ASSESSMENT OF ANTIFUNGAL POTENTIALS OF SOME AQUEOUS PLANT EXTRACTS AND FUNGICIDES AGAINST *ALTERNARIA ALTERNATA*

Waheed Anwar, Muhammad Saleem Haider*, Muhammad Aslam, Muhammad Shahbaz**, Salik Nawaz Khan and Ayesha Bibi*

ABSTRACT

Present study was carried out in Institute of Agricultural Sciences, Punjab University, Lahore during 2010. Efficacy of aqueous leaf extracts of four allelopathic tree species i.e. *Azadirachta indica* (L.), *Datura alba* (L), *Eucalyptus* sp. (L) and *Melia azedarach* (L)) and different fungicides (halonil, acrobat and mancozeb) were studied against *Alternaria alternata* (Fr.) causal agent of leaf spot disease by using disc plate method. The fungus was exposed to different concentrations (1, 2, 3 & 4% w/v) of plant extracts and tested fungicides in the followed doses 20, 40 and 60 ppm. The growth of *A. alternata* was highly inhibited after the use of 4% extracts of *A. indica* and *M. azedarach* where respectively an inhibition rate of 29.14 and 29.1% was observed. *A. indica* extracts which are cheap and environmentally safe, exhibited considerable efficacy in the control of *A. alternata* may be considered as promising outlet in plant protection against this widespread pathogen.

Keywords: Allelopathic trees; *Alternaria alternata* (Fr,); plant extract; growth inhibition; Lahore; Pakistan.

INTRODUCTION

The genus alternaria contains one of the best known and economically important fungi i.e. *Alternaria alternata*. Diverse and ubiquitous species of fungi including acute and opportunistic plant pathogens affecting the majority of cultivated plants are the peculiarities of this group. *A. alternata* is the causal agent of late blight on pistachios (*Pistacia vera*) and is distributed throughout the pistachio growing areas of California. In early summer the latent infection occurs on leaves and fruits, which becomes severe in late July and early August when fruit development and maturity takes place (17).

***Institute of Agricultural Sciences, University of the Punjab, Lahore, **Directorate General Pest Warning and Quality Control of Pesticides Punjab, Pakistan.**

Severe defoliation, deterioration of nut quality, stained shells and appearances of infected kernels in early splits are the disease symptoms, under favorable conditions, which also reduce the marketable yields (12).

The surplus use of agrochemicals gives rise to unenviable biological effects on animals and human beings (19) because these synthetic fungicides pose more carcinogenic risk than other pesticides (3). Therefore, in the recent years the development of bio-pesticides has been focused as a viable pest control strategy. Natural products of plant origin are considered as one of the potential source for new pesticides. Antifungal activity of essential oils and plant extracts has been observed against a vast range of fungi (1, 23, 13).

It has been efficiently proved that blight diseases could be economically controlled through chemicals. Hence the people are becoming more concious about the environmental issues, there is a dire need for the development of alternative management system either to reduce dependence on pesticides or to encumber the pathogen for which naturally occurring chemical compounds should be explored (20, 11). Natural compounds derived from plants contribute a lot to fight against pathogens (22). Antifungal and cytotoxic properties have been observed in several plant families, which include acanthaceae, amranthaceae, apiaceae and magnoliaceae (16, 18).

Various studies done in Pakistan brought out broad spectrum prospects of using plant extracts as biological control agents against plant pathogenic fungi (5, 8, 2, 9). Examination of various plants extracts e.g. *Parthenium hysterophorus* (7), *Cicer arietimum* (6) and *Magnolia grandiflora* (2) etc. has been done in order to find out environmentally safe alternatives for controlling plant diseases.

Present study was designed to investigate the antifungal activity of aqueous leaf extract of *Melia azedarach* L., *Azadirachta indica* (L.), *Datura alba* (L)*, Eucalyptus* sp. (L) and chemicals fungicides acrobat, halonil and mancozeb against *Alternaria alternata* (Fr).

MATERIALS AND METHODS

Procurement and maintenance of target fungal species

Culture of *Alternaria alternata* was obtained from FCBP (first fungal culture bank of Pakistan), Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus Lahore and maintained on malt extract agar (MEA) medium.

Collection of plant materials

Healthy and fresh leaves of allelopathic tree species *Eucalyptus* sp., *Melia azedarach*, *Azadirachta indica* and *Datura alba* were collected from the premises of botanical garden of University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan in March, 2010. Leaves were thoroughly washed with detergent to remove dust. Washed leaves were dried in an electric oven at 30°C for 72 hours and crushed to make powder. Similarly fungicides acrobat, halonil and mancozeb (100%), were obtained from, Directorate General of Pest Warning and Quality Control of Pesticides, Punjab, Lahore and used for solution preparation.

Preparation of aqueous extract

Twenty grams of dried leaf powder of each of the test tree species were soaked for 24 hours in 100 ml of distilled sterilized water. A double layered muslin cloth with whatman No. 1 filter paper was used in the further experiment immediately for the filtration of the extract. Similarly, doses of 20, 40 and 60 ppm of Acrobat, Halonil and Mancozeb solutions were prepared by dissolving 0.3, 0.4, 0.6 µg of each fungicide in 1000 ml of water.

Aqueous extract bioassay

Sterilization of malt extract agar was done by autoclaving at 121°C. Eighty ml of malt extract media was poured in 100 ml plates and was allowed to cool at room temperature. Final volume of each plate was raised up to 100 ml by adding appropriate amount of stock solution and distilled water in order to make 5, 10 and 15% (w/v) media. Twenty ml of distilled water was added in the control medium to make its final volume up to 100 ml. Similarly 3 ml of each concentration of halonil, acrobat and mancozeb were added in the respective plates. Actively growing discs of *A. alternata* were prepared by using a presterilized cork borer having 5mm diameter and were then transferred to presterilized flasks aseptically. Flasks were incubated at 25°C for 7 days in an incubator. Each treatment was in triplicate. After seven days, diameter of fungal colony in each plate was measured. Percentage reduction in fungal growth was recorded after seven days by estimating the colony size of fungus.

Treatments

T_1 = control	T_2 = 01 % aqueous extract of *Melia azedarach* (L.)
T_3 = 02 % Aqueous extract of *Melia azedarach* (L.)	T_4 = 03 % aqueous extract *Melia azedarach* (L.)
T_5 = 04 % aqueous extract of *Melia azedarach* (L.)	T_6 = 01 % aqueous extract of *Azadirachta indica* (L.)
T_7 = 02 % aqueous extract of *Azadirachta indica* (L.)	T_9 = 04 % aqueous extract of *Azadirachta indica* (L.)
T_8 = 03 % aqueous extract *Azadirachta indica* (L.)	T_{10} = 01 % aqueous extract of *Datura alba* (L.)
T_{11} = 02 % aqueous extract of *Datura alba* (L.)	T_{12} = 03 % aqueous extract *Datura alba* (L.)
T_{13} = 04 % aqueous extract of *Datura alba* (L.)	T_{14} = 01 % aqueous extract of *Eucalyptus* (L.)
T_{15} = 02 % aqueous extract of *Eucalyptus* (L.)	T_{16} = 03 % aqueous extract *Eucalyptus* (L.)
T_{17} = 04 % aqueous extract of *Eucalyptus* (L.)	T_{18} = 20 ppm halonil
T_{19} = 40 ppm halonil	T_{20} = 60 ppm halonil
T_{21} = 20 ppm acrobat	T_{22} = 40 ppm acrobat
T_{23} = 60 ppm acrobat	T_{24} = 20 ppm mancozeb
T_{25} = 40 ppm mancozeb	T_{26} = 60 ppm mancozeb

Data Collection and Statistical Analysis

The experiment was done by using CRD. Computer software Microsoft Excel was used to compute the standard errors of means of three replicates. All the results were subjected to ANOVA followed by mean separation through Duncan's multiple range test (21) using computer software CO-STAT**.**

RESULTS AND DISCUSSION

All plant products significantly reduced the fungal growth as against control treatment (T_1) (Fig 1)**.** Among these T_5 reduced growth 29.1%, T_9 inhibited growth to 29.14%, T_{13} caused 9.41% growth inhibition and T_{17} inhibit 7.17 % growth of *Alternaria alternata*. They have showed a significant decrease in the colony growth of fungi. Statistically analyzed results clearly indicated the moderate fungi toxicity of *A. indica* and *M. azedarach* extract to control mycelia growth of *A. alternata*. It has also been studied earlier that the plant extracts, viz *Calotropis procera*, *Eucalyptus globulens*, *Jatropha multifida Azadirachta indica, Allium sativum* were significantly pronounced in reducing mycelia growth of *A. alternata* L. (10). Similar antifungal properties of ethanolic leaf extracts of *M. azedarach* against *Rhizoctonia bataticola, Fusarium chlamydosporum, Alternaria alternata*, *Trichoderma viride* and *Aspergillus niger* have recently been reported by Aqil and Ahmad (2003). Recently Hasan *et al.,* (15) reported that aqueous extracts of bulb of *Allium sativum, A. cepa,* rhizome of *Zingiber officinale* leaves of *Adhatoda vestica, Lawsonia alba, Azadirachta indica, Achyranthes aspera,* stem of *Cuscuta reflexa,* root of *Vicia rosea* and seeds of *Nigella sativa* significantly reduced the incidence of seed-borne fungi of wheat viz., *Bipolaris sorokiniana*, *Fusarium* sp., *Aspergillus* sp.*, Penicillium* sp and *Rhizopus* sp.

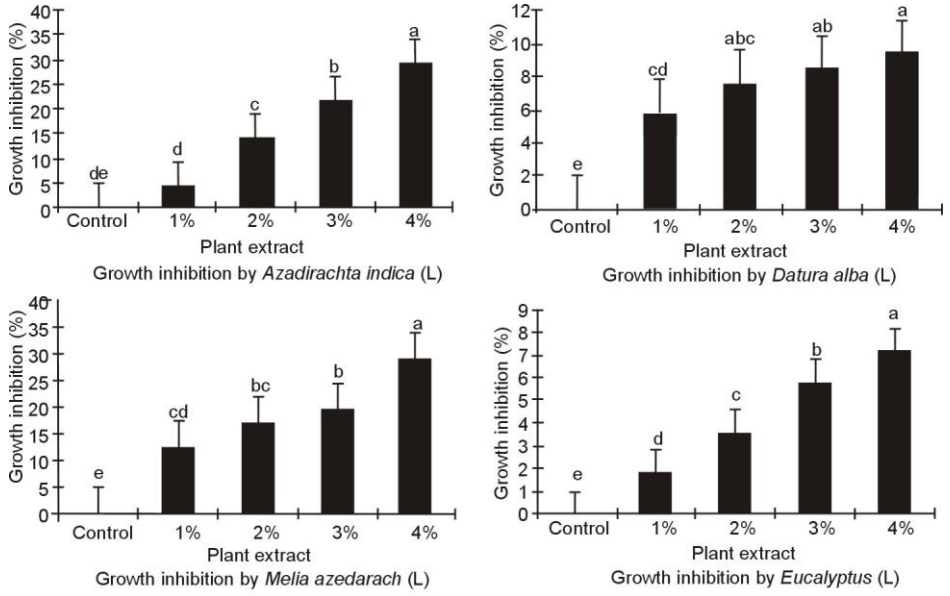

Fig. 1. Evaluation of *Alternaria alternata* growth inhibition by different plant extracts.

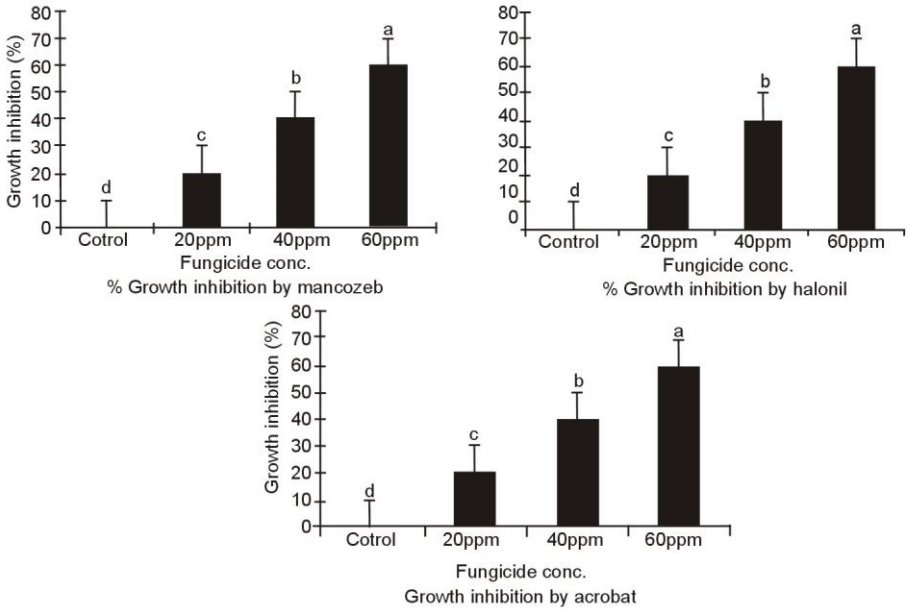

Fig. 2. Evaluation of *Alternaria alternata* growth inhibition by different fungicides.

Analysis of variance shows that the effect of different doses of tested chemicals fungicides (halonil, acrobat and mancozeb), significantly reduced the fungal growth. At higher concentrations i.e. 60 ppm halonil was the most

effective fungicide inhibiting 83.8 % of the fungal growth among the three synthetic products used. When comparing data obtained in this study, plant extracts showed the same percentage of inhibition rate with lower concentrations of chemical fungicides. This signifies the importance of aqueous extract plants as potential agent to be manipulated for biological control of *A. alternata* as they are equally effective as fungicide. Biological method of disease control should be preferred if specific formulation is effective against the pathogen. Gondal *et al.,* (14) found that different concentration of mancozeb affects the growth of Alternaria sp. Further studies are required to observe the exact effect of different fungicides on different isolates causing leave spot on different plants.

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