*Pak. J. Bot.*, 42(4): 2911-2919, 2010.

# *IN VITRO* ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANT DIFFUSATES AGAINST *ALTERNARIA SOLANI, RHIZOCTONIA SOLANI* AND *MACROPHOMINA PHASEOLINA*

**AQSA ASLAM[1], FARAH NAZ[2], MUHAMMAD ARSHAD[1], RAHMATULLAH QURESHI[1*] AND C.A. RAUF[2]**

*[1]Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
*[2]Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*

**Abstract**

The present paper reports the antifungal activity of plant diffusates from 5 indigenous medicinal plant species of Potohar region viz., *Adhatoda zeylanica, Azadirachta indica, Capparis decidua, Dodonaea viscosa* and *Salvadora oleoides*. Antifungal activity was tested against 3 pathogens attacking commercial crops viz., *Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina*. All selected medicinal plants exhibited considerable distinction in radial mycelial growth of tested pathogens. Overall, *Dodonaea viscosa* appeared significantly the most effective and suppressed the radial mycelial growth of the *Alternaria solani* and *Rhizoctonia solani*, whereas, *Adhatoda zeylanica* exhibited maximum inhibition (77.44%) against *Macrophomina phaseolina*. However, *Salvadora oleoides* exhibited minimum inhibition against all tested pathogens. It was also observed that radial mycelial growth of selected pathogens reduced at an increase of plant diffusates concentration. Among 5 concentrations of plant diffusates, the highest inhibition in radial mycelial growth of all 3 pathogens was observed at 100 and 200g/l respectively, as compared to control, while minimum inhibition was recorded at 10g/l in all plant diffusates. It may be concluded from the present investigation that *Dodonaea viscosa* can be utilized for the management of fungal diseases caused by *Alternaria solani*, *Macrophomina phaseolina* and *Rhizoctonia solani*.

## Introduction

Biological screening of plant extracts is carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using diffusates from different plants (Gerresten & Haagsma, 1951; Kumar *et al*., 1979; Naidu & John, 1981).

Many studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which showed antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antimicrobial potential (Mothana & Lindequist, 2005; Bajpai *et al*., 2005; Wojdylo *et al*., 2007).

The trend towards the environmental friendly pesticides with alarming levels of pest resistance to commonly used pesticides has led to search new antimicrobial agents from various sources including medicinal plants. The major characteristics of such biopesticides are that they should have minimal toxic effects to human and other organisms, rapid degradation and often a narrow spectrum of the activity (Loper *et al*., 1991).

*Corresponding author: rahmatullahq@yahoo.com

*Alternaria* genus is cosmopolitan in occurrence. The members of this species like *A. alternata, A. solani, A. porri, A. dauci, A. helianthi, A. carthami* and *A. macrospore* causing different diseases in their respective hosts (Rotem, 1998). Among them, *A. solani* causing early blight of potato (*Solanum tuberosum)* and tomato (*Lycopersicon esculentum)* is the most destructive (Reni & Roeland**,** 2006) of field crops. It causes diseases on foliage (blight), basal stems of seedlings (collar rot and damping off), stems of adult plants (stem lesions), fruits (fruit rot) of tomato and may also infect egg plant and pepper. This disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants (Dater & Mayee, 1985).

*Macrophomina phaseolina* causing charcoal rot is cosmopolitan in distribution and is potential threat to crop production in arid regions (Hoes, 1985). It is soil inhabiting funguses, an important root pathogen and causes dry root rot / stem canker, stalk rot or charcoal rot of over 400 plant species (Mahrshi, 1986). It has a wide host range and is responsible for causing losses of more than 500 cultivated and wild plant species (Indera *et al*., 1986). So far in Pakistan, 67 economic hosts of *M. phaseolina* including cotton, rice, maize, cucurbits, okra, and wheat have been reported (Mirza & Qureshi, 1978; Shehzad *et al*., 1988). Wide host range of *M. phaseolina* suggested that it is non-host specific fungus. Charcoal rot is of great economic importance in arid areas of the world and reported to be the major limiting factor for sunflower production. It caused decrease in yield upto 90% in Pakistan (Mirza, 1984).

*Rhizoctonia solani,* an important destructive soil borne pathogen has detrimental effects on agricultural and horticultural crops by pre-emergence and post-emergence damping-off, root rot, and stem canker (Khouray & Alcorn, 1973). There are over five hundred hosts in United States of America alone (Farr *et al*., 1989). Its host plants included alfalfa, peanut, soybean, lima bean, cucumber, papaya, eggplant, corn and many more. It is known to cause a large number of distinct diseases on a wide variety of plants (Sinclair & Backman, 1989). It can affect potato plants from planting to harvest by inhibition in eyes germination, killing of underground sprouts, stem canker and stolon canker resulting in subsequent yield reduction (Banville, 1989).

The most common method for controlling these pathogens is the use of fungicides but the development of resistance in pathogenic fungi to common fungicides and increasing residual hazardous effects on human health and environmental pollution has given a thrust to search for new plant derivatives that can obstruct the fungal pathogencity. Use of natural products for the management of fungal diseases in the plants is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment. Many higher plants and their constituents have been successful in plant disease control and proved to be safe and nonphytotoxic; unlike chemical fungicides. Three weedy plants namely *Lantana camara* and *Capparis decidua* has been used for this purpose (Sharma & Kumar, 2009).

Plant diffusates are not only easy to prepare but are also non-polluting and low-priced as compare to commercial fungicides. Keeping in view, 5 plant diffusates were evaluated for their antifungal activity against *Alternaria solani*, *Macrophomina phasiolina, Rhizoctonia solani*. These selected pathogens cause yield losses in numerous economically important crops during vegetative growth. The objective of present study was to determine the *In Vitro* antifungal activity of selected medicinal plant diffusates against *Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina*.

## Materials and Methods

This work was conducted in Department of Plant Pathology and Botany, PMAS-Arid Agriculture University Rawalpindi during 2008-09 to determine the antifungal activity of *Azadirachta indica, Capparis decidua, Adhatoda zeylanica, Dodonaea viscosa* and *Salvadora oleoides* against three selected fungal pathogens viz., *Rhizoctonia solani, Macrophomina phasiolina* and *Alternaria solani* in water and ethanol by employing food poisoning technique (Naz *et al*., 2006).

**Isolation of pathogens:** Three pathogens viz., *Rhizoctonia solani, Macrophomina phaseolina* and *Alternaria solani* were selected for this experimental work. Pure culture of *Macrophomina phasiolina* was brought from Crop Disease Research Program (CDRP) Mycology Lab., National Agriculture Research Centre (NARC) while other two pathogens were isolated from infected potato and tomato, with visible symptoms of black scurf of potato and early blight of tomato respectively. Diseased samples were surface sterilized with 5% Chlorox for one minute and washed three times with sterilized distilled water.

**Preparation of pure culture:** Pathogens were isolated with the help of sterilized forceps and plated on sterilized potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g and distilled water to make the volume 1 liter, which was sterilized in a gas operated autoclave at 15 pounds pressure per square inch (PSI) for 20 minutes. Plates were incubated at 25ºC and observed daily for emergence of colonies. Sub-culturing was done from single spore to obtain pure culture.

**Collection and preservation of plants samples:** Fresh leaves of *Azadirachta indica, Capparis decidua, Adhatoda zeylanica, Dodonaea viscosa, Salvadora oleoides* were collected from Pind Dadan Khan. These were washed with tap water and air dried for one day to eliminate surface moisture. Then leaves were packed into envelop and kept in oven at 60°C temperature until dried. Dried leaves were grinded separately in an eclectic grinder to obtain powder which was than kept in plastic bags for further use.

**Preparation of extracts:** Hundred gram of the dried powdered plant were soaked separately in 500ml of 98% ethanol. These mixtures were refluxed followed by agitation at 200 rpm (revolution per minute) for 1 hour. The ethanolic extracts were squeezed and then filtered by muslin cloth. The extracts were placed into a wide tray to evaporate ethanol and added with water to make plant extracts (Rahber, 1986).

**Food poison technique:** Diffusates were added in Potato dextrose agar (PDA) @ 10, 50, 100 and 200 g $L^{-1}$ and poured into Petri dishes. PDA medium added only with ethanol and water served as control. Each Petri dish was inoculated with 5 mm plug of pure isolate taken from margins of actively growing culture of pathogen. Then Petri plates were incubated at 25° ± 2°C.

Mycelial growth was recorded when the growth of three selected pathogens were completed in the control treatment. Each treatment was repeated five times. Mean radial mycelial growth of each plant diffusates was recorded and data were subjected to statistical analysis. Radial mycelial growths on different diffusates were transformed into inhibition percentage by using the following formula (Naz *et al*., 2006):

$$\text{Inhibition percentage} = 100 - \frac{\text{Mycelial growth on diffusates}}{\text{Mycelial growth on control}} \text{ X } 100$$

**Statistical analysis:** Data regarding two parameters (viz., concentration, pathogen and medicinal plants) were taken following the procedure and analyzed statistically using MSTATC program with completely randomized design (CRD). Inhibition of radial mycelial growth was examined using analysis of variance (ANOVA) and means were separated by the test of least significant difference (LSD 0.05). Results helped in finding out the best treatment for disease management (Naz *et al*., 2006).

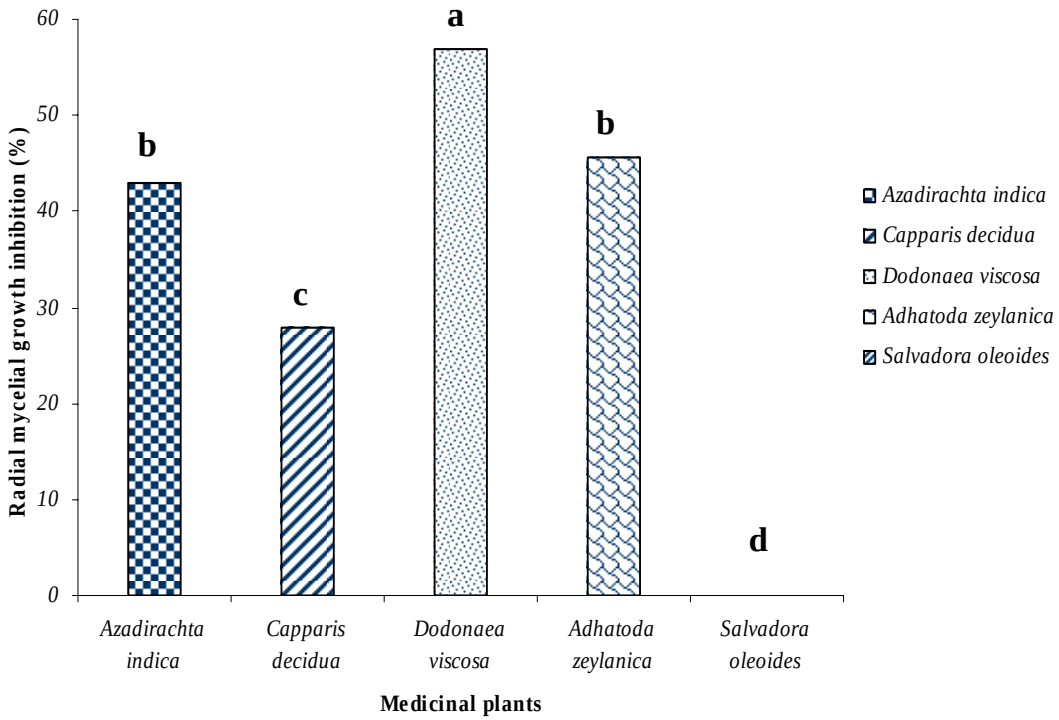
## Results and Discussion

**Inhibition in radial mycelial growth (rmg) of *Alternaria solani* induced by five medicinal plants:** Highest Inhibition in radial mycelial growth % induced by *Alternaria solani* was exhibited b*y Dodonaea viscosa* (56.96%) followed by *Adhatoda zeylanica* (45.59%), *Azadirachta indica* (42.90%), *Capparis decidua* (27.87%) and *Salvadora oleoides* (01%) irrespective of the solvents (Fig. 1). Statistically, there was no significant difference found in inhibition of radial mycelial growth between *Adhatoda zeylanica* (45.59%) and *Azadirachta indica* (42.90%), whereas significant difference in inhibition of radial mycelial growth was found between *Dodonaea viscosa* (56.96%) and *Adhatoda zeylanica* (45.59%). While no inhibition in radial mycelial growth was exhibited by *Salvadora oleoides* (Fig. 1). Results were found cognizant with previous findings of Hassanein *et al*., (2008) that the extract obtained from *Azadirachta indica* completely suppressed the growth of *Alternaria solani* on PDA *In vitro.*

Statistically, significant increase in radial mycelial growth of *Alternaria solani* was observed with an increase in concentration of the diffusates (Fig. 2). Among 5 concentrations, the highest inhibition in radial mycelial growth was 55.72% at 200g/l followed by 48.60% at 100g/l, 44.51 at 50g/l and lowest inhibition was recorded 24.49 at 10g/l as compared to control. Similar results were recorded from preliminary investigations by Hassanein *et al*., (2008) reporting antifungal activity of *Azadirachta indica* leaf extract against *Alternaria solani*.

**Effect of different plant diffusates on radial mycelial growth of *Macrophomina phaseolina*:** Maximum inhibition in radial mycelial growth % induced by *Macrophomina phasiolina* was exhibited b*y Dodonaea viscosa* (52.06%) followed by *Adhatoda zeylanica* (25.09%), *Salvadora oleoides* (17.92%), *Azadirachta indica* and *Capparis decidua* (0.01%) irrespective of the solvents (Fig. 3). Statistically, there was no significant difference in inhibition of radial mycelial growth between *Adhatoda zeylanica* and *Salvadora oleoides*, whereas significant difference in inhibition of radial mycelial growth was observed between *Dodonaea viscosa* and *Adhatoda zeylanica*. No inhibition in radial mycelial growth was recorded in *Azadirachta indica* and *Capparis decidua*.

Rate of increase in radial mycelial growth inhibition was regressed against 5 different concentrations of plant diffusates. Linear equation was used for the analysis. The equations for the fitted lines are presented along with original data (Fig. 4). Among 5 concentrations, the highest inhibition in radial mycelial growth was 27.85% at 200g/l followed by 26.64% at 100g/l, 21.15% at 50g/L and the lowest inhibition was recorded 20.30% at 10g/l as compared to control. Previously, Fabry *et al*., (1996) reported that different concentrations of plant extracts had different effects on radial mycelial growth of the pathogen. The present findings correlates with the findings of Farooq (2002), who evaluated the effects of different concentrations of plant extract of *Achillea millefolium* on linear growth of *Macrophomina phaseolina*. His results also legitimated our findings that the growth rate of pathogen decreases by increasing the concentration of plant extract.




* Means followed by the same letters are not significantly different according to LSD test (p=0.05) LSD = 6.62

Fig. 1. Inhibition in radial mycelial growth induced by five different medicinal plants against *Alternaria solani*.

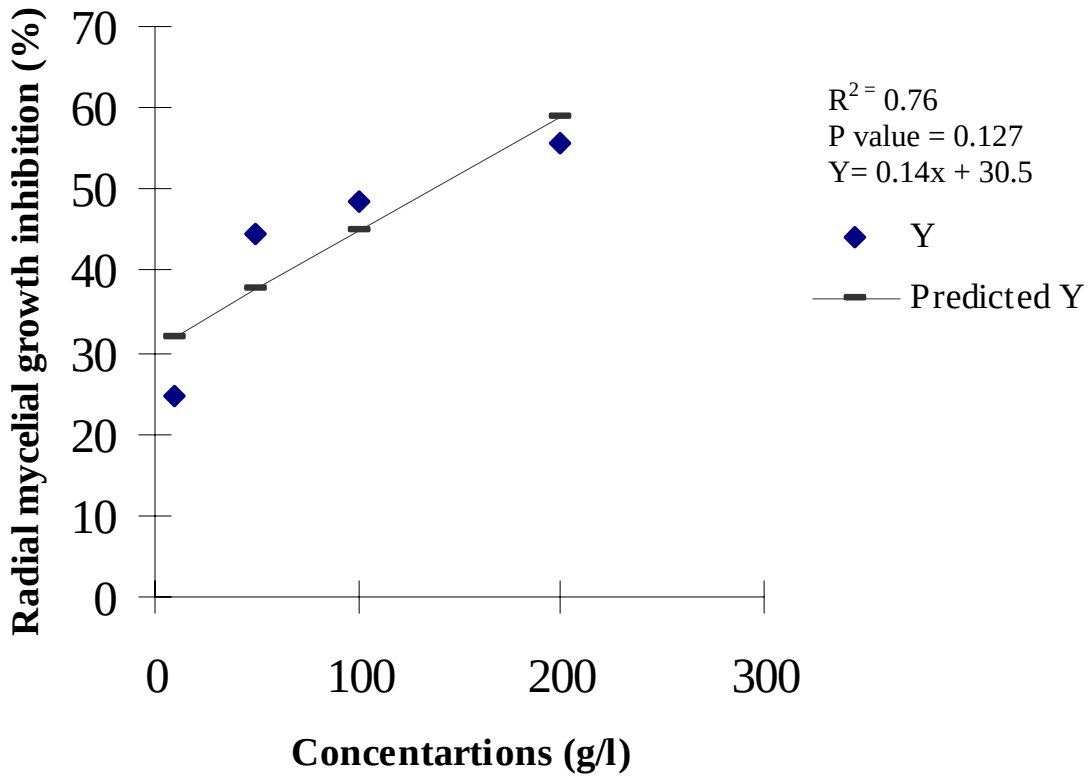



Fig. 2. Effect of different concentrations of some medicinal plants extract on inhibition of radial mycelial growth of *Alternaria solani*.

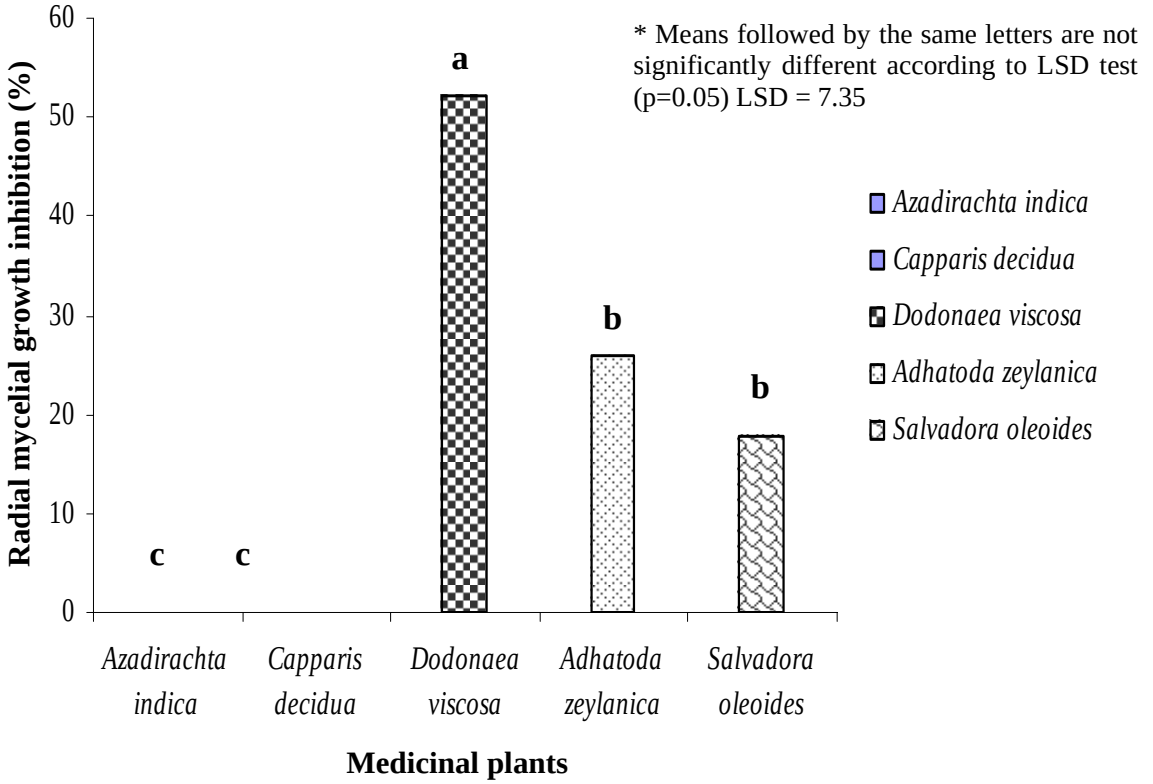



Fig. 3. Inhibition in radial mycelial growth induced by five different medicinal plants against *Macrophomina phasiolina*.

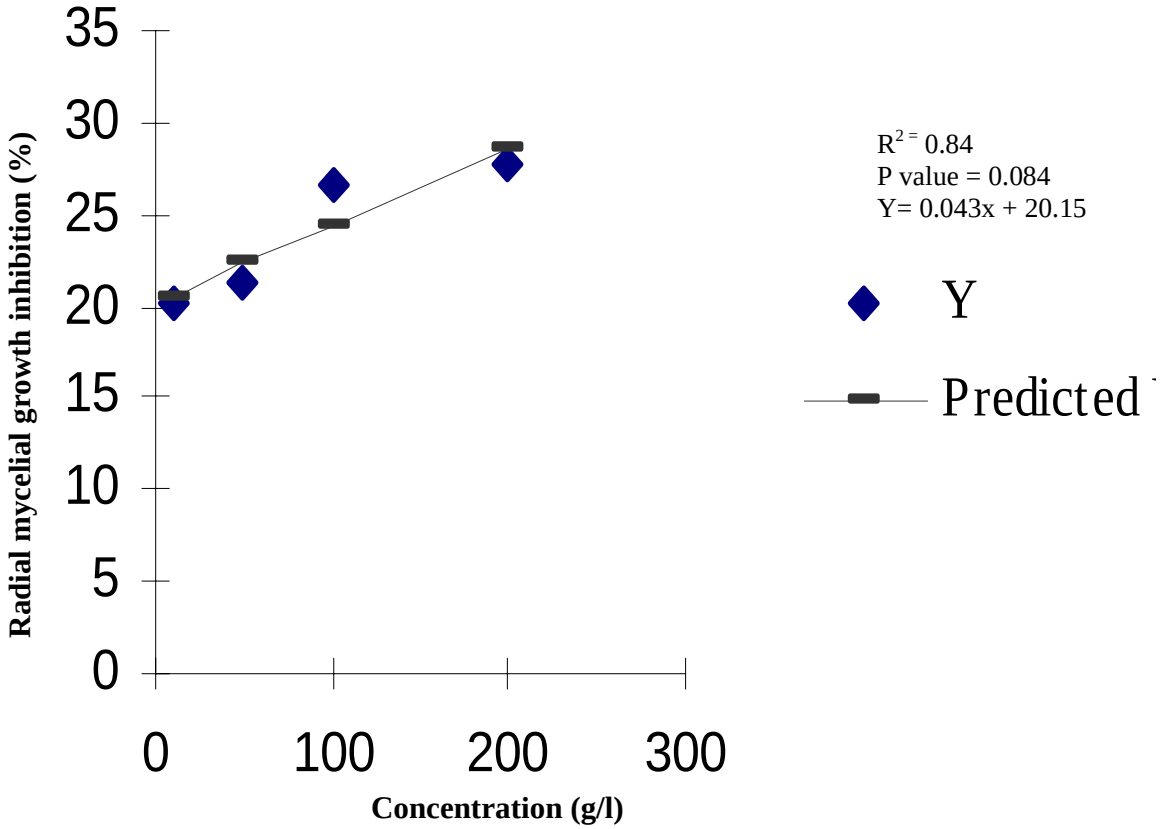



Fig. 4. Effect of different concentrations of some medicinal plant extarct on inhibition of radial mycelial growth of *Macrophomina phasiolina.*

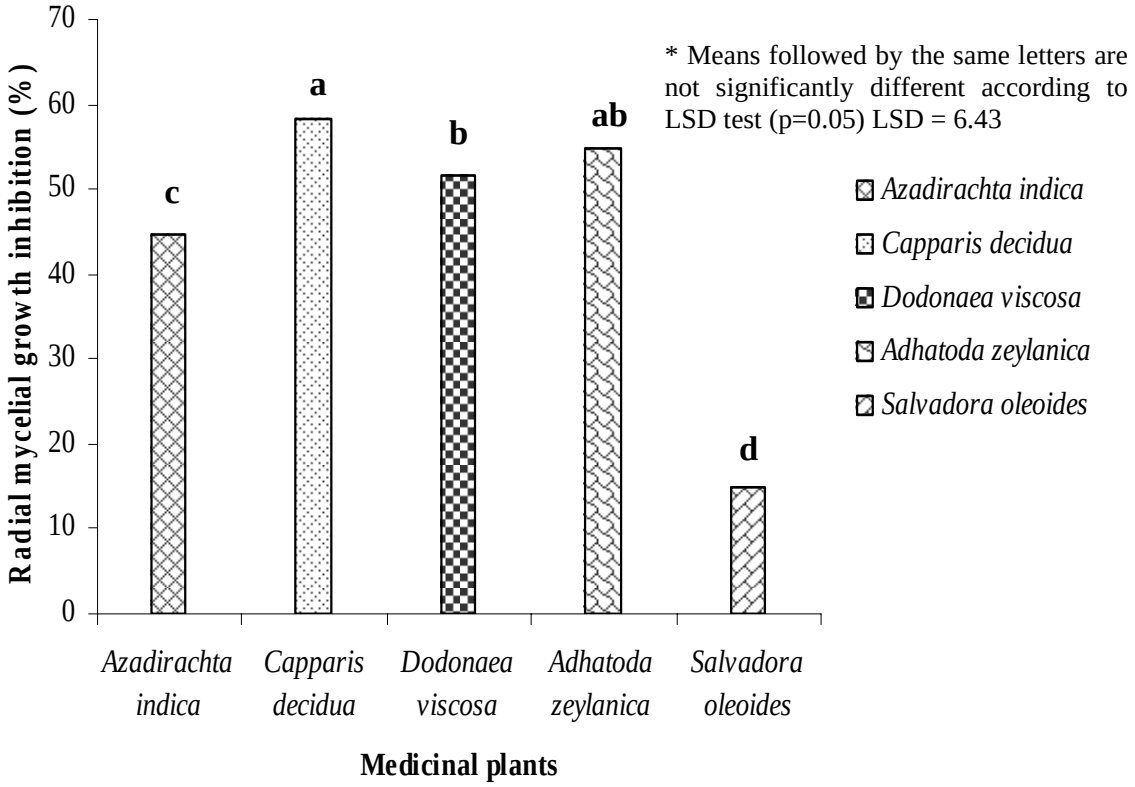



Fig. 5. Inhibition in radial mycelial growth induced by five different medicinal plants against *Rhizoctonia solani*.

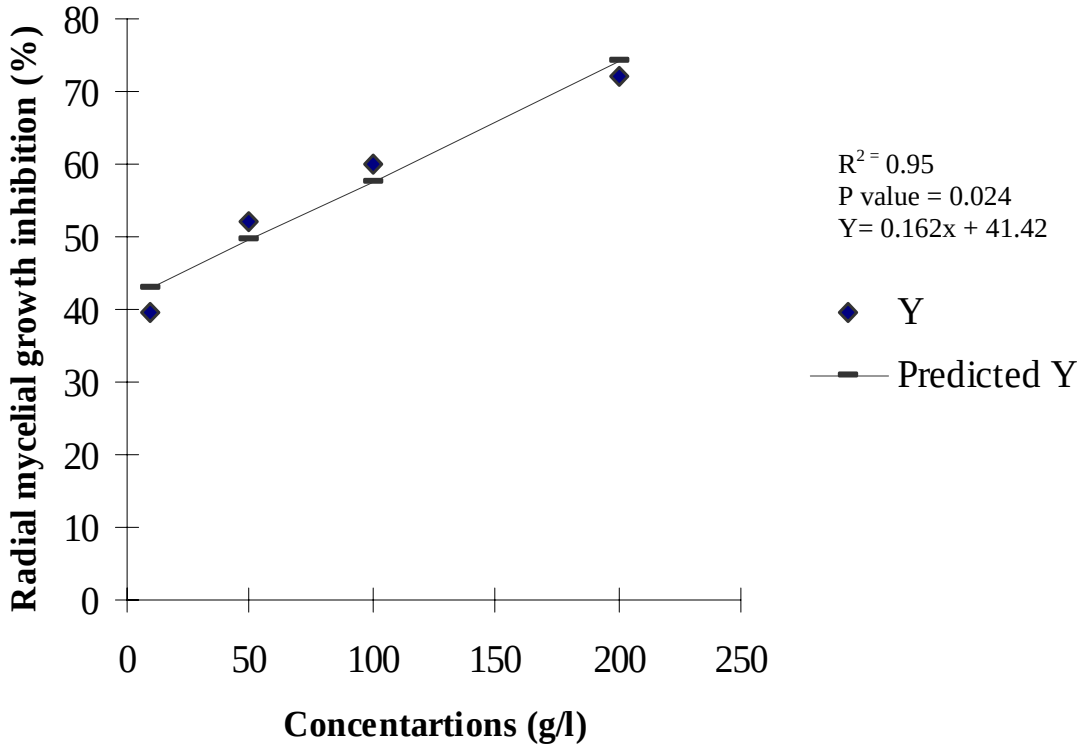



Fig. 6. Effect of different concentrations of some medicinal plant extract on inhibition of radial mycelial growth of *Rhizoctonia solani*.

**Inhibition in radial mycelial growth (rmg) of *Rhizoctonia solani* induced by five medicinal plants:** For biological screening of plant extracts, 5 plant species were evaluated for their antifungal activity against *Rhizoctonia solani*. Of the diffusates tested, *Capparis decidua* appeared significantly the most effective and exhibited 58.27% inhibition in radial mycelial growth of the pathogen followed by *Adhatoda zeylanica* (54.86%), *Dodonaea viscosa* (51.54%), *Azadirachta indica* (44.73%) and *Salvadora oleoides* (14.91%) regardless of solvent (Fig. 5). The differential sensitivity of mycelial growth of the fungus to various bio-pesticides may be due to their chemical configuration and active ingredient, slower or faster absorption and detoxification after absorption on account of metabolic activity of the fungus (Viyas, 1984). The results from these preliminary investigations into exploring the possible use of plant diffusates for the management of diseases induced by *Rhizoctonia solani* appeared to be encouraging with regards the mycelial growth of the fungus. Antifungal activity of *Azadirachta indica* has already been reported to have inhibitory effects on *Rhizoctonia solani* (Sivakadadacham, 1988; Sharma & Jnandaik, 1994).

The current results demonstrate that selected medicinal plant diffusates effectively suppressed the radial mycelial growth of *Rhizoctonia solani.* The highest inhibition (72.27%) was recorded at 200g/l concentration in radial mycelial growth of pathogen, followed by 100g/l (60.20%), 50g/l (52.11%), whereas the least inhibition of radial mycelial growth (39.72%) was observed at 10g/l. Statistically, the rate of increase in radial mycelial growth inhibition was regressed against five different concentrations of plant diffusates. Linear equation for the fitted lines is presented along with original data and fitted lines (Fig. 6). Inhibition in radial mycelial growth of the fungus gradually increases with an increase in concentration of the plant diffusates. Naz *et al*., (2006) reported the antifungal activity of *Azadirachta indica* against *Rhizoctonia solani*. The study also revealed that increasing in the concentration of plant diffusates diminishes the radial mycelial growth of fungal pathogen *In vitro*.

**References**

Bajpai, M., A. Pande, S.K. Tewari and D. Prakash. 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int. J. F. Sci. Nutrition*, 96(4): 287-291.

Banville, G.J. 1989. Yield losses and damage to potato plants caused by *Rhizoctonia solani Kuhn. Am. J.*, 66: 821-834.

Dater, V.V. and C.D. Mayee. 1985. Chemical management of early blight of tomato. *J. Maha Agri. Univ*., 10(3): 278-280.

Fabry, W., P. Okemo and R. Ansorg. 1996. Fungistatistic and fungicidal activity of East African medicinal plants. *Mycoses*, 36: 67-70.

Farooq, A. 2002. *Effects of medicinal plant extract on the growth rate, Sclerotial production and biomass of Macrophomina phaseolina* (M.Sc. Thesis), Univ. Azad Jammu and Kashmir Muzaffarabad, pp. 43.

Farr, D., F. Bills, G.P. Chamris and A.Y. Rossman. 1989. *Fungi on plants and plant products in the United States*. APS Press, St. Paul, Minnesota, pp. 1252.

Gerretsen, F.C. and N. Haagsma. 1951 Occurrence of antifungal substances in *Brassica repa, Brassica olleracea* and *Beta vulgaris. Nature* (London), 168-659.

Hassanein, N.M., M.A. Abou Zeid, K.A. Youssef and D.A. Mahmood. 2008**.** Efficacy of leaf extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) against early blight and wilt diseases of tomato. *Aust. J. Basic Appl. Sci.*, 2(3): 763-77.

Hoes, J.A. 1985. *Macrophomina* causal agents of charcoal rot of sunflower and other crops. *Agric. Canada Research station, Modern Manitoba.*

Indera, K.S., T.C.C. Machado and J.B. Sinclair. 1986. Histopathology of soybean seed infection by *Macrophomina phaseolina. Phytopathol*., 76: 532-535.

Khouray, F.Y. and S.M. Alcorn. 1973. Influence of *Rhizoctonia solani* on the susceptibility of cotton plants to *Verticillium albo-atrum* and on root carbohydrates. *Phytopathol*., 63: 352-358.

Kumar, B.P., M.A.S. Charya and S.M. Reddy. 1979. Screening of plants extracts for antifungal properties. *New Botanist*, 6: 41-43.

Loper, J.E., M.D. Henkels, R.G. Roberts, G.G. Grove, M.J. Willett and T.J. Smith. 1991. Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. *Plant Disease*, 75: 287-290.

Mahrshi, R.P. 1986. A report on three pigeonpea diseases in Rajasthan. *Int. Pigeonpea News Letter*, 5: 32-34.

Mirza, J. and M.S.A. Qureshi. 1978. *Fungi of Pakistan*. Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan, pp. 34.

Mirza, M.S. 1984. Occurrence of sunflower diseases in Pakistan in 1980-83. *Proc. National Sunflower Workshop, PARC*., pp. 31-32.

Mothana, R.A.A. and U. Lindequist. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. *J. Ethnopharmacol*., 96: 177-181.

Naidu, V.D. and V.T. John. 1982. *In vitro* inhibition of rice fungal pathogens by extracts from higher plants. *Int. Rice Res. Newsletter*, 6: 12.

Naz, F., C.A. Rauf, I.U. Haque and I. Ahmad. 2006. Management of *Rhizoctonia solani* with plant diffusates and chemicals. *Pak. J. Phytopathol*., 18(1): 36-43.

Rahber, M.H. 1986. Control of *Phakopsora grewia* with plant diffusates. *Pak. J. Bot.*, 18: 329-333.

Reni, C. and V.E. Roeland. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *J. Phytopathol*., 72(13): 335-347.

Rotem, J. 1998. *The genus Alternaria: Biology, Epidemiology and Pathogenicity. Am. Phytopathol*., Society Press, St. Paul, Minnesota.

Sharma, B. and P. Kamar. 2009. *In vitro* antifungal potency of some plant extracts against *Fusarium oxysporum. Int. J. G. Pharmacy*, 3(1): 63-65.

Sharma, V.N. and C.L. Jandaik. 1994. Effect of some plant materials in controlling different moulds in *Agaricus bisporus* (Lang) Imb. *J. Ind. Mycol. and Pl. Pathol*., 24(30): 183-185.

Shehzad, S., A. Sattar and A. Ghaffar. 1988. Addition to the hosts of *Macrophomina phaseolina*. *Pak. J. Bot.*, 20: 151-152.

Sinclair, J.B. and P.A. Backman. 1989. *Compendium of Soybean Diseases*. 3rd Ed. Am. Phytopathol, Society. St. Paul. MN, USA.

Sivakadadcham, B. 1988. Green manure for the control of soil-borne pathogens. *Tropical Agriculturist*, 114: 163-164.

Viyas, S.C. 1984. *Systematic fungicides*. Tata Mc Grahh hill co. limited. New Delhi, pp. 36.

Wojdylo, A.J., Oszmianski and R. Czemerys. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *F. Chem*., 105: 940-949.

(Received for publication 31 December 2009)