



Official publication of Pakistan Phytopathological Society  
**Pakistan Journal of Phytopathology**

ISSN: 1019-763X (Print), 2305-0284 (Online)

<http://www.pakps.com>



**SCREENING OF PEAS (*PISUM SATIVUM*) VARIETIES/ LINES AGAINST FUSARIUM WILT (*FUSARIUM OXYSPORUM* F. SP. *PISI*) AND *IN VITRO* EVALUATION OF FUNGICIDES AGAINST MYCELIAL GROWTH OF PATHOGEN**

<sup>a</sup>Syed K. Husnain\*, <sup>b</sup>Sabir H. Khan, <sup>c</sup>Muhammad Atiq, <sup>c</sup>Nasir A. Rajput, <sup>d</sup>Waseem Abbas, <sup>e</sup>Muhammad Mohsin

<sup>a</sup> Barani Agricultural Research Institute, Chakwal, Pakistan.

<sup>b</sup> Plant Pathology Research Institute, Faisalabad, Pakistan.

<sup>c</sup> University of Agriculture, Faisalabad, Pakistan.

<sup>d</sup> Vegetable Research Institute, Faisalabad, Pakistan.

<sup>e</sup> National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan.

**ABSTRACT**

Twenty Peas (*Pisum sativum* L.) varieties/ lines were evaluated against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *pisi* by sowing them in sick plot during the year of 2016-17 at the Plant Pathology Research Institute, Faisalabad. Each cultivar/line was planted in a single row of three meter length, with plant to plant and row to row distances of 15cm and 30 cm respectively and replicated thrice by following Randomized Complete Block Design (RCBD). Out of these twenty varieties/ lines 13 including check variety Olympia were found highly susceptible ranging from 53.2 to 83.5% plant mortality. Six varieties/lines were susceptible ranging from 30.3 to 44.1 % plant mortality. Only a single variety Garrow performed as moderately resistant by showing 21% plant mortality in the field. Efficacy of five fungicides against *Fusarium oxysporum* f. sp. *pisi*, at various concentrations was evaluated in-vitro and significant variations among treatments was observed. In general there was a significant decrease in mycelial growth of the fungus with an increase in concentration of fungicides. Tilt (Propiconazol), Daconil (Chlorothalonil) and Crest (Carbendazim) were the most effective fungicides in inhibiting the growth of the fungus in descending order. The Tilt almost 90% inhibited the growth @ 50µg/ml concentration, Daconil and Crest exhibited intermediate effectiveness. Topsin-M (Thiophanate-methyl) and Score (Difenoconazole) were the least effective fungicides.

**Keywords:** Screening, peas germplasm, wilt, *Fusarium oxysporum*, Fungicides evaluation,

**INTRODUCTION**

Peas (*Pisum sativum* L.) a vegetable /pulse crop belongs to family leguminosae mainly cultivated in winter throughout the tropics including Burma, India, Ethiopia, Morocco, Columbia, Ecuador, Peru, and Pakistan. (Nawab *et al.*, 2008, Javaid *et al.*, 2002). Its significance can be estimated from the fact that peas are ranked as fourth important legume crop and in the world 40% of total pulses trade relies over peas (Hulse, 1994). They

*Submitted: December 31, 2018*

*Revised: May 4, 2019*

*Accepted for Publication: May 29, 2019*

\* Corresponding Author:

Email: [kamiluaf@gmail.com](mailto:kamiluaf@gmail.com)

© 2017 Pak. J. Phytopathol. All rights reserved.

are a rich source of protein (15.5 to 39.7%) carbohydrates, vitamins, minerals and fibers, not only consumed as a pulse but also used as a fresh vegetable. In Pakistan peas are cultivated on an area of 82.8 thousands hectares and production 57 thousands tones (FAO, 2011). Among the total cultivated area under peas, 71.2% is present in Punjab province, which is followed by 12.8% in KPK, 11.3% in Baluchistan and 4.7% in Sindh (Khokhar, 2014). Optimum temperature for the growth of peas ranges from 10°C to 21°C, increase in temperature adversely affect the vegetative growth and pollination. (Davies *et al.*, 1985).

Several pathogenic fungi attack on peas in which most destructive are *Ascochyta pisi*, *Botrytis cinerea*, *Cladosporium pisicola*, *Erysiphe polygoni* *Sclerotinia*

*sclerotiorum*, *Peronospora pisi*, and *Pythium species*. (McPhee, 2003). Among various devastating diseases *Fusarium* wilt is an acute disease of peas in Pakistan caused by *Fusarium oxysporum* f. sp. *pisi* which is both seed as well as soil borne pathogen. (Pande *et al.*, 2007). *Fusarium oxysporium* causing pea wilt has been reported in every pea growing area of the world (Hagedorn, 1984). The Pathogen with two pathotypes and eight pathogenic races causes partial to complete loss of pea crop. In case of wilting pathotype plant death followed by chlorosis, flaccidity and vascular discoloration appear within 20 days after inoculation Whereas in yellowing pathotype vascular discoloration with plant death took place between 40 days (Haware and Nene, 1982). Fungus can survive on crop residues upto 6 years (Haware *et al.*, 1978). Presence of disease inoculums and cultivation of susceptible variety causes huge losses to crop. Symptoms on plant include chlorosis that cause leaflets to curl downward and flaccid. The plants show wilting and develop a yellowish brown color. Lower subterranean portion of the stem turns larger than normal and below and above ground vascular system become light yellow to brick-red color (Kraft, 1994). *Fusarium* wilt root rot and near wilt disease severely restrict the productivity of pea crop and, may induce 10-50% crop loss every year in Pakistan. (Person *et al.*, 1997; Khan *et al.*, 2002).

Detection of new varieties is important, because prevailing resistant variety may become susceptible to new physiological races of the pathogen. Present study was conducted to evaluate newly developed genotypes of peas for resistance against wilt in order to identify the source of resistance. The results of this study will be useful for breeders to design effective resistance breeding program in peas.

In-vitro evaluation of fungicides provides useful information about their efficacy against pathogen in very Disease rating scale

Disease Rating	% Infection	Disease response
1	0-10% plant wilted	Highly Resistant
3	11-20% plant mortality	Resistant
5	21-30% plant mortality	Moderately Resistant
7	31-50% plant mortality	Susceptible
9	More than 50% plant mortality	Highly Susceptible

**DATA ANALYSIS**

The data collected were analyzed according to Randomized Complete Block Design (RCBD). The analysis

short time and prevent from economic loss by minimizing the risk of side effects on crop and environment. So it must be sustained to measure the toxicity of fungicides against a particular pathogen.

**MATERIAL AND METHOD**

**Sick Plot Preparation:** Sick plot was prepared and maintained by incorporating disease plant debris followed by repeated cultivations of susceptible variety. The pathogen *Fusarium oxysporum* was isolated from diseased plants and was maintained on potato dextrose agar (PDA) medium at 25± 2°C. For mass multiplication of pathogen the half boiled sorghum seeds were used as substrate in 2% sucrose solution and then inoculated with fungal discs under a septic condition and incubated for 21 days at room temperature. To increase inoculum potential the cultured inoculum was incorporated in wilt sick plot. (Nene *et al.*, 1981)

**Screening of germplasm:** During November 2016, twenty varieties/lines of peas obtain from Vegetable Research Institute, Faisalabad and Pulses Research institutes, Faisalabad were sown in research area of Plant Pathology Research Institute Faisalabad. The source of origin of the varieties/lines was local. Each line was planted in a single row of three meter length, with plant to plant distance 15 cm and row to row 30 cm, replicated thrice by following Randomized Complete Block Design (RCBD). Olympia was used as susceptible check variety. All agronomic practices were same for all varieties/ lines.

**Data Collection:** According to Ahmad *et al.*, 2010 disease incidence was recorded in mid of March 2015 at reproductive stage and calculated by formula given below.

$$\text{Wilt incidence (\%)} = \frac{\text{Number of wilted plant}}{\text{Total number of plant}} \times 100$$

The level of susceptibility and resistance of each test line/variety was determined by using following 1-9 rating scale suggested by. Iqbal *et al.* (2005).

of variance (ANOVA) and the differences among means were analyzed by applying LSD test at 5% level of probability. (Steel *et al.*, 1997)

**IN VITRO EVALUATION OF FUNGICIDES FOR THE DETERMINATION OF THEIR COMPARATIVE EFFICACY AGAINST THE MYCELIAL GROWTH OF *Fusarium oxysporium*:**

The sensitivity of mycelial growth of *F. oxysporium* against five fungicides (Thiophanate methyle, Difenconazole, Carbandazim, Propiconazol, Chlorothalonil) was evaluated at various concentrations i.e. 5, 10, 20, 50 µg/ml using modified Borum and Sinclaris, technique (Borum and Sinclaris, 1968). Weighed quantity of each fungicide at different doses was amended into the flask containing sterilized media separately to obtain required concentration. PDA without fungicides was used as control. 25 ml of the medium was poured in each of the 90 mm (diameter) petriplate, after solidification 5mm agar plugs containing *F. oxysporium* mycelium were taken from 10 days old PDA culture plates using sterilize cork borer and placed in the center of each petriplate. The inoculated petriplates were incubated at 22 ± 2°C and linear mycelium growth of *F. oxysporium* in mm was recorded after 7, 14 and 21 days of incubation. Growth inhibition of the pathogen was calculated by the formula given by Sunder *et al.*, 1995.

$$\text{Percent inhibition} = \frac{A - B}{A} \times 100$$

Here, A = Colony growth in control plates; B= Colony growth in fungicide treated plates and data were analyzed statistically to see the differences among various treatments.

**DATA ANALYSIS**

The data collected were analyzed according to Complete Randomized Design. The analysis of variance (ANOVA) and the differences among means was analyzed by applying LSD test at 5% level of probability (Steel *et al.*, 1980).

**RESULTS**

According to the present study among 20 varieties/lines of peas thirteen (Metor, FS-2187, 2002-20, 5180, CLIMAX, 2001-40, 9800-10, Pea-09, 6173, R1-70P11-1G, R1-5PF4000E, Olympia, 92000-1) were highly susceptible to the disease with more than 50% plant mortality in descending order. Among these highly susceptible varieties /lines first eleven varieties exhibit greater disease incidence as compared to the check variety. Six varieties /lines (Winner, R13-DP09-22C, R1-1DP-09-33A, R12-DP-09-08B, R1-6N0267F, R14DP09-07D) were rated as susceptible with 31 to 50 % plant mortality. Only a single variety Garrow responded as moderately resistant with 21% plant mortality. Not, even a single variety/line found resistant against the disease (Table 1 and Figure 1).

Table 1. Disease incidence data of peas varieties/lines against *Fusarium oxysporum* f. sp. *pisi*.

Sr.#	Variety	Adjacent mean	Difference among varieties	Disease responses
1	Metor	83.5	A	HS
2	FS-2187	82.7	A	HS
3	2002-20	82.6	A	HS
4	5180	82.5	A	HS
5	CLIMAX	82.3	A	HS
6	2001-40	79.9	A	HS
7	9800-10	77.0	AB	HS
8	Pea-09	74.1	ABC	HS
9	6173	73.2	ABC	HS
10	R1-70P11-1G	63.3	BCD	HS
11	R1-5PF4000E	61.0	CD	HS
12	Olympia	54.6	DE	HS
13	92000-1	53.2	DEF	HS
14	Winner	44.1	EFG	S
15	R13-DP09-22C	42.1	EFG	S
16	R1-1DP-09-33A	41.6	EFG	S
17	R12-DP-09-08B	39.6	FG	S
18	R1-6N0267F	35.0	G	S
19	R14DP09-07D	30.3	GH	S
20	Garrow	21.0	H	MR

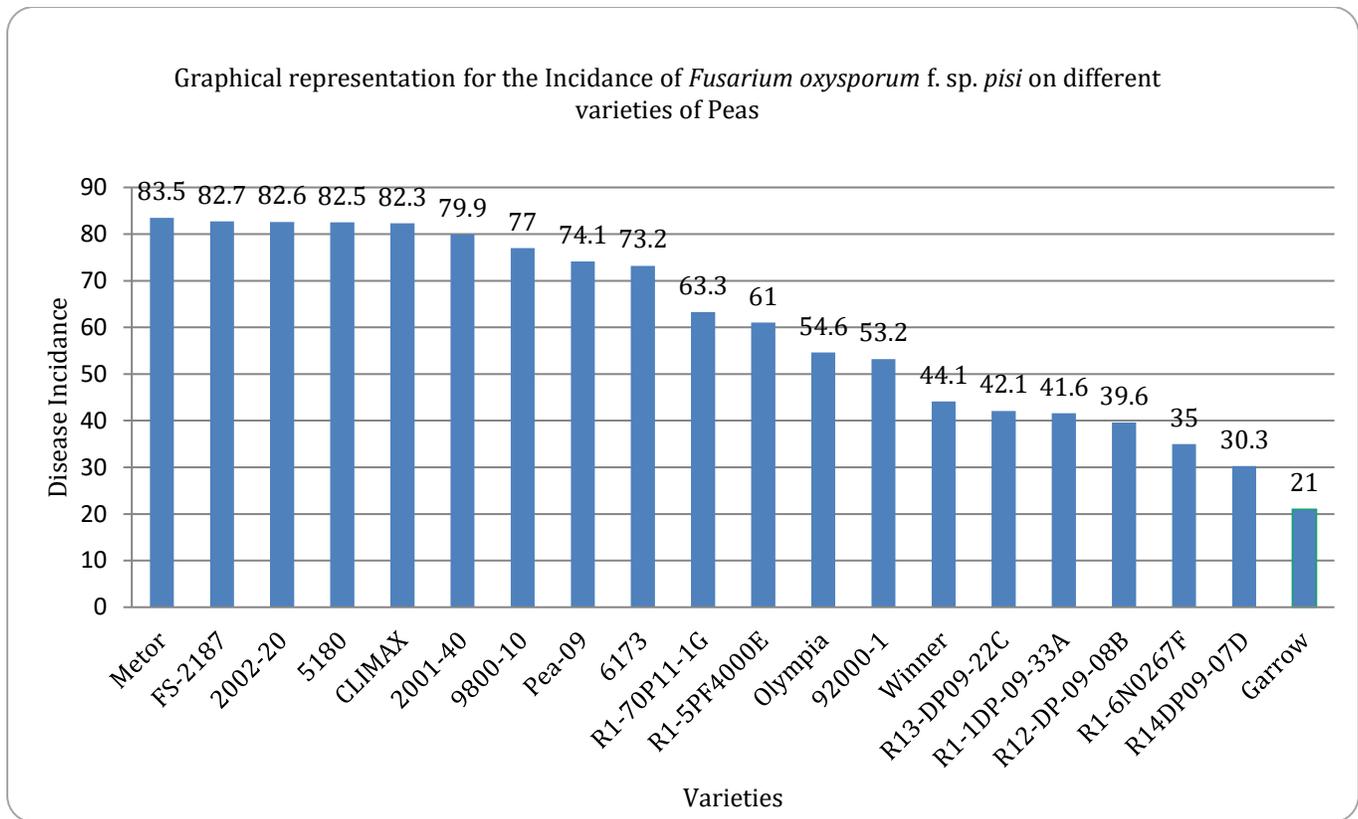


Figure 1: Evaluation of pea varieties against *Fusarium oxysporum* f. sp. *pisi* under field conditions

The data of present study reveals that most of the commercial varieties and lines of peas are susceptible or highly susceptible to the *Fusarium oxysporum* f. sp. *pisi*. In current situation there is a dire need to develop resistant genetic material against the disease by incorporating resistant exotic germplasm in breeding programme. Meanwhile use of systemic seed dressing fungicide may also be recommended to the farmer to combat the problem.

**IN VITRO EFFECT OF SOME FUNGICIDES AT DIFFERENT CONCENTRATION ON MYCELIAL GROWTH OF *Fusarium oxysporium*:** In-vitro testing of fungicides revealed that the sensitivity of *Fusarium oxysporium* mycelium varied greatly against five fungicides. In general there was a significant decrease in mycelial growth of the fungus with an increase in fungicides concentration.

When growth of the fungus (*Fusarium oxysporium*) in response to various fungicides at different concentration after incubation period of 7 days was examined, it was observed that Tilt, Score, Topsin-M, Daconil, and Crest reduced the mycelial growth as compared to control.

The most effective fungicides for inhibiting the growth of fungus in descending order were Tilt, Daconil, Crest,

Topsin-M, and Score caused 89.16, 85.86, 70.18, 50.03 and 35.39 percent reduction in mycelial growth respectively.

However after incubation period of 14 days Tilt, Daconil, Crest, Topsin-M and Score caused 78.99, 74.53, 62.59, 41.76 and 23.09 percent reduction in mycelial growth of fungus respectively.

Similarly, after incubation period of 21 days Tilt, Daconil, Crest, Topsin-M, and Score caused 72.40, 69.04, 57.57, 36.93 and 19.73 percent reduction in mycelial growth respectively (Table 3 and Figure 3).

Thus the most effective fungicide in inhibiting the mycelial growth of fungus was Tilt at the dosage rate of 50µg/ml which causes 89.16% reduction while Daconil came out to be second effective fungicide which causes 85.57% reduction in mycelial growth of *Fusarium oxysporium*, followed by Crest and Topsin-M which were intermediate in their effect on fungal growth.

However, Score proved to be least effective fungicide against *Fusarium oxysporium* at the dosage rate of 50µg/ml which cause 19.72% reduction of the mycelial growth of the fungus (Table 2 and Figure 2).

At all dosage rates tested the effectiveness of fungicides was statistically significant.

Table 2. Efficacy of different concentrations of various fungicides against *Fusarium oxysporum* after 7, 14 and 21 days

Fungicides	Mean colony growth (mm) at various concentration (µg/ml) after 7 days				Mean colony growth (mm) at various concentration (µg/ml) after 14 days				Mean colony growth (mm) at various concentration (µg/ml) after 21 days			
	5%	10%	20%	50%	5%	10%	20%	50%	5%	10%	20%	50%
Tilt (propiconazole)	11.46j	7.8l	5.0 o	2.4 q	19.5 i	14.16k	9.07 n	7.06 o	27.0 h	19.9 k	13.8 n	12.0 o
Daconil (Chlorothalonil)	12.57i	9.0 k	5.8 n	3.13 p	20.57h	16.50j	11.0m	8.56 n	28.5 g	22.5 j	16.0m	13.5 n
Crest (Carbendazim)	13.53h	11.26j	8.53 k	6.60m	23.53g	20.0hi	16.50j	12.57l	31.5 f	27.5gh	24.0 i	18.5 l
Topsin-M (Thiophanate-methyl)	17.16d	15.17f	13.27h	11.06j	28.57d	25.57f	23.53g	19.57i	39.0 c	34.5 e	32.2 f	27.5gh
Score	19.80b	18.06c	16.36e	14.30g	31.67b	30.57c	27.57e	25.57f	43.0 a	41.3 b	37.5 d	35.0 e
Control (Difenoconazole)	22.13a	22.13a	22.13a	22.13a	33.6 a	33.6 a	33.6 a	33.6 a	43.6 a	43.6 a	43.6 a	43.6 a

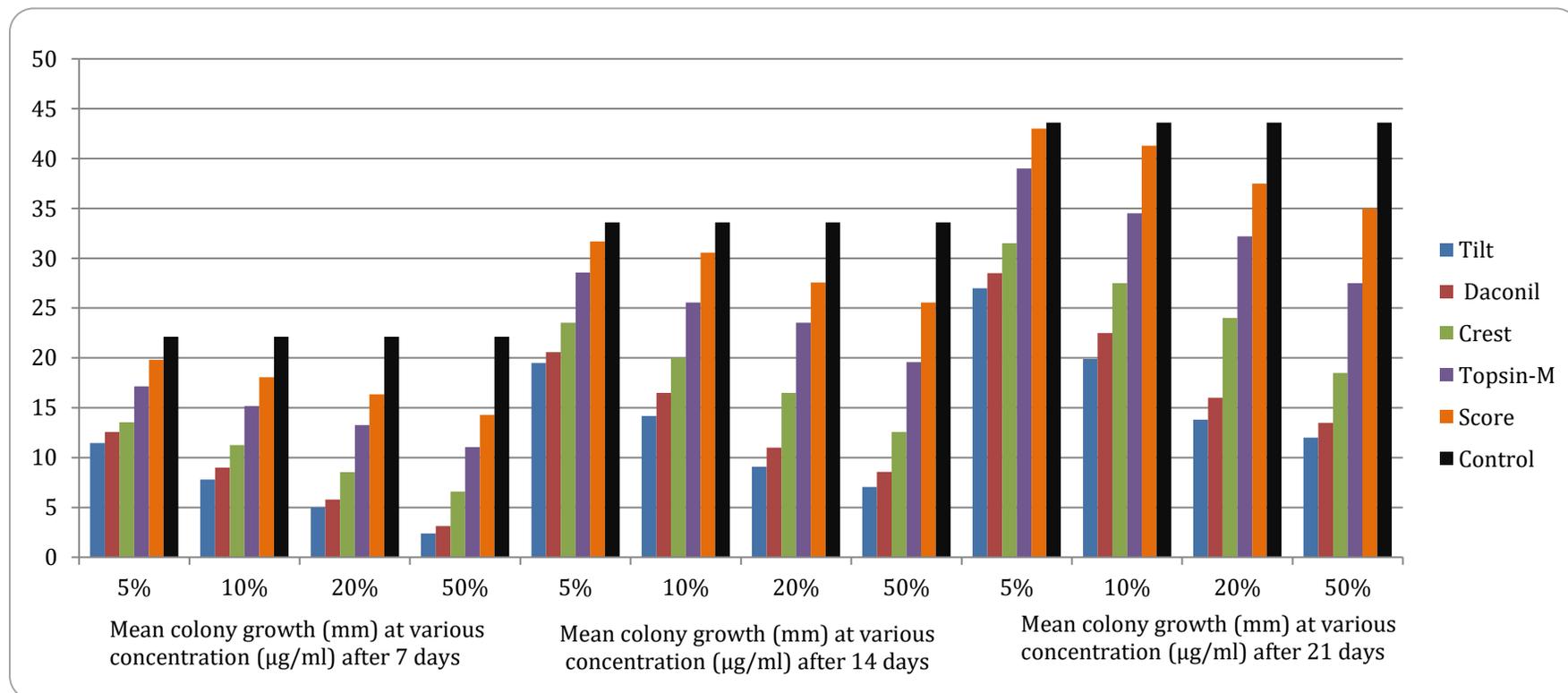


Figure 2: Efficacy of different concentrations of various fungicides against *Fusarium oxysporum* after 7, 14 and 21 days

Table 3. Percent decrease over control of different concentrations of various fungicides against *Fusarium oxysporium* after 7, 14 and 21 days.

Fungicides	Active ingredient	7 days				14 days				21 days			
		5%	10%	20%	50%	5%	10%	20%	50%	5%	10%	20%	50%
Tilt	Propiconazole	51.78%	35.24%	22.59%	10.84%	58.03%	42.14%	26.99%	21.01%	61.92%	45.64%	31.65%	27.52%
Daconil	Chlorothalonil	56.80%	40.66%	26.20%	14.14%	61.22%	49.10%	32.73%	25.47%	65.36%	51.60%	36.69%	30.96%
Crest	Carbendazim	61.13%	50.88%	38.54%	29.82%	70.02%	59.52%	49.10%	37.41%	72.24%	63.07%	55.04%	42.43%
Topsin-M	Thiophanate-methyl	77.54%	68.54%	59.96%	49.97%	85.02%	76.10%	70.02%	58.24%	89.44%	79.12%	73.85%	63.07%
Score	Difenoconazole	89.47%	81.60%	73.92%	64.61%	94.25%	90.98%	82.05%	76.10%	98.62%	94.72%	86.00%	80.27

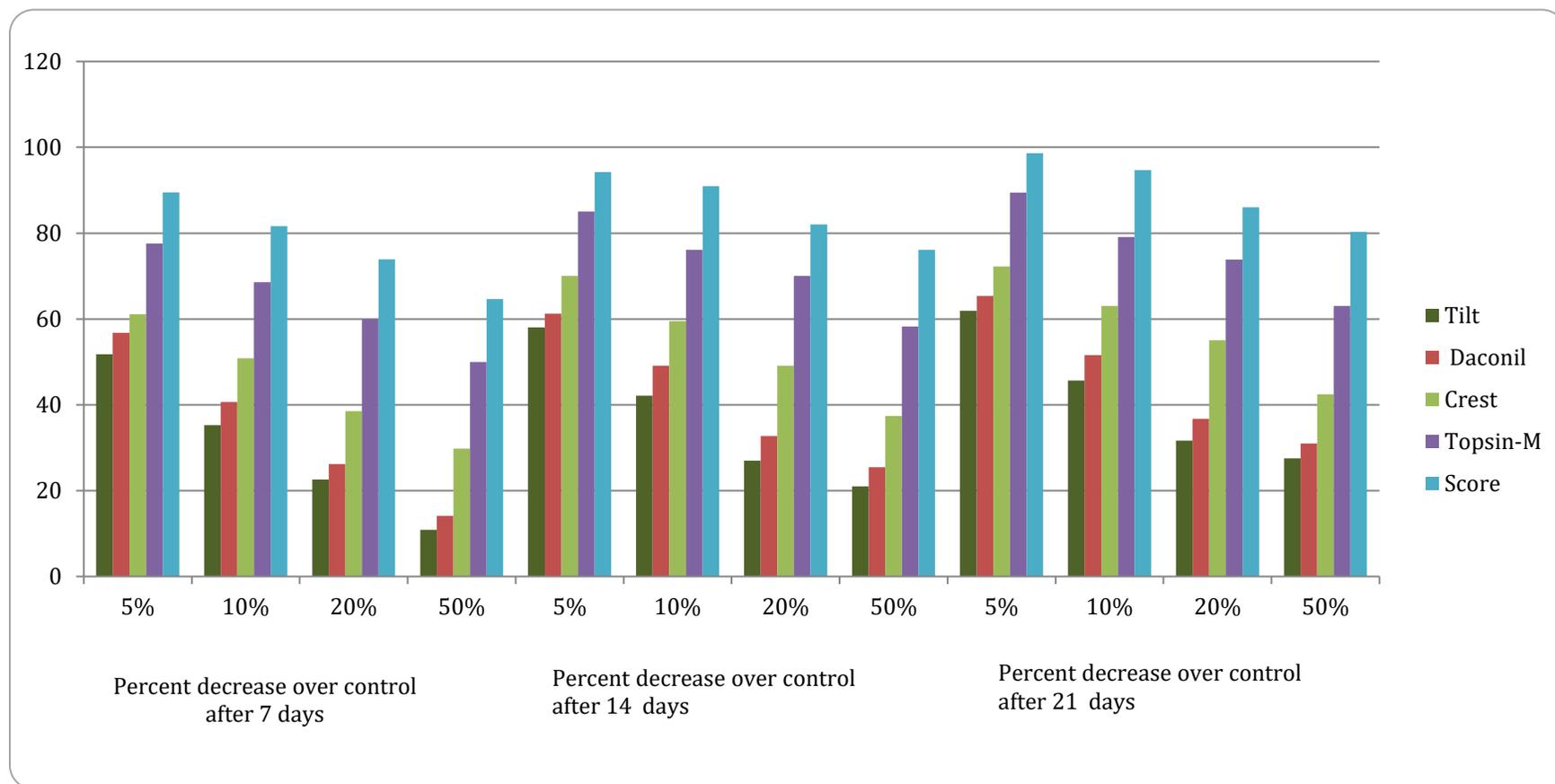


Figure 3: Percent decrease over control of different concentrations of various fungicides against *Fusarium oxysporium* after 7,14 and 21 days.

## DISCUSSION

In current study most of the varieties were found highly susceptible so available germplasm of peas is not suitable for the further breeding purpose. Similar study was conducted Khan *et al.*, 2016 and found 25 varieties moderately susceptible. Hannan *et al.*, 2014 also evaluate the peas germplasm against Fusarium wilt and observed the various response. The effectiveness of fungicides tested in inhibiting mycelial growth of *Fusarium oxysporum* varied a great deal and there was in general, a significant increase in the inhibition of mycelial with an increase the fungicides concentration or in other way, there was significant decrease in mycelial growth with an increase in concentration of each of the test fungicides. The most effective fungicide in inhibiting the growth of the fungus in descending order was Tilt, Daconil and Crest. The Tilt almost 90 percent inhibited the growth at 50µg/ml concentration while Topsin-M and Score were the least effective. Results of current study are similar and differ at the same time to the Ilyas *et al.*, 1992 who reported that Benomyl, Topsin-M, (Thiophenate-methyl), Tilt (propeconazole) and Rhizolax were the most effective in inhibiting the *F. oxysporum f.sp. ciceris*. Daconil (85 percent) also inhibited the fungal growth at 50µg/ml concentration. The effectiveness of Daconil as non-systemic fungicide with high fungi toxicity to several pathogen and nonpathogenic fungi is also reported by (Vyas, 1984). Crest (70.58 percent) proved to be the third effective fungicide to inhibit the fungus growth even at 10µg/ml it inhibited the growth of fungus almost half, results are also similar to (Maitlo *et al.*, 2014) who reported the efficacy of Crest (carbendazim) even at very low concentration (1ppm) against the fungus.

Topsin-M and Score were the least effective fungicides in habiting the mycelial growth of Fusarium. So present study contradict to the Hannan *et al.*, 2014 and Khan *et al.*, 2016. The low toxicity of these compounds to the test fungus may be due their continues use in the field as compared to the other tested fungicides. Present study reveals that all fungicides show at least inhibitory effect on the mycelial growth of pathogen even at lowest concentration. Keeping in view the incidence and severity of disease all these chemicals could be used in rotation for the management of the disease.

## REFERENCE

Ahmad, M. A., S. M. Iqbal, N. Ayub, Y. Ahmad and A. Akram. 2010. Identification of resistant sources in chickpea

against Fusarium wilt. Pak. J. Bot, 42: 417-426.

- Borum, D. E. and J. Sinclair. 1968. Evidence for systemic protection against *Rhizoctonia solani* with vitavax in cotton seedlings. Phytopathology, 58: 976-&.
- Davies, D., G. Berry, M. Heath and T. Dawkins. 1985. Pea (*Pisum sativum* L.). Grain Legume Crops. Collins, London, UK: 147-198.
- Food and Agriculture Organization of the United Nations. 2011
- Hagedorn, D. 1984. Compendium of pea diseases. 57 p. Am. Phytopathol. Soc., St. Paul, Minnesota, USA.
- Hannan, A., S. T. Sahi, I. Ahmed and A. A. Choudhry. 2014. Differential impact of *Fusarium oxysporum f. sp. pisi* on resistance source of pea genotypes and its chemical management. Pakistan Journal of Phytopathology, 26: 91-96.
- Haware, M. P. 1978. Eradication of *Fusarium oxysporum f. sp. ciceri* Transmitted in Chickpea Seed. Phytopathology, 68: 1364.
- Haware, M.P. and Nene, Y.L., 1982. Races of *Fusarium oxysporum f.sp. ciceri*. Plant disease, 66 (9), pp.809-810.
- Hulse, J. H. 1994. Nature, composition, and utilization of food legumes. Expanding the Production and Use of Cool Season Food Legumes. Springer Netherlands, pp. 77-97.
- Ilyas, M., M. Iqbal and K. Iftikhar. 1992. Evaluation of some fungicides against *Fusarium oxysporum f. sp. ciceris* and chickpea wilt. Pakistan Journal of Phaytopahtology, 4: 5-8.
- Iqbal, S. M. 2005. Screening of chickpea genotypes for resistance against Fusarium wilt. Mycopath (Pakistan).
- Javaid, I. A., A. Ghafoorm and R. Anwar. 2002. Evaluation of local and exotic pea *Pisum sativum* germplasm for vegetable and dry grain straits. Pak. J. Bot, 34: 419-427.
- Khan, I., S. Alam and A. Jabbar. 2002. Selection for resistance to Fusarium wilt and its relationship with phenols in chickpea.
- Khan, S. A., A. Awais, N. Javed, K. Javaid, A. Moosa, I. U. Haq, N. A. Khan, M. U. Chattha and A. Safdar. 2016. Screening of pea germplasm against *Fusarium oxysporum f. sp. pisi* and *in vitro* management through chemicals. Pakistan Journal of Phytopathology, 28: 127-131.
- Khokhar, M. 2014. Production status of major vegetables

- in Pakistan, their problems and suggestions. Agric. Corner, 9.
- Kraft, J. M. 1994. Fusarium wilt of peas (a review). Agronomie, 14: 561-567.
- Maitlo, S., R. Syed, M. Rustamani, R. Khuhro and A. Lodhi. 2014. Comparative efficacy of different fungicides against fusarium wilt of chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany, 46: 2305-2312.
- McPhee, K. 2003. Dry pea production and breeding. Food, Agri Environ, 1: 64-69.
- Nawab, N. N., G. M. Subhani, K. Mahmood, Q. Shakil and A. Saeed. 2008. Genetic variability, correlation and path analysis studies in garden pea (*Pisum sativum* L.). J. Agric. Res, 46: 333-340.
- Nene, Y., M. Haware and M. Reddy. 1981. Chickpea diseases: resistance-screening techniques.
- Pande, S., J. N. Rao and M. Sharma. 2007. Establishment of the Chickpea Wilt Pathogen *Fusarium oxysporum* f. sp. *ciceris* in the Soil through Seed Transmission. The Plant Pathology Journal, 23: 3-6.
- Persson, L., L. Bødker and M. Larsson-Wikström. 1997. Prevalence and pathogenicity of foot and root rot pathogens of pea in Southern Scandinavia. Plant Disease, 81: 171-174.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, a biometrical approach. McGraw-Hill Kogakusha, Ltd.
- Sundar, A. R., N. Das and D. Krishnaveni. 1995. *In-vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of Castor. Indian Journal of Plant Protection, 23: 152-155.
- Vyas, S. C. 1984. Systemic fungicides. Systemic fungicides.