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In Vitro Studies on the Growth Inhibiting Potential of Some Botanical Extracts Against *Macrophomina phaseolina*

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ABSTRACT

Natural plant extracts are valuable source of numerous fungitoxic compounds that can substitute synthetic fungicides. In current studies, six plant extracts viz., *Zingiber officinalis*, *Eucalyptus camaldulensis*, *Azadirachta indica*, *Allium cepa*, *Cassia fistula* and *Allium sativum* in three dose levels standard dose (S.D), S/2 and S/3 were tested against colony growth of *Macrophomina phaseolina* under in vitro conditions. Among all the treatments ginger extract at its standard dose was found highly effective followed by eucalyptus and neem. Percentage colony growth inhibition of (50 %), (38 %) and (29 %) was recorded to produce by ginger, eucalyptus and ginger respectively as compared to control treatment where no growth inhibition was recorded. Extract of *Allium sativum* was found least effective phytochemical where pathogenic fungal growth inhibition was (24.5 %). Concentration comparison of three dose levels showed that (S.D) was highly effective where mean colony growth of *M. phaseolina* was (3.72 cm) followed by (4 cm) @ S/2 and maximum diameter was (4.36 cm) @ S/3 concentration.

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INTRODUCTION

Macrophomina phaseolina belongs to the subdivision "Deuteromycota" and class "Coelomycetes" (Wheeler 1975) is a fungal factor of prime importance. It is alarming threat to the crops in arid region of the world (Hoes 1985). This pathogen is reported (Mayek-Perez *et al*., 2002) to infect a wide range of major crops including, common bean, maize, sorghum, soybean, sesame and cotton while 67 host species of this pathogen have been recorded from Pakistan (Shehzad *et al*., 1988). It is non-host specific fungus. High degree of variability in morphology and physiology of the fungus is found when isolated from different parts of the same plant by Dhingra and Sinclair (1973). Fungus is soil and seed borne. Black color sclerotial bodies are the primary mean of fungal survival according to Kaisar *et al*. (1988).

Pathogen as reported by Gaetan *et al*. (2006) produces pycnidia only under specific environmental conditions such as low moisture level and high temperature. Fungus has ability to survive in dry soil condition for more than 10 months and the disease severity caused by this fungus directly relates with the availability of viable sclerotial bodies in soil and mycillial bodies are not primary source on infection (Meyer *et al*., 1974). Various cultural, regulatory, physical, chemical, and biological measures were purposed to handle the disease, but all were effective when applied as precautionary measures (Kata, 2000). Fungicide applications are the solution to control

such type of fungal pathogens according to Rauf (2000). Chemical control is very effective but most of these chemicals produce harmful effects on human health. Moreover, these chemicals are very expensive, and their continuous use induce resistance in target pathogen against that chemical (Akhtar and Siddiqui, 2008). Due to increasing cost of chemicals and environmental complications, importance should be given to biological control (Agrios, 2005). Green plants are important source of natural pesticides and reservoir of chemo therapeutants (Mahajan and Das, 2003). The data about the plant by-products possessing antimicrobial effects against several pathogens is available (Dorman and Deans, 2000; Parameshwari and Latha, 2001; Bylka *et al*., 2004; Shimpi and Bendre, 2005; Kilani, 2006). Recently, many studies have been done on plant derived compounds as these are alternative to fungicides and are also environmentally safe to use (Bajwa *et al*., 2001). In present studies, anti-fungal potential of selected plant extracts in three concentrations viz., S.D, S/2 and S/3 were evaluated against test fungus under aseptic condition and data on colony growth reduction was recorded.

METHODS AND MATERIAL

Isolation of *Macrophomina phaseolina*

Mung bean plants with characteristic symptoms of charcoal rot were collected from Auyb Agriculture Research Institute, Faisalabad. Infected samples were put in polythene bags and were preserved at 4 °C until further use. Samples were cut into small pieces of 2-3 cm length, surface sterilized with 1% $Hgcl_2$ for 2-3 minutes and washed thoroughly with distilled water. These pieces were placed on Petri dishes containing Potato Dextrose Agar (PDA) medium (20 mL/dish). Plates were incubated at 28 °C for 5-7 days. Pathogen was identified based on colony characters, microscopy, and consulting with relevant literature (Mukadam *et al*., 2006).

Preparation of plant extracts

Modified method (Okigbo *et al*., 2009) was utilized to prepare crude botanical extracts of collected plants (Safaida, Neem, Amltas, Onion, Garlic and Ginger). Fresh plant parts (leaves, rhizome and bulbs) were washed thoroughly with tap water, surface sterilized with 1 % solution of sodium hypochlorite and rinsed twice with distilled water. Extracts were prepared by macerating 75 g of leaves or fruits in 25 mL of distilled water. After grinding, the extracts were passed separately through three folds of maslin cloth for filtration and through Whatman No.1 filter paper (Shafique *et al*., 2005). This concentration was considered as (S.D) standard dose (Ilyas *et al*., 1997) and from these different concentrations viz., S/2 and S/3 were prepared.

Evaluation of botanical extracts against *M. phaseolina*

Efficacy of botanical extracts in various concentrations was studied against *M. phaseolina* in dual culture method. Both pathogen and extracts were applied opposite to each other in Petri dishes having solidified PDA media. Three replications of nine Petri plates were maintained. Plates in control contain pathogen and distilled water. These plates were incubated at 27±2 °C and data about colony growth inhibition was recorded after 72, 96 and 120 hours.

Statistical analysis

Data to the experiments was analyzed according to statistical procedure. Least Significant Differences (LSD) test was used to compare the mean values (Steel *et al*., 1997). Data analysis was done through SAS software (SAS, 1990).

RESULTS

Plant extracts of Ginger (*Zingiber officinalis*), Safaida (*Eucalyptus camaldulensis*), Neem (*Azadirachta indica*), Onion (*Allium cepa*), Amaltas (*Cassia fistula*) and Garlic (*Allium sativum*) were evaluated in three concentrations viz., standard dose (S.D), S/2 and S/3. Colony growth inhibition of *M. phaseolina* was recorded after 72, 96 and 120 hours of treatment application. Ginger extracts (*Zingiber officinalis*) in its highest concentration were found highly effective followed by safaida (*Eucalyptus camaldulensis*) and neem (*Azadirachta indica*) as compared to control where there was no application of extracts. While among all the treatments, garlic extract (*Allium sativum*) in all its concentrations was found least effective phytochemical. Results are given in (Figure 1).

Data on percentage colony growth inhibition was recorded after 72, 96 and 120 hours. It was observed that among all the tested plant extracts ginger was found highly effective in percentage colony growth inhibition. Maximum percentage colony growth inhibition after 120 hours of treatment application was recorded (50 %) in *Zingiber officinalis* treatment followed by *Eucalyptus camaldulensis* (38 %) and *Azadirachta indica* (29 %) as compared to control where no colony growth inhibition

was recorded. Extract of *Allium sativum* was found least effective where colony growth inhibition of *M. phaseolina* was only (24.5 %). Percentage colony growth inhibition is given in (Figure 2).

Concentration comparison of liquid botanical extracts has shown in (Figure 3). Data shows that all the plant extracts in their standard concentrations (S.D) were found significantly effective against the mycelial growth of *M. phaseolina* followed by S/2 concentration. While results of S/3 were very much closer to the control plates in which only distilled water was added against pathogenic fungi. Mean fungal colony growth @ standard concentration was (3.72 cm) followed by (4 cm) @ S/2 and maximum diameter was (4.36 cm) @ S/3 concentration.

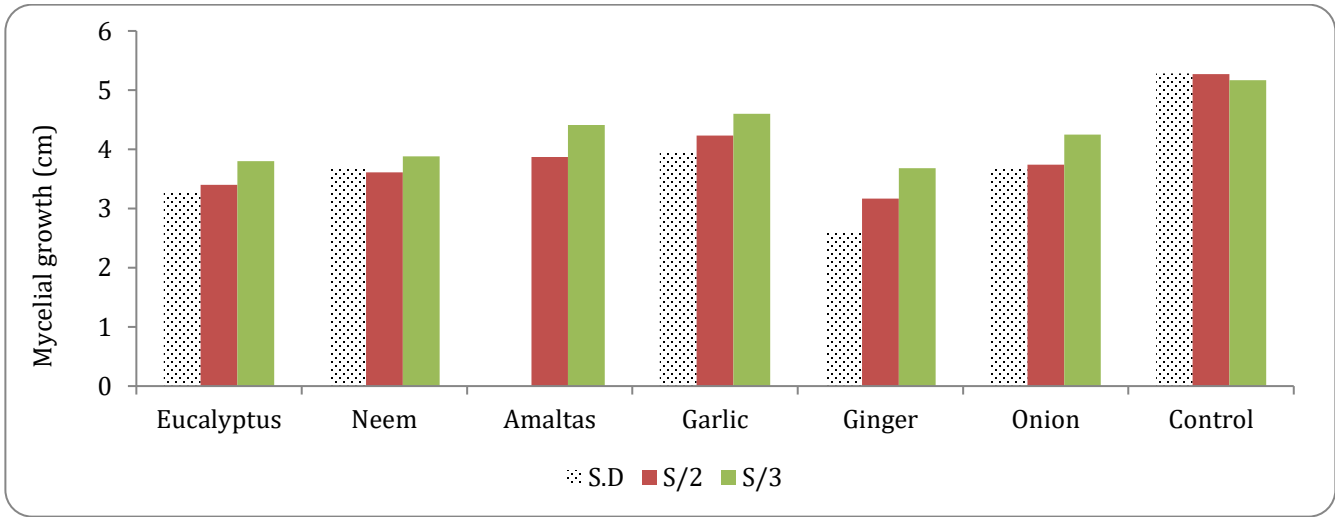

Figure 1. Comparative effect of Extract * Concentration on colony growth reduction.

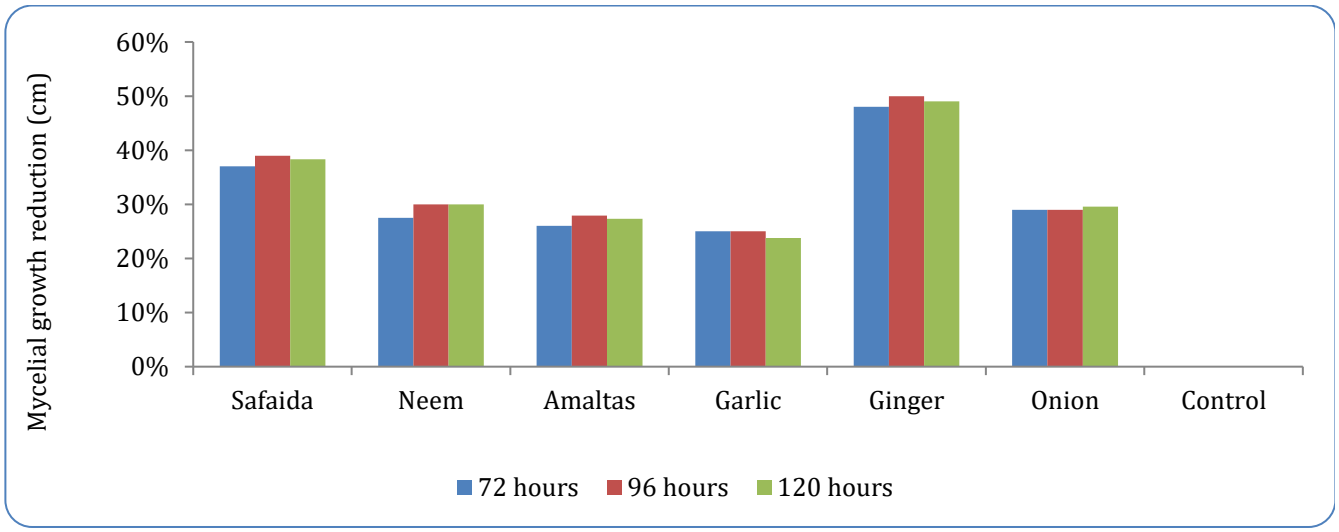

Figure 2. Comparative effect of Extract * Hours on growth reduction of *M. phaseolina*.

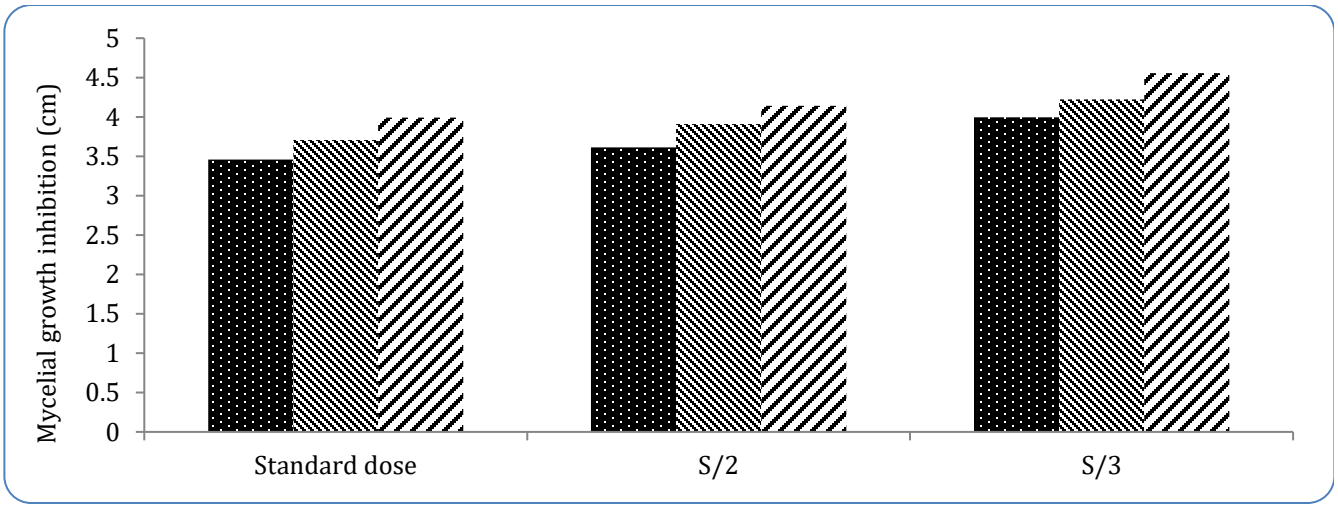

Figure 3. Comparison of concentrations of botanical extracts against *M. phaseolina.*

Data of concentration comparison recorded after 48, 72 and 96 hours has revealed that standard doses (S.D) of plant extracts were effective against the pathogenic fungi. S/3 doses were statistically least effective in inhibiting the fungal growth. Standard doses were recorded efficient because these contain active ingredients in higher amount as compared to other concentrations. Fungal colony growth was 3.45, 3.70 and 3.99 cm in standard doses which is significantly lower than mycelial growth of pathogenic fungus in S/3 that was 3.99, 4.22 and 4.55 cm, respectively.

DISCUSSION

In present study six plant extracts viz., *Zingiber officinalis*, *Eucalyptus camaldulensis*, *Azadirachta indica*, *Allium cepa*, *Cassia fistula* and *Allium sativum* in three concentrations (standard dose, S/2 and S/3) were tested to study their anti-fungal activities against rotting fungi *M. phaseolina* under *in-vitro* conditions. Data recorded have shown that ginger extract in all its three concentrations was found significantly effective in controlling the mycelial growth of *M. phaseolina* while liquid extract of garlic (bulb) was found least effective against fungi.

Maximum inhibition of the fungus is observed at highest concentration level of extracts and results are supported by the findings of Onwuliri and Wonang (2005) who reported *Z. officinale* and *A. sativum* even at low concentration possess antibacterial properties against certain bacteria. However, Shelef (1983) found that among all the applied botanical treatments Cinnamon and Clove extracts were found highly effective while extracts of black pepper, red chilies and ginger showed least effective anti-microbial activity whereas in present study ginger in all its concentrations was found highly effective in suppressing the colony growth of *M. phaseolina*. Dawar *et al*. (2007) examined that growth of *Rhizoctonia solani*, *Fussarium solani* and *M. phaseolina* was significantly controlled @ 5 % w/v extract of *Eucalyptus* sp. In our studies, eucalyptus extract was found less effective than ginger extract against *M. phaseolina*. Inouye *et al*. (2001) reported that essential oils of *Euclayptus* possess remarkable antiseptic potential against various bacterial, viral, and fungal infections. Abid *et al*. (1992) concluded that among various organic substances, neem cake was found much effective against the growth of root infecting fungi. Results of present study are in accordance with the findings of Lawson (1996) who reported about strong anti-microbial efficacy of ginger extract in controlling the mycotoxin producing ability of *Aspergillus parasiticus* due to the presence of "gingerol" compound in ginger. It is the need of the time to develop plant extract-based formulations which are safe to human health and environmentally safe in controlling the root infecting fungi.

CONCLUSIONS

It was concluded that among all the tested plant extracts, ginger extract in all its three concentrations was found highly effective in controlling *M. phaseolina* while extract of garlic was found least effective.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.