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DIFFERENTIAL IMPACT OF *FUSARIUM OXYSPORUM* F.SP. *PISI* ON RESISTANCE SOURCE OF PEA GENOTYPES AND ITS CHEMICAL MANAGEMENT

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A B S T R A C T

Pea wilt caused by *Fusarium oxysporum* f.sp*. pisi* has significant impact on reduction of pea yield. Present study was an attempt to find resistant sources in pea varieties/advance lines efficacy of commercially available fungicides against *Fusarium oxysporum* f.sp. *pisi.* For this purpose twelve genotypes were sown at the experimental area of Department of Plant Pathology, University of Agriculture, Faisalabad, during 2012-2013 to be screened against wilt disease under natural conditions. The data regarding disease incidence was recorded three times. Only one variety (Jumbo) was found highly resistant. While the advance lines (26709 and 26711) were found resistant to disease. Two lines (26702 and 26714) were moderately resistant and six lines (18372, 18374, 26710, 26712, 26715 and 26723) showed susceptible response. Moreover Climax was found highly susceptible. After screening, *in-vitro* study was conducted for isolation and identification of pathogen. Pathogenicity test was confirmed through Koch's postulates. For evaluation of chemicals firstly eight fungicides (Ridomil, Copper Oxychloride, Carbendazim, Nativo, Alliete, Mancozeb, Topsin-M and Difenoconazole) were tested with three concentrations (400, 600 and 800ppm). Among eight, four fungicides (Alliete, Nativo, Topsin-M and Difenoconazole) gave significantly good results as compared to control. These four fungicides were further applied to manage the disease under field conditions. The results revealed that Topsin-M significantly manage the disease where only 31% of wilt incidence was recorded. The systemic mode of infection may be a support to show better performance. Moreover Topsin M. followed by Aliette, Nativo and Difenoconazole respectively. Therefore, Topsin M. may be recommended for controlling wilt disease to pea growers.

Keywords: *Pisum sativum* L., *Fusarium oxysporum* f.sp*. pisi,* fungicides, screening, wilt.

INTRODUCTION

Pea (*Pisum sativum* L.) is an important leguminous vegetable crop grown throughout the world as winter annuals which requires cool and humid climate (Smart, 2000). In Pakistan, it was grown over 10,000 hectors with total production of 71,792 tons and an average yield of 4.9 t/ha (Anonymous, 2011). Pea grains are rich source of protein and also known as common man's meat, this nutritional delicious vegetable is available in market throughout the year.

Regardless of other constraints in pea production, diseases are major factor which influence yield of pea grains. Among all pathogens *Fusarium oxysporum* f.sp. *pisi* is dominant which can cause partial to complete losses of crop (Basu *et al.,* 2004; Persson *et al*., 2007). It is a major soil born pathogen in Pakistan which is widely distributed throughout the country. At present there are no efficient, economical and useful ways to manage Fusarium wilt. Farmer always prefers chemical control for quick and easy method. Although pea wilt has been controlled by several fungicides individually or in combinations in few parts of world but some solid and economical management is lacking. Previously screening data was used to study the susceptible and resistant genotypes and relationship between genetic diversity and geographic patterns (Nisar *et al*., 2006).

As above mentioned Fusarium wilt is prevalent in Pakistan, hence a research plan was designed to check the differential response of *Fusarium oxysporum* on pea varieties/advance lines and its control by using newly available fungicides.

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MATERIALS AND METHODS

Field Trials: Seeds of twelve varieties were obtained from Vegetable Research Institute, AARI, Faisalabad. Field trial was sown in the Research area of Department of Plant Pathology, University of Agriculture, Faisalabad. Each variety/line was planted on a single bed with plant to plant distance of 10 cm. Augmented design was used for screening while Randomized Complete Block Design (RCBD) for management of *Fusarium* wilt with all agronomic practices except disease management.

Experiment was conducted under natural conditions. As the favorable temperature for the disease development is 30^0C (Hagedorn, 1991), so keeping in view these facts, incidence of pea wilt disease was checked routinely and field survey was done regularly.

Present study was conducted after Charchar and Kraft (1989) where if disease incidence (DI) was 0-10% the crop response was considered highly resistant, while between 11-20% the crop rating was resistant whereas between 21-30% it was moderately resistant. The crop was declared susceptible when DI was 31-50% and when DI is above 50% crop is considered highly susceptible.

$$Disease\ incidence = \frac{Number\ of\ infected\ plant}{Total\ number\ of\ plants} X100$$

Isolation, Purification and Identification of Pathogen: The *Fusarium oxysporum* was isolated from infected pea roots which were received from farmer's fields. Roots were taken and cut into small pieces with sterilized scissors and then surface sterilized with 70% ethanol for 60 seconds. The roots were rinsed twice in sterile distilled water before transferring to sterilized filter paper in Petri plates for drying. Then sterilized infected parts were plated on Petri plates containing potato dextrose agar (PDA) medium. For transferring these infected roots parts, forceps were sterilized by dipping in methylated spirit and flaming several times. All Petri plates were incubated at 25 ^{0}C for 7 days and observed daily. The isolated pathogen was identified microscopically following the method of Altinok (2005) where sickle shaped macro conidia were confirmed with 3-5 septation and typical creamy colony developed on PDA.

Figure 1. *Fusarium oxysporum*

Figure 2. Macro conidia of *F. oxysporum*

Figure 3. Mycelium of *F. oxysporum*

Pathogenicity Test: Pathogenicity test was done for host specificity. The *F. oxysporum* f.sp. *pisi* was collected from wilted pea plant and multiplied on PDA. A spore suspension was prepared by washing spores from mycelia with sterile distilled water, followed by filtration through muslin cloth and adjustment of the spore concentration to 1 × 10^6 spores/mL with haemocytometer. The inoculation was done at the root zone of plants by drenching of spore suspension (Rahman *et al.,* 2011). Pathogenicity is then assessed from symptoms exhibited. Then the pathogen was reisolated and microscopic examination as for host specificity testing (Harun and Fatma, 2012).

Management experiment

***In-vitro* management:** Fungicides were suspended in sterilized distilled water to achieve final concentration of 400, 600 and 800ppm, then added to petri plates containing autoclaved PDA and spread on medium with the help of glass rod. Mycelial plugs of single-spore isolates of *F. oxysporum* were then transferred to the centre of the fungicide-amended media. The dishes were incubated for 7 days at 25 °C, after which colony diameters were measured (two perpendicular measurements per colony). The mycelial growth on the fungicide-amended PDA was compared with the growth of the pathogen on unamended PDA plates that served as controls. Each treatment were replicated five times. The *in-vitro* studies were conducted twice (Chakraborty *et al.,* 2009).

***In-vivo* management:** Highly susceptible pea variety (Climax) was cultivated for management trial where four fungicides i-e. Aliette, Native, Topsin-M and and Difenoconazole were applied @ recommended doses. The experiment was run under natural condition with artificial inoculation by disc of fully grown *F. oxysporum* on PDA and control treatment was sprayed by water.

Table 1. List of Varieties/advance lines

Sr. No.	Variety/advance line
1	018372
2	018374
3	026702
4	026709
5	026710
6	026711
7	026712
8	026714
9	026715
10	026723
11	Climax
12	Jumbo

Table 2. List of Fungicides.

Sr. No.	Fungicide	Active ingredient
1	Topsin M.	Thiophanate methyl 70% (WP)
2	Difenoconazol	Difenoconazol 25% W/V
3	Aliette	Phosytile aluminium 80% W/W
4	Nativo	Teboconazol 50% W/W
5	Ridomil	Metelaxyl 8% W/W Mancozeb 64% W/W
6	Copperoxychloride	Copper oxychloride 77%
7	Carbendazim	Carbendazim 50%
8	Mancozeb	Mancozeb 80%

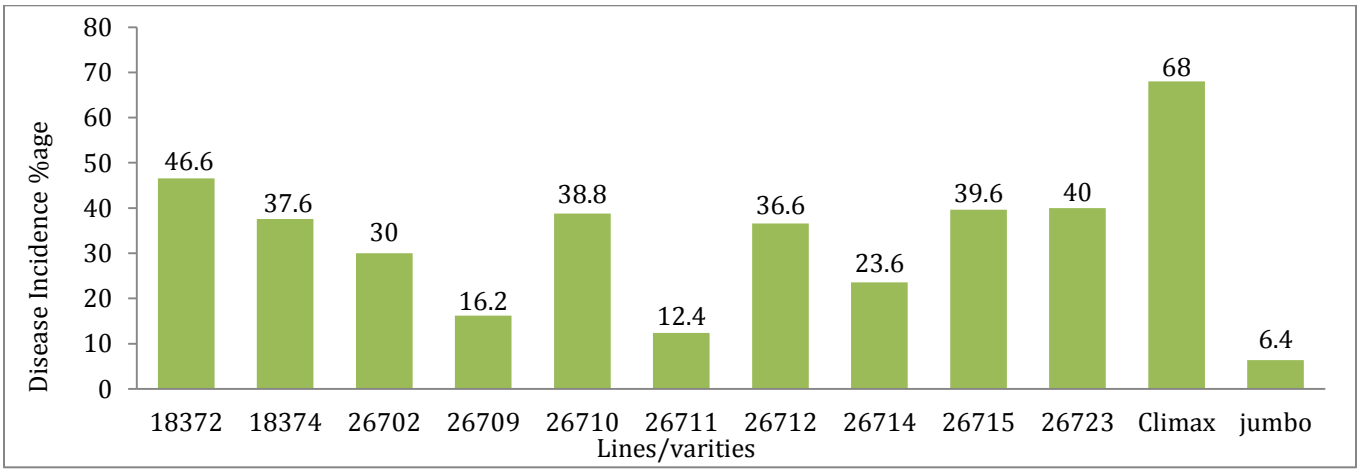
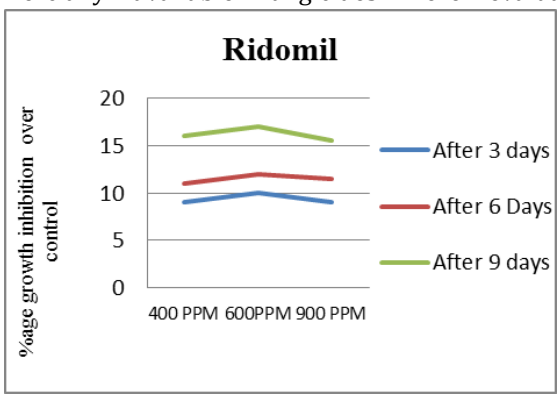
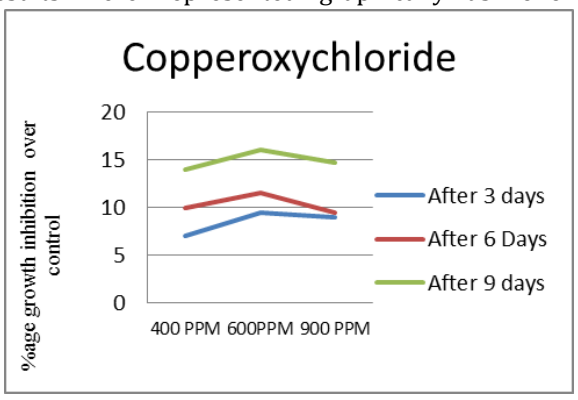


Figure 4. Pea genotypes screened against Fusarium wilt.

RESULTS

According to disease rating scale one variety (Jumbo) was found highly resistant. While the advance lines (026709and 026711) were found resistant to disease. Two lines (026702 and 026714) were moderately resistant and six lines (018372, 018374, 026710, 026712, 026715 and 026723) showed susceptible response. Moreover Climax was found highly susceptible. During management trials different commercially available fungicides were evaluated against *F. oxysporum* under laboratory conditions using poisoned food technique. Three concentrations (400, 600 and 800ppm) were tested for each chemical. Among all fungicides Topsin-M gave maximum reduction (22.5%) in fungal growth over control as compared to other fungicides. Mancozeb and carbendazim were found least effective. Among all fungicides Topsin M, Difenoconazol, Aliette and Nativo performed better results therefore these are also tested in field trial. Results were represented graphically as following,





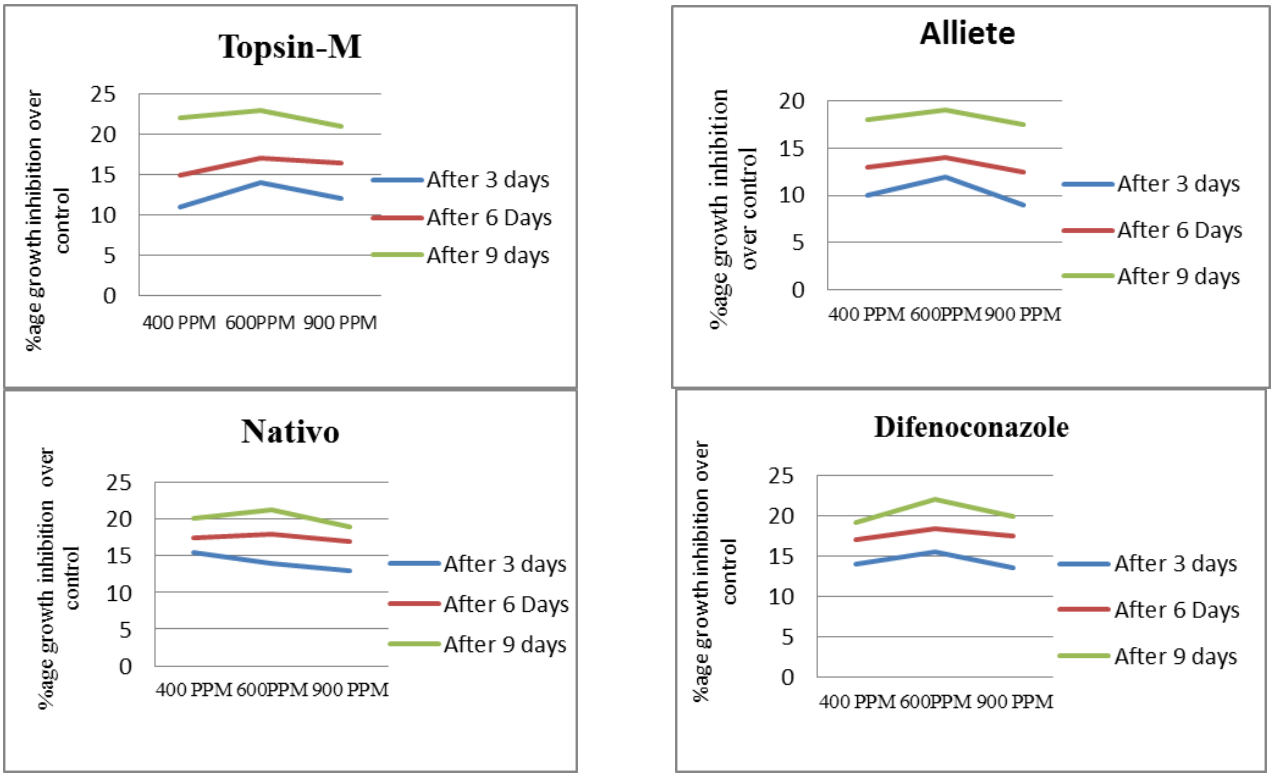


Figure 4. *In-vitro* management of *Fusarium* wilt.

Four fungicides were applied in field trials to evaluate their effectiveness against wilt disease with recommended doses. Again Topsin M. showed best result after 21 days 31% disease incidence was recorded.

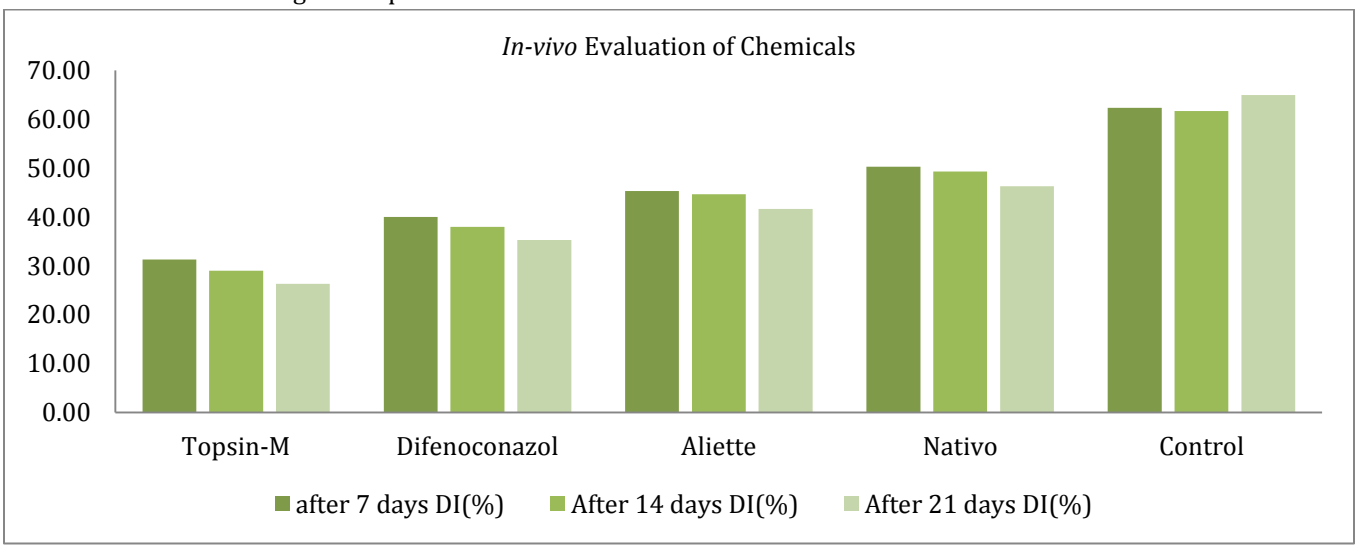


Figure 5. Incidence of *Fusarium* wilt against fungicides.

DISCUSSION

Pea wilt causes qualitative as well as quantitative losses to crop. Present study was an attempt to find resistant source in pea varieties/advance lines. Conventional breeding can be an option for development of resistant cultivars if resistant sources are available. Among all genotypes, one (Jumbo) was highly resistant with low disease incidence (6.4%) and it could be used in breeding programs for development resistance against disease. One variety (Climax) was found highly susceptible to Fusarium wilt with disease incidence upto 68.0% so, it was not preferred for commercial use.

Experiments were conducted under natural conditions. At present there are no efficient, economical and useful ways to manage Fusarium wilt. The recommended management practices are rotation of crops, use resistant varieties, soil sterility, solarization and use of different fungicides. As soil sterility through chemicals is not successful because under favorable environmental conditions macro conidia of the *Fusarium oxysporum* can be re-colonized (Yucel *et al*., 2007). Although many soil borne diseases have been managed by use of certain bacteria which are beneficial to plants and inhabitant of rhizosphere of the plants in recent times (Thomshaw, 1996). These bacteria are beneficial to plant by means of stimulating the growth of plants (Bloemberg and Lugtenberg, 2001) but proper application of biocontrol agents is not easy hence farmer always prefer chemical control for quick and easy method. Harpal and Singh (2001) controlled pea wilt by using several fungicides individually and in combinations i.e. Formaldehyde and Thimet as soil application. Fungicides that are used as seed treatment i.e. Baviston and Captan. Combination of Thimet and Captan as seed treatment proved most effective in reducing plant mortality and increasing the crop yield by using Thimet as soil application but these chemicals are not available in market now and fungicides with new chemistry was evaluated. In this experiment results revealed that Topsin M proved most effective in reducing the disease incidence as compared to other fungicides because of its systemic mode of action. It was observed that mycelial growth of pathogen was reduced by all chemicals. Topsin-M proved to be most effective against pathogen as compared to all fungicides evaluated *in-vitro* and *in-vivo* conditions. The results of performed experiments revealed that strategic use of fungicides should be considered as an element of integrated management of Fusarium wilt in pea crop.

CONCLUSION

Finally it was concluded that Topsin-M (Thiophanate methyl 70% WP) exhibited better performance against pea wilt out of tested fungicides and may be further use for disease management while Jumbo was the only variety which was resistant against *Fusarium oxysporum* f.sp. *pisi.*

REFERENCES

Altinok H.H., 2005. First report of Fusarium wilt of eggplant caused by *Fusarium oxysporum* f.sp *melongenae* in Turkey. Plant Pathology 54:577-579.

Anonymous, 2011. Economic Survey of Pakistan, Ministry of Finance, Government of Pakistan.

Basu, P.K., R. Crete, A.G. Donalldson, C.O. Gourley, J.H. Hass, F.R. Harper, C.H. Lawrence, W.L. Sesman, H.N.W. Toms, S.I. Wong, and R.C. Zimmer. 2004. Prevalence and severity of diseases of processing peas in Canada, 2001-2003. Can. Pl. Dis. Survey, 53: 49-57.

Bloemberg G.V. and B.J.J. Lugtenberg. 2001. Molecular basis of plant growth promotion and biocontrol by Rhizobacteria. Current Opinion of Plant Biology 4: 343-350.

Chakraborty M.R., N.C. Chatterjee and T.H. Quimio, 2009. Integrated management of Fusarial wilt of eggplant (*Solanum melongena*) with soil solarization. USA. Micologia Aplicada International 21: 25-36.

Charchar, M. and J.M. Kraft, 1989. Response of near-isogenic pea cultivars to infection by *Fusarium oxysporum* f. sp*. pisi* races 1 and 5. Can. J. Plant Sci. 69:1335-1346.

Hagedorn, D.J., 1991. Handbook of Pea Diseases A1167. University of Wisconsin- Extension, Madison.

Harpal, S. and I. singh, 2001. Chemical control of pea stem fly (*Ophiomyia phaseoli*) and wilt complex of peas. Agric. Sci. Digest 21(3): 192-193.

Harun B. and S.D. Fatma, 2012. Pathogenic variability of *Fusarium oxysporum* f.sp. *ciceris* isolates from chickpea in Turkey. Pak. J. Bot. 44: 821-823.

Nisar, M., A. Ghafoor, M.R. Khan and A.S. Qureshi, 2006. Screening of *Pisum sativum* L. germplasm against *Erysiphe pisi* syd. Acta Biological Cracoviensia Series Botanica 48/2: 33-37. Islamabad, Pakistan.

Persson, L.L. Bodker and M.L. Wikstrom, 2007. Prevalence and pathogenicity of foot and root rot pathogen of pea in Southern Scandinavia. Pl. Dis. 81: 171-174.

Rahman M.A., F. Ali and K.M.A. Hossain, 2011. Shoot and Root Growth and Yield of Eggplant Cultivars as Affected by Wilt Disease. J. Exp. Sci. 2: 11-15.

Smart, J. 2000. Grain Legumes: Evolution and Genetic Resources. Cambridge University Press, Cambridge, UK.

Thomshaw L.S., 1996. Biological control of plant pathogens. Current Opinion 77: 343-347.

Yucel S., Y.H. Elekcioolu, C. Can, M.A. Soout and A. Ozarslandan, 2007. Alternative Treatments to Methyl Bromide in the Eastern Mediterranean Region of Turkey. Turk. J. Agric. 31: 47-53.