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IN-VITRO EVALUATION OF HOMEO-FUNGICIDES AND METHANOLIC PLANT EXTRACTS AGAINST MYCELLIAL GROWTH OF *FUSARIUM OXYSPORUM* F.SP. *PISI* CAUSING WILT DISEASE IN PEA

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A B S T R A C T

Fungitoxic effects of three homeo-fungicides *viz;* Rigrous, Ventage, Vampire and six methanolic plant extracts *viz;* Garlic Extract, Ginger Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extract and Parthenium Leaf Extract at nine different concentrations *viz*; 5, 10, 20, 50, 100, 150, 300, 500 and 1000 µg/ml were tested *in vitro* by applying poisoned food technique method at an incubation period of nine days at 25 ± 2°C. Mycellial growth of the fungus in response to various homeo-fungicidal at different concentrations was compared Vampire proved to be the best as it has reduced the mycelial colony (83.4 percent) which was followed by Rigrous (82.2 percent), while Vantage proved to be the least effective (76.5 percent) at highest concentration (1000 µg/ml). None of the other homeo-fungicides have completely checked the mycelial growth of *F. oxysporum* f.sp. *pisi*. The experiment was arranged in randomized complete design (CRD) However, when growth of the fungus in response to various methanolic plant extracts and their concentrations were compared, Neem leaf extract proved to be the best as it had given the maximum colony reduction (60.5 percent) followed by Ginger extract (57.7 percent), while Parthenium leaf extract proved to be the least effective (25.4 percent) at highest concentration (1000 µg/ml). None of the tested Plant extracts and their concentrations tested has completely checked the mycelial growth. There was an overall trend of reduction in mycellial growth of fungus with an increase in the concentration of homeo-fungicides and methanollic plant extracts.

Keywords: Wilt disease *Fusarium oxysporium*, homeo-fungicide, plant extracts.

INTRODUCTION

Pea (*Pisum sativum)* which is commonly known as garden pea belongs to family Leguminosae. It is important contributor to developing farming systems in which low-input are required as this crop add nitrogen by fixing atmospheric nitrogen in the roots of pea plants. It also serves as opportunity crop which reduces the use of inputs (Smýkal *et al.*, 2012). Leguminosae family comprises of 18,000 species categorizing in 650 genera, which provides it the status of third largest family among the flowering plants (Lewis *et al.*, 2005). According to With reference to economical point of view, it is the second most imperative family after grass family (*Poaeceae)* which accounts almost 27% of crops of the world (Graham, *et al.*, 2003).

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In Pakistan, total area under cultivation of pea crop during 2011-12 was 15.8 thousand hectares and production was 105.0 thousand tons with an average yield of 587 kg per hectare (Pak. Economic Survey, 2013). Out of which 7456 tons was exported with earnings of 518 Million Rs., which holds the biggest figure in the export income among the vegetables export. During the same year, production share of Punjab, Sindh, K.P.K. and Bloachistan was 71.2, 4.7, 12.8 and 11.3 thousand tons, respectively (Pak. Economic Survey, 2013).

In spite of all above mentioned plant's high efficiency for nutrient usage and environmental aspects, the yield of pea crop is decreasing day by day. This is mainly due to the attack of many soil and air borne pathogens. Pea is an early winter season crop and its climatic conditions favour the attack and invasion of the soil and air borne pathogens. A number of pathogens including *Ascochyta*

foot rot, bacterial blight, *Rhizoctonia* seedling blight, *Pythium* blight, Powdery mildew, Downy mildew; *Aphanomyces* root rot and *Fusarium* wilts (Hadwiger, 2008). Out of these diseases *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *pisi* is the major damaging factor to the production of pea.

The major losses are caused by fungal pathogens. Root rot and wilts caused by *Fusarium* sp., are the major cause of losses in pea production among other fungal pathogens. This pathogen is capable of causing partial to complete loss of crop and it is also capable of causing huge damages to other such crops (Basu *et al.*, 1973, 1976; Persson *et al.*, 1997).

Fusarium wilt cause significant yield loss to winter crop and it is more prevalent to heritage varieties, causing yield losses up to 50%. No registered fungicide is available for the control of wilt (Powell, 1993). *Fusarium* wilt always remained a major threat to pea production as this disease is unpredictable. It occasionally appears and causes heavy crop losses. Need of the hour is to find out the effective and potential ways, by which, we can control this pathogen and be able to stop the economic losses caused by this pathogen. Present study is designed to check *in vitro* the efficacy of homeo-fungicides and methanolic plant extracts at different concentration to control the *Fusarium oxysporium* f.sp. *pisi*.

MATERIALS AND METHODS

The sensitivity of mycelial growth of *Fusarium oxysporum* against different homeo-fungicides (Rigrous, Ventage and Vampire) and methanolic plant extracts (Garlic Extract, Ginger Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extractand Parthenium Leaf Extract) were evaluated at different concentrations that are i.e. 5, 10, 20, 50, 100, 150, 300, 500 and 1000 µg/ml by using modified Borum and Sunclair's technique (1968).

A measured quantity of each of the homeo-fungicide and methanolic plant extract was amended to autoclaved garden pea seed meal agar medium for obtaining required concentrations. Garden pea seed meal agar without fungicide served as control. Twenty five milliliter of amended and non-amended medium was poured in each of the four 90mm diameter Petri plates. After solidification 5mm agar plugs containing *Fusarium oxysporum* f.sp. *pisi* mycelium were cut from edge of 10 days old culture plates and was placed in the center of each Petri dish with the help of sterilized needle. The inoculated etri plates were incubated at 25 ± 2°C. Radial growth of *Fusarium oxysporum* f.sp. *pisi* was recorded in (mm) after every 24 hours for 9 days of incubation and final data (after 9 days) was analyzed statistically to see the difference among various treatments.

RESULTS AND DISCUSSION

Evaluation* of homeo-fungicides against mycellial growth of *Fusarium oxysporum* f.sp. *pisi: Fungitoxic effects of three homeo-fungicides *viz;* Rigrous, Ventageand Vampire at nine different concentrations *viz*; 5, 10, 20, 50, 100, 150, 300, 500 and 1000 µg/ml were tested *in vitro* by applying poisoned food technique. Analysis of variance shows highly significant results homeo-fungicides, their concentration and interaction between fungicides and fungicide concentrations. On the other hand there was a continuous trend that with increase in the concentration of homeo-fungicides decrease was seen in the mycellial growth of the *Fusarium oxysporum* f.sp. *pisi* (Table 1).

The results obtained on the fungitoxicity of homeo-fungicides against *Fusarium oxysporum* f.sp. *pisi* in *vitro* are presented in (Table 1). The fungitoxicity of three homeo-fungicides varied greatly among each other and their concentrations (Table 1). In general, there was a significant decrease in mycellial growth of the fungus with an increase in fungicidal concentration. However, when growth of the fungus in response to various fungicidal concentrations at an incubation period of nine days at 25 ± 2°C compared Vampire proved to be the best as it had given the maximum control (83.4 %) followed by Rigrous (82.2 %) on 1000 µg/ml concentration. While Vantage proved to be the least effective (76.5 %) at highest concentration (1000 µg/ml). None of the tested fungicides have completely checked the mycelial growth of *Fusarium oxysporum* f.sp. *pisi*. There was an overall trend that the mycellial growth of *Fusarium oxysporum* f.sp. *pisi* is reduced with an increase in the concentration (Table 1).

Evaluation of Plant Extracts against mycellial growth of *Fusarium oxysporum* f.sp. *pisi:* Fungitoxic effects of six methanolic plant extracts *viz;* Garlic Extract, Ginger Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extract and Parthenium Leaf Extract at nine different concentrations *viz*; 5, 10, 20, 50, 100, 150, 300, 500 and 1000 µg/ml were tested *in vitro* by applying poisoned food technique. Analysis of variance shows highly significant results of plant extracts, their concentration and interaction between plant extracts and their concentrations. Mycellial growth of *Fusarium oxysporum* f.sp. *pisi* (Table 2) was decreased with the increase in the concentration of plant extracts.

Table 1 Effect of homeo-fungicides on percent reduction in mycellial growth of *Fusarium oxysporum* f. sp. *pisi* at different concentrations.

Concentration	Homeo-Fungicides			
	Rigurous	Ventage	Vampire	Control
5 µg/ml	36.7 cd	36.7 cd	46.9 de	6.8
10 µg/ml	39.5 cde	39.5 cde	42.4 de	6.2
20 µg/ml	30.3 bcd	18.6 abc	82.9 f	6.8
50 µg/ml	78.5 f	30.4 cd	11.2 abc	6.2
100 µg/ml	65.5 ef	37.1 cd	82.3 f	6.8
150 µg/ml	65.2 ef	75.7 f	77.6 f	6.2
300 µg/ml	78.1 f	69.3 ef	73.4 f	6.8
500 µg/ml	76.6 f	69.2 f	77.6 f	6.2
1000 µg/ml	82.2 f	76.5 f	83.4 f	6.8

The results obtained on the fungitoxicity of methanolic plant extracts against *Fusarium oxysporum* f.sp. *pisi* in *vitro* are presented in (Table 2). The fungitoxicity of six methanolic plant extracts varied greatly among each other and their concentrations (Table 2). In general, there was a decrease in mycellial growth F*usarium oxysporum* f.sp*. pisi* with an increase in concentration of methanolic plant extracts. However, when growth of the fungus in response to various methanolic plant extract concentrations after an incubation period of nine days at 25 ± 2°C was compared, Neem leaf extract proved to be the best as it had given the maximum control (60.5 %) followed by Ginger extract (57.7 %) on 1000 µg/ml concentration. While Parthenium leaf extract was proved to be the least effective (25.4 %) at highest concentration (1000 µg/ml). None of the tested Plant extracts and their concentrations tested has completely checked the mycelial growth of *Fusarium oxysporum* f.sp. *pisi*. Although there was an overall trend of reduction in mycellial growth of *Fusarium oxysporum* f.sp. *pisi* with an increase in the concentration of methanolic plant extracts (Table 2).

DISCUSSIONS

The fungitoxicity of three homeo-fungicides varied greatly among each other and their concentrations (Table 1). Vampire proved to be the best as it had given the maximum control (83.4 %) followed by Rigrous (82.2 %) on 1000 µg/ml concentration, while Vantage proved to be the least effective (76.5 %) at highest concentration (1000 µg/ml). None of the tested homeo-fungicides have completely checked the mycelial growth of *Fusarium oxysporum* f.sp. *pisi*. There was an overall trend that the mycellial growth of *Fusarium oxysporum* f.sp. *pisi* was reduced with an increase in the concentration (Table 1).

Incessant and extensive use of the synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity (Lynch, 1983; Bull *et al*., 2002; Landa 1997; Pavlou *et al*., 2002; Datnoff *et al*., 1995 and Bell *et al*., 1988). Considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms (Alabouvette, 1990). Strains of several bacterial species such as *Bacillus*, *Pseudomonas* and recently the *Rhizobium* group were found to effectively control various soil-borne plant pathogenic fungi under greenhouse and field conditions (Prusky *et al*., 1994).

Fungitoxic effects of six methanolic plant extracts *viz;* Garlic Extract, Ginger Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extract and Parthenium Leaf Extract at nine different concentrations was tested *in vitro* by applying poisoned food technique. Analysis of variance shows highly significant results of plant extracts, their concentration and interaction between plant extracts and their concentrations. Mycellial growth of *Fusarium oxysporum* f.sp*. pisi* was decreased with the increase in the concentration of plant extracts.

Neem leaf extract proved to be the best as it had given the maximum control (60.5 %) followed by Ginger extract (57.7 %) on 1000 µg/ml concentration. While Parthenium leaf extract was proved to be the least effective (25.4 %) at highest concentration (1000 µg/ml). None of the tested Plant extracts and their concentrations tested has completely checked the mycelial growth of *Fusarium oxysporum* f.sp. *pisi*. Although there was an overall trend of reduction in mycellial growth of *Fusarium oxysporum* f.sp. *pisi* with an increase in the concentration of methanollic plant extracts (Table 2).

Table 2. Effect of methanolic plant extracts on percent reduction in mycellial growth of *Fusarium oxysporum* f.sp. *pisi* at different concentrations.

Concentration	Garlic Extract	Ginger Extract	Akk Leaf Extract	Neem Leaf Extract	Moringa Leaf Extract	Parthenium Leaf Extract
5 µg/ml	23.0 cdefghi	30.9 defghij	28.3 defghij	24.1 cdefgh	11.6 abc	5.0 ab
10 µg/ml	25.0 efghijk	26.6 cdefgh	28.5 defghij	13.7 abcde	12.7 abcd	8.0 abc
20 µg/ml	37.4 fghijklm	37.1 fghijklm	33.1 defghij	32.9 fghijkl	21.9 cdefg	17.0 abcde
50 µg/ml	31.0 fghijklm	41.7 ghijklm	33.4 fghijkl	35.7 fghijklm	35.5 fghijkl	23.7 bcdef
100 µg/ml	39.9 fghijklm	53.3 klm	33.7 fghijkl	43.0 fghijklm	39.5 fghijkl	28.3 cdefgh
150 µg/ml	43.4 fghijklm	51.3 jklm	41.7 fghijklm	42.2 fghijklm	46.0 fghijklm	28.4 defghij
300 µg/ml	44.0 ghijklm	52.2 klm	40.8 fghijkl	54.8 ghijklm	40.7 fghijklm	39.2 fghijkl
500 µg/ml	44.2 hijklm	49.6 hijklm	37.8 fghijkl	57.8 lm	48.9 hijklm	38.0 defghij
1000 µg/ml	45.9 ijklm	57.7 lm	50.3 fghijklm	60.5 lm	55.3 jklm	41.2 fghijklm

Means sharing same letter does not differ significantly at $P < 0.05$

These results are also reported by Shukla and Dwivedi (2012). Who are strongly agree to the results and enforces use of plant extracts instead of synthetic fungicides. Jamal-u-ddin hajano *et al*., 2012, stated the efficacy of Neem leef extract. But according to their findings, Neem leaf extract stood next to garlic extract which give them best efficacy. None of the homeo-fungicides and methanolic plant extracts had checked the mycelial growth of *Fusarium oxysporum* f.sp. *pisi* completely. Therefore, further studies should be carried out to establish the effectiveness of homeo-fungicides and methanolic plant extracts in combination *in vitro* and *in vivo.*

REFERENCES

Alabouvette, C. 1990. Biological control of *Fusarium* wilts pathogens in suppressive soils. Biological Control of Soil borNPlant Pathogens. 27-43.

Anonymous. 2012. Economic Survey of Pakistan. Governement of Pakistan.

Basu, P. K., N. J. Brown, R. Crete, C. O. Gourley, Johnston, H. S. Pepin and W. L. Seaman. 1976. Yield loss conversion factors for *Fusarium* root rot of pea. Canada. Plant Dis. Survey. 56: 25-32.

Basu, P. K., R. Crete, A. G. Donaldson, C. O. Gourley, J. H. Haas, F. R. Harper, C. H. Lawrence, W. L. Sesman, H. N. W. Toms, S. I. Wong and R. C. Zimmer. 1973. Prevalence and severity of diseases of processing peas in Canada. Canada Plant Dis. Survey. 53: 49-57.

Bell A. A., J. C. Hubbard, L. Liu, R. M. Davis and K. V. Subbarao. 1998. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows of celery. Plant Dis. 82:322-8.

Borum DF, Sinclair JB (1968). Evidence for systemic protection against *Rhizoctonia solani* with Vitavax in cotton seedlings. Phytopathol., 58: 976-980.

Bull C.T., K. G. Shetty, K. V. Subbarao. 2002. Interactions between Myxobacteria, Plant Pathogenic Fungi and Biological Control Agents. Plant Dis. 86. 889-96.

Datnoff, L. E., S. Nemec and K. Pernezny. 1995. Biological control of *Fusarium* crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Contr. 5:427-31.

Graham, P. H. and C. P. Vance. 2003. Legumes: Importance and constraints to greater use. *Plant Physiol.* 131:872–7.

Hadwiger, L.A. 2008. Pea_*Fusarium solani* interactions contributions of a system towards understanding disease resistance. Phytopathol. 98:372-379.

Jamal-u-ddin Hajano, A. Mubeen lodhi*, M.A. Pathan, M.A. khanzada and G.S. Shah. 2012. *In-vitro* evaluation of fungicides, plant extracts and Bio-controlagents against rice blast pathogen *Magnaporthe oryzae* couch. *Pak. J. Bot*., 44(5): 1775-8.

Landa B. B. l. 1997. Antagonistic Activity of

Bacteria from the Chickpea Rhizosphere against *Fusarium oxysporum* f.sp. *ciceris* Phytoparasitica 25(4):305-18.

Lewis, G., B. Schrirer, B. Mackinder and M. Lock. 2005. *Legumes of the World*; Royal Botanical Gardens: Kew, UK.

Lynch, J. M. 1983. Microbiological Factors in Crop Productivity. Soil Biotechnology, Blackwell Scientific Publications.

Pavlou G. C., D. J. Vakalounakis and E. K. Ligoxigakis. 2002. Control of root and stem rot of cucumber, caused by *Fusarium oxysporum* f. sp. r*adicis cucumerinum*, by grafting onto resistant rootstocks. Plant Dis. 86:379-382.

Persson, L., L. Bodker and M. Larsson-Wikstrom. 1997. Prevalence and pathogenicity of foot and root rot pathogens of pea in Southern Scandinavia. Plant Dis 81(2): 171-4.

Powell, K. 1993. *Biological control the answer for sustainable agriculture*. Mycologist. 7: 75-8.

Prusky, D., S. Freeman, R. J. Rodriguez and N. Keen. 1994. A nonpathogenic mutant strain of Colletotrichum magna induces resistance in avocado fruits to C. gloeosporioides. Mol. Plant-Microbe Interact. 7:326-33.

Shukla, A. and S.K. Dwivedi. 2012. Bioefficacy of plant extracts against *Fusarium* species causing wilt in pulses. IOSRJEN. 2(1), pp. 136-44.

Smýkal, P., G. Aubert, J. Burstin., *et al*. 2012. Pea (*Pisum sativum* L.) in the Genomic Era. *Agronomy. 2*, 74-115.