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Inhibitory Potential of Selected Fungicides and Plant Extracts Against Mycelial Growth of *Fusarium oxysporum* f. sp. *ciceri* Causal Agent of Chickpea Wilt

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ABSTRACT

Chickpea wilt is one of the major limiting factors, for low yield of chickpea. In Pakistan chickpea wilt causes 10-50% losses every year. At all concentrations, Carbendazim and Benomyl proved most effective while Acrobat was least effective in suppressing the mycilial growth of the *Fusarium oxysporum* f. sp. *ciceri*. However, higher concentration of Acrobat was also slightly effective. Among the plant extracts, higher concentrations of Sufaida and Neem proved to be effective while onion failed to control the colony growth of *Fusarium oxysporum* f.sp. *ciceri* at all concentrations.

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INTRODUCTION

A Chickpea wilt ranks second most severe problem after the Gram blight in Pakistan (Khan *et al*., 2002). The disease is common particularly in the districts of Jhang, Layyah, Khushab, Bhakkar and Mianwali. It is more prevalent in lower latitudes (0-30 ^{o}N) where growing season is relatively dryer and warmer than in the higher latitudes (30-40 ^{o}N). Fusarium wilt epidemics cause significant annual losses of chickpea yields (Halila and Strange, 1996, Jalali *et al*., 1992) that may reach 100 % under conditions favorable for disease (Anjaiah *et al*., 2003, Halila and Strange 1996, Navas-Cortes and Jimenez-Diaz 2000). Earlier wilting caused more loss than late wilting. Seeds harvested from wilted plants were lighter and duller than those from healthy plants (Haware and Nene, 1980).

In order to avoid the plant diseases and to defend the crop plants against pathogen, chemical control methods are also in practice. In view of expensive pesticides and their perilous consequence, use of biodegradable substances like plant extracts, is gaining importance during last three decades for disease control (Grainge and Ahmed, 1988). The use of chemicals has helped in the increase of yield but one of the major problems with the constant use of chemicals is development of resistance in the pathogen and contamination of the environment with very toxic substances (Okigbo, 2004).

Fungitoxicants of plant origin are environmentally safe and non-phytotoxic. The extracts of these plant materials can be easily prepared by farmers (Okigbo and Nameka, 2005). Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should include use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998). There is a need to explore the efficacy of different fungicides and plants extracts to manage the disease below economic threshold level in the absence of resistant germplasm.

METHODS AND MATERIAL

The sensitivity of mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* against different fungicides: Acrobat, Antracol, Benomyl, Carbendazim and Mancozeb were evaluated at different concentrations that are i.e. 50, 100 and 150 µg/ml. These fungicides were tested in vitro to evaluate their effect on colony growth of isolated fungi, by using inhibition zone technique. All fungicides were used at above ppm (parts per million) concentrations. Prior the PDA medium pouring 0.5 ml of autoclaved medium was poured in each sterilized plate (9 cm). After solidification, the plates were inoculated by placing 5mm discs of 7 days old PDA cultures of isolated fungi. Three replicated plates were used for each concentration of every fungicide while distilled water without fungicides as control. The inoculated plates were incubated at 25 °C and data on the radial colony diameter were recorded after 3, 6 and 9 days of incubation in cm and were analyzed statistically to see the difference among various treatments. The efficacy of different plant extracts against isolated fungal pathogen was studied, by using inhibition zone technique. Two adjacent holes were made with the help of sterilized cork borer at equal distance in each petri plate.

In order to made equal distance between the holes marking was done on the back side of each petri plate with the help of permanent marker. In vitro, five plant extracts *Allium cepa* (Onion), *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Eucalyptus camaldulensis* (Sufaida) and *Zingiber officinale* (Ginger) extracts were tested to check their efficacy for the selection of the most effective one at different dose rate Fresh leaves of *Azadaracta indica* and *Eucalyptus camaldulensis*, Bulbs of *Allium cepa* and *Allium sativum* and rhizome of *Zingiber officinale* were collected from local market. Collected plant materials were surface sterilized with 0.1% sodium hypochlorite and repeatedly washed in sterile water and cut into small pieces. A 50 % w/v stock solution of *A. indica*, *E. camaldulensis*, *A. cepa*, *A. sativum* and *Z. officinale* was prepared by soaking the crushed plant materials in sterilized water for 24 hours at room temperature, passing through muslin cloth and finally through Whatman filter paper No.1.

The lower concentrations of S/2, and S/3 w/v were prepared by adding appropriate quantity of sterile water into the stock solution. The diluted plant extracts were heated to 40-50 °C for 10 min. to avoid contamination. The extract was stored at 4 °C to avoid contamination and prospective chemical alterations. After solidification, the plates were inoculated by placing 5mm discs of 7 days old PDA cultures of isolated fungi. Three replicated plates were used for each concentration of every aqueous plant extract, while distilled water in place of any extract was used as control. The effective plant extracts were determined by calculating colony diameter of 3 days interval i.e. 3, 6 and 9 days.

RESULTS

Fungitoxic effects of five fungicides viz. Acrobat, Antracol, Benomyl, Carbendazim and Mancozeb at three concentrations 50 ppm, 100 ppm and 150 ppm were tested in vitro by applying inhibition zone technique. The fungitoxicity of five fungicides varied greatly. In general, there was a significant decrease in mycellial growth of the fungus with an increase in fungicidal concentration. The most effective fungicides in inhibiting the growth of the fungus were Carbendazim followed by Benomyl as they inhibit the mycelial growth efficiently. Mancozeb and Antracol were less and equally effective in inhibiting the growth of fungus as they reduce mycelial growth less effectively than Carbendazim and Benomyl. Acrobat was the least effected one fungicide. The comparative efficacy of all the fungicides is shown in the Figure 1.

Fungitoxic effects of five plant extracts viz. *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Eucalyptus camaldulensis* and *Zingiber officinale* at three concentrations SS, S/2 and S/3 were tested in vitro by applying inhibition zone technique. The fungitoxicity of five plant extracts varied greatly. In general, there was a significant decrease in mycellial growth of the fungus with an increase in plant extracts concentration However, when growth of the fungus in response to various plant extracts concentration at an incubation period of 3, 6 and 9 days at 25 °C was compared. The order of fungitoxicity of all the extracts was found in following order *Eucalyptus camaldulensis*, *Azadirachta indica*, *Allium sativum*, *Zingiber officinale* and *Allium cepa* respectively (Figure 2).

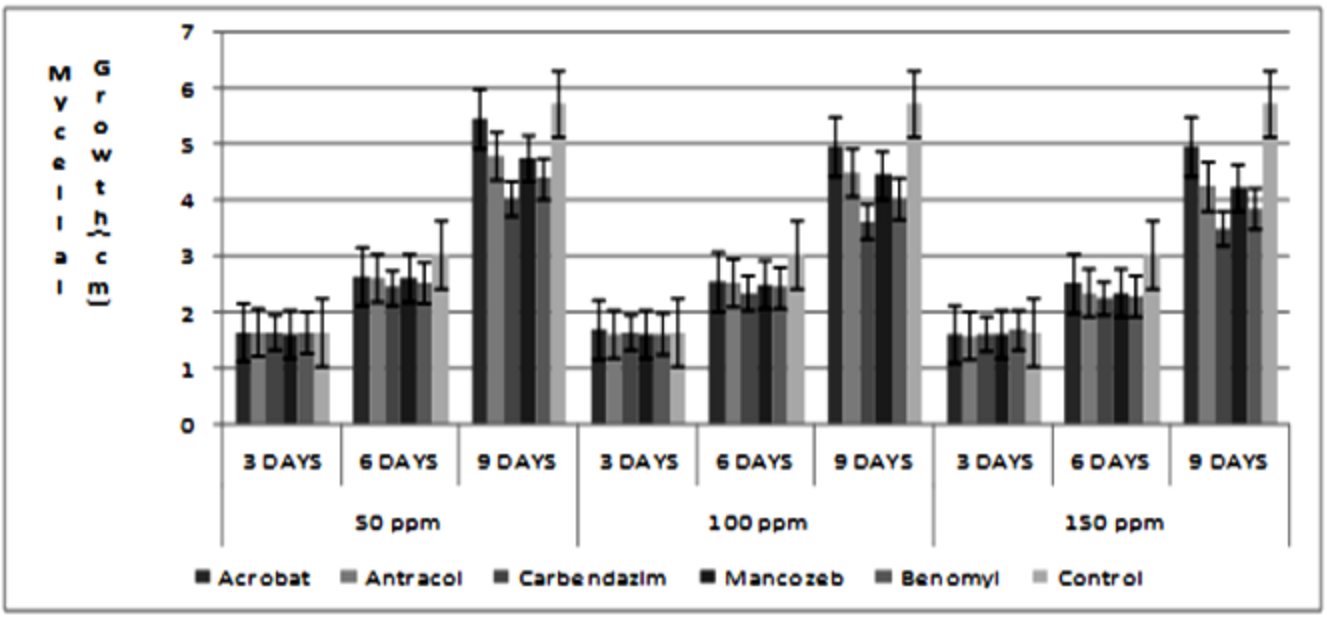

Figure 1. Comparison of fungitoxicity of various fungicides.

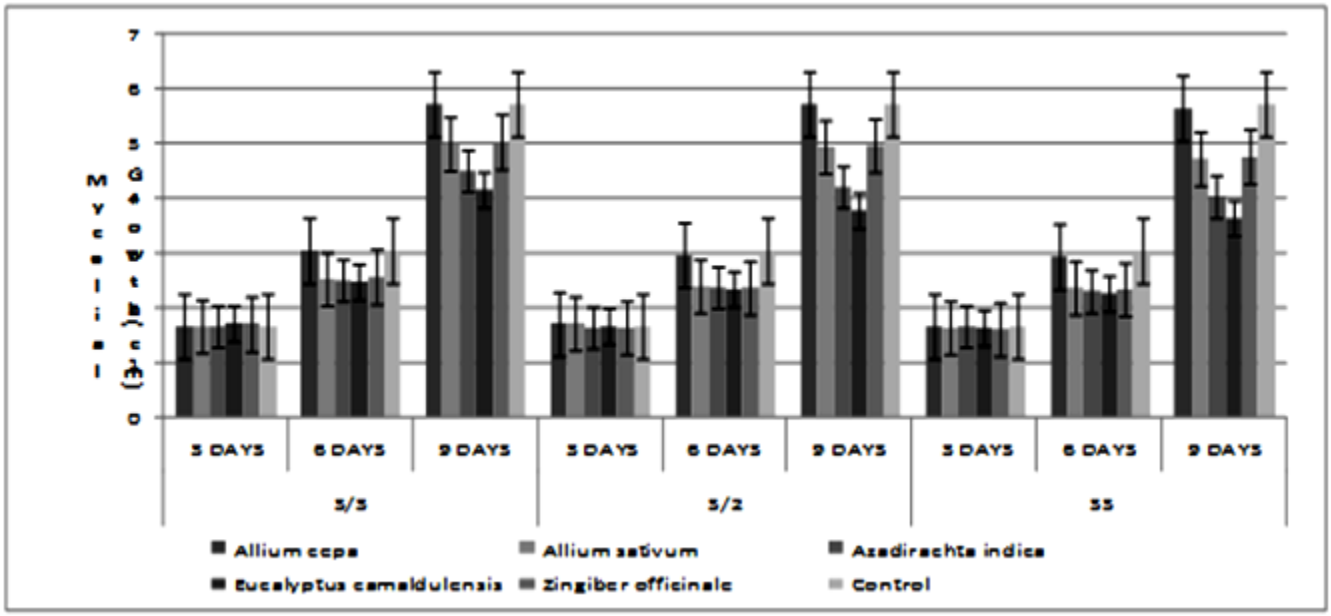

Figure 2. Comparison of fungitoxicity of plant extracts.

DISCUSSION

Preliminary evaluation of the comparative effect of fungicides on the mycelial growth of *F. oxysporum* revealed that the effectiveness of fungicides in inhibiting the mycelial growth of the pathogen varied a great deal and there was, in general, a significant increase in the inhibition of mycelial growth with an increase in fungicide concentration. The effectiveness of fungicides in inhibiting the growth of fungus, in descending order Carbendazim, Benomyl, Mancozeb, Antracol and Acrobat. The effectiveness of Carbendazim and Benlate in controlling chickpea wilt has been reported by another worker as well (Cother, 1977; Haware *et al*., 1978; Shukla and Bharagava, 1978; Gosh and Sinha, 1981; Haware and Nene, 1981; Mani and Sethi, 1984; Jimenez-Diaz and Troperoeasas, 1985).

Similarly, among three fungicides, Carbendazim (50 WP) and Benomyl (50WP) was proved to be the most effective

against *F. oxysporum* f. sp. *ciceri* at 0.1 %, 0.2 % and 0.3 % (Mukhtar, 2007). Nikam, *et al*., (2007) studied three fungicides alone and in combination in vitro and in pot culture under glass house against *F. oxysporium* f. sp. *ciceri* viz., Carbendazim 0.2 % (2 g / kg seed), Captan 2.0 (2 g / kg seed) and Thiram 3.0 % (3 g/kg seed). Chemical seed treatment with Carbendazim (0.1 %) + Thiram (0.15 %) against *Fusarium oxysporium* f. sp. *ciceri* was proved to be the most effective. Our result quiet coincides with the results of these scientists as in our results Carbendazim and Benomyl were proved to be the most effective.

Analysis of Variance shows that effect of test chemicals, different applied doses significantly reduced the fungal growth. At higher concentration, Carbendazim and Benomyl and were the most effective fungicides among the five fungicides used. Acrobat was least effective even in higher concentration. Similarly, Cother (1977) obtained effective control of gram wilt by seed treatment with Benomyl, Captan and Thiram. Gupta *et al*. (1997) screened 6 fungicides against *F. oxysporium* f. sp. *ciceri* and reported Carbendazim @100 mg/ml as most effective in inhibiting the growth of fungus in vitro. However, at low concentration, test fungicides showed equally effective behavior. Chemical Seed treatment with Thiram (0.15 %) + Carbendazim (0.1 %) were found most effective against *F. oxysporium* f. sp. *ciceri* (Nikum *et al*., 2007). Carbendazim 0.2 % was effective among three treatments (Figure 2).

Incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity (Gassner *et al*., 1997; Harris *et al*., 2001; Campos *et al*., 2005). The present study signifies the importance of aqueous extracts plants as potential agent to be manipulated for biological control of the pathogen as they are equally effective as the fungicides.

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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.

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