against *Macrophomina phaseolina*. The efficacy of eight different plant species, *Zingiber officianale*, *Aloe indica*, *Lawsonia inermis*, *Chenopodium album*, *Allium cepa*, *Piper betel*, *Murraya koenigii*, *Parthenium hysterophorus* aqueous extracts including oil cakes of mustard and neem were evaluated by using of poisoned food technique *in vitro* for their inhibitory effect on mycelial growth of *M. phaseolina* causing stem rot of jute (*Corchorus olitorius* and *C. capsularis*). The rhizome extract of *Z. officinale* produced maximum growth inhibition (74.59%) of the pathogen followed by leaf extracts of *A. indica* (63.57%), and leaf extract of *L. inermis* (60.17%) at the concentration of 10%. The maximum mycelial growth inhibition (52.40%) was recorded with neem cake (*Azadirachta indica*) at the concentrations of 20% followed by 42.61% and 29.60% with concentration of 15% and 10%, respectively. However, maximum mycelial growth inhibition (19.42%) was recorded with mustard cake (*Brassica juncea*) at the concentration of 20% followed by 16.64% and 12.20% at the concentration of 15% and 10% respectively. In general mycelial growth inhibition was dose dependent and it was maximum in case of neem cake than mustard cake. The present study revealed that, these plant extracts and oilcake extracts could be exploited for the possible control of deadly pathogen *M. phaseolina*. Accordingly, this is an important proactive measure in preventing the spread of the stem rot disease through a more ecofriendly approach.

Keywords: Jute, *Macrophomina phaseolina*, Oilcake extracts, Phytoextracts

INTRODUCTION

Phytochemicals derived from various bio-active plant species offer a promising and natural source of safer agrochemicals (Isman, 2006). There are many botanical products commercialized and reported as antifungal compounds that are present among higher plants, and are well-known entities for disease resistance. These phytochemicals are biodegradable, protective, curative and selective in toxicity (Chowdhury *et al*., 2008). Therefore, plant extracts stands as an alternative source for controlling plant diseases. Plant extracts are eco-friendly possess structural diversity, complexity and are frequently impregnated with halogenated atoms (Duke *et al*., 2000). Presently more than 200 species of plant pathogens are reported to be resistant to chemical pesticides (Varma and Dubey, 1999). A serious setback off these chemicals is environmental pollution that resulted in stand-by mode. Thus, there is urgent need for the development of alternative disease control tactics that are effective coupled with eco-friendly nature. These phytoextracts are cost effective, relatively safe for farmers who can't rely on synthetic pesticides. Jute (*C. olitorius* and *C. capsularis*) is an important bast fiber crop. Its fiber is used for making bags, decorative, textiles and geotextiles and its sticks are also used for fuel, door panels of automobiles and for making false ceiling boards. It suffers from various diseases resulting in yield loss and deterioration in fiber quality. The diseases compel crop to suffer are seedling blight, root rot, leaf blight, stem rot (*M. phaseolina*), anthracnose and leaf mosaic (Roy *et al*., 2008). Among them *M. phaseolina* is devastating. It attacks any part of the plant at any stage of growth from seed germination to harvest and causes infection up to the tune of 35-60%. Widespread host range and abundant growth of the pathogen makes the chemical control strategy an unsuccessful approach inevitably. Earlier workers have reported the effect of plant extracts of various plant species to inhibit the growth of *M. phaseolina in vitro* (Upadhyay and Gupta, 1990; Dubey and Dwivedi, 1991). Phytoextracts of garlic (*Allium sativum*) proved to be effective against charcoal rot caused by *M. phaseolina* in sorghum (Datar, 1999). Phytoextracts of eleven plant species evaluated against *M. phaseolina* of green gram revealed that the onion bulb extract produced

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maximum mycelial growth inhibition followed by acacia, ginger, neem, garlic and karanj extracts (Tandel *et al*., 2010). Reported that amongst various oil cakes evaluated *Brassica juncea* cake exhibited maximum inhibition of mycelial growth of *M. phaseolina* causing root rot in okra (Jha *et al*., 2000). The present study was ascertained to investigate the antifungal activities of leaf extracts of various plant species and oil cakes which are readily available, affordable and environmentally safe, on the mycelial growth of *M. phaseolina* under *in vitro* conditions.

MATERIALS AND METHODS

Phytoextracts : The investigation was carried out at Crop Protection Division, Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore. Pure culture of *M. phaseolina* was obtained from the CRIJAF, Plant Pathology laboratory. Aqueous extracts were obtained from plant parts such as leaves, rhizome by using standard protocols as mentioned (Ezhilan *et al*., 1994). Healthy fresh leaves and rhizome (50 gm.) of eight different plant species were collected and surface sterilized with 0.02 per cent mercury chloride and repeatedly washed with sterilized water. The sterilized leaves along with 50 ml of sterilized distilled water were minced with the help of a grinder. The phytoextracts were first passed through four layers of muslin cloth and then filtered through Whitman's filter paper No.41 into 150 ml conical flasks. The flasks were plugged with non-absorbent cotton and tightly wrapped with aluminum foil and autoclaved at 121°C at 15psi pressure for 20 minutes. The effect of phytoextracts of various plant species were tested *in vitro* by poisoned food technique to know their inhibitory effect on the mycelial growth of *M. phaseolina*. Autoclaved extracts were individually added into sterilized potato dextrose agar (PDA) plates at the concentration of 10 per cent and mixed |thoroughly at the time of pouring into sterilized petri plates. The petri plates were inoculated aseptically after solidification of the media by placing 5 mm diameter mycelial disc at the center, cut aseptically with cork borer from eight days old pure culture of *M. phaseolina*. Each treatment was replicated thrice. Control was maintained without adding extracts. The plates were incubated at 27±2°C temperature till the complete growth coverage in control plate. The colony diameter in each treatment was measured on the fifth day after inoculation and the percent growth inhibition (PGI) of the pathogen was recorded using formula given by Vincent (1947)

Oil cakes: The neem cake and mustard cake aqueous extracts were prepared following standard protocol (Dhingani *et al*., 2013). 40gm of each organic material was suspending in 150 ml sterilized distilled water in a conical flask and kept aside 15 days. The flasks were shaken every day for thoroughly mixing and dissolution of the content. After 15 days, the extracts were first passed through four layers of muslin cloth and then filtered through Whitman's filter paper No.41 into 150 ml conical flasks and tightly wrapped with aluminum foil and autoclaved at 121°C at 15psi pressure for 20 minutes. The autoclaved extracts were individually added in previously sterilized molten potato dextrose agar medium at the concentration of 10% 15% and 20% at the time of pouring in petri plates and mixed thoroughly and allowed it to solidify. Each treatment was replicated thrice. After placing the 5mm discs of actively growing eight day old culture of *M. phaseolina* all the plates were incubated at 27±2°C temperature till the complete growth coverage in control plate. The colony diameter in each treatment was measured on the fifth day after inoculation and the percent growth inhibition (PGI) of the pathogen was recorded. The statistical analysis was subjected to one-way ANOVA.

RESULTS

Phytoextracts: The results presented in (Table 1) revealed that all the plant extracts at the concentration of 10 per cent inhibited the mycelial growth of the *M. phaseolina* in comparison to control, except *M. koenigii*, *P. hysterophorus*. The rhizome extract of *Z. officinale* (22.36mm), leaf extracts of *A. indica* (32.05mm) and *L. inerm*is (35.05mm) allowed minimum mycelial growth of the pathogen followed by leaf extract of *C. album* (42.35mm), bulb extract of *A. cepa* (63.12mm), and leaf extract of *P. betel* (74.19mm), whereas, the leaf extracts of *M. koenigii* (81.05mm) and *P. hysterophorus* (86.07mm) were least effective enabling maximum mycelial growth. At the minimal concentration strenght of 10% rhizome extract of *Z. officinale* (74.59%) produced maximum mycelial growth inhibition of the pathogen followed by leaf extracts of *A. indica* (63.57%), *L. inermis*

Table 1. Effect of various phytoextracts at the concentration of 10% on the growth of *M. phaseolina in vitro.*

Plant species	Average colony diameter of pathogen (mm)	Growth inhibition (%)
Z. officinale	22.36	74.59
A. indica	32.05	63.57
L. inermis	35.05	60.17
C. album	42.35	51.87
A. cepa	63.12	28.27
P. betel	74.19	15.69
M. koenigii	81.05	7.89
P. hysterophorus	86.07	2.19
Control	88.00	-
SEm±	0.80	
C. D. at 5%	2.42	
C.V. %	2.40	

Table 2. Effect of different oil cakes extracts on the growth of *M. phaseolina in vitro.*

Organic extracts	Concentration (%)	Average colony diameter of pathogen (mm)	Growth inhibition (%)
Neem cake	10	7.705 (58.40)*	29.60
	15	6.811 (45.39)	42.61
	20	6.049 (35.60)	52.40
Mustard cake	10	8.763 (75.80)	12.20
	15	8.509 (71.36)	16.64
	20	8.342 (68.58)	19.42
Control		9.434 (88.00)	-
SEm.±		0.73	
C. D. at 5%		1.03	
C.V. %		2.58	

* Figures in the parentheses indicates arc-sine transformed values.

(60.17%), *C. album* (51.87%), bulb extract of *A. cepa* (28.27%) and leaf extract of *P. betel* (15.69%). The leaf extracts of both *M. koenigii* (7.89%) and *P. hysterophorus* (2.19%) were least effective in inhibiting the mycelial growth of the pathogen.

Oil cakes: The extracts of two oil cakes at different concentration were evaluated for their inhibitory effect on *M. phaseolina*. The neem cake extract at the concentration of 20% allowed minimum growth (35.60mm) of the pathogen followed by neem cake extract 15% (45.39mm), neem cake extract 10% (58.40mm) and mustard cake extract 20% (68.58mm). On contrary, mustard cake extract at 15% concentration of (71.36mm) and 10% (75.80mm) also reduced the growth but not at par as neem cake extract at similar concentration (Table-2). The maximum mycelial growth inhibition (52.40%) was recorded with neem cake extract at the concentration of 20% followed by 42.61% and 29.60% with concentration of 15% and 10%, respectively. Likewise, the maximum mycelial growth inhibition (19.42%) was recorded with mustard cake extract at the concentration of 20% followed by 16.64% and 12.20% at the concentration of 15% and 10% respectively. In general mycelial growth inhibition was dose dependent and it was higher in case of neem cake extract than mustard cake extract.

DISCUSSION

The findings in the present studies are in corroboration with those described earlier by other workers viz., Datar (1999) who studied the fungicidal effect of botanicals on *M. phaseolina* and reported that of varied rhizome and bulb extracts evaluated, *A. sativum* and *A. cepa* were found most effective in inhibiting mycelial growth of *Rhizoctonia bataticola*. Jha *et al*. (2000) reported that among oil cakes tested, *B. juncea* cake exhibited maximum inhibition of mycelial growth (51.8%) at 5 per cent concentration against *M. phaseolina* causing root rot of okra. Kane *et al*. (2002) reported that crude extract of *A. sativum*, *Eucalyptus globulens* and *Z. officinale* were effective in inhibiting the mycelial growth of the *R. solani* to the extent of cent per cent. Mishra *et al*. (2005) evaluated 7 aqueous plant extracts (*Calotropis gigantea, Vinca rosea, Ocimum sanctum, A. indica, Eucalyptus citriodora, A. cepa* and *Z. officinale*) against *R. solani* in green gram *in vitro* and found that highest inhibitory action (86.11%) was recorded by *Z. officinale*. Tandel, *et al*. (2010) evaluated phytoextracts of eleven plant species against *M. phaseolina* of green gram and revealed that the onion bulb extract produced maximum inhibition (98.14%) followed by extract of acacia, ginger, neem, garlic and karanj. Rahiman *et al*. (2013) identified "lawsone" ($C_{10}H_6O_3$), a naturally-occurring naphthoquinone which is responsible for its fungicidal activity in *L. inermis*. This results is similar to the study investigated by Dhingani, *et al*., (2013). Several reports have stated the antifungal activity of plant extracts against many phytopathogenic fungi, Parveen *et al*. (2014). When results compared with other findings, it was found that, the *Z. officianale*, *A. indica*, *L. inermis* and *A. indica* cake extract supplemented with PDA medium did not allow the growth of the pathogen whereas the remaining plant extracts did not exhibit significant inhibition against *M.phaseolina* at same concentration (10%). Thus, it cannot be considered as a promising fungicide for control of *M. phaseolina*. From this present study it is clear that the antifungal activity of crude plant extracts at same concentration depend on presence of fungicidal components in each plant extract. While, in oil cake extracts, it were found to be dose dependent and effectively reduced the pathogen growth at higher

concentration of the extract as compared to lower concentration.

Conclusion

It is concluded that the aqueous leaf extracts and oil cake extracts of different plants like, *Z. officinale*, *A.indica*, *L. inerm*is and *A. indica* cake extracts were effective in reducing the growth of *M. phaseolina* causing stem rot disease in jute. Hence, these cost effective, eco-friendly natural products can be used as alternative to hazardous fungicides.

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