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SCREENING OF PEA GERMPLASM AGAINST FUSARIUM OXYSPORUM F. SP. PISIAND IN VITROMANAGEMENT THROUGH CHEMICALS

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ABSTRACT

The present study was designed to evaluate response of pea (*Pisum sativum* L.) germplasm against *Fusarium oxysporum* f.sp. *pisi* associated with pea wilt disease. A total of 32 different varieties of pea were screened. Among these, 25 were moderately susceptible, 6 susceptible and 1 was highly susceptible. *In vitro* evaluation of the antifungal effect ofsix different chemical formulations viz.,Raxil Ultra (Tebuconazole), Topsin-M(Thiophanate Methyl), Score (Difenconazole), Derosil (Carbendazim), Hombre (Imidacloprid+Tebuconazole) and Divident Star (MetalaxylM+Difenconazole) was conducted at different concentrations i.e., 5,10,20,25,50 and 100ppm against mycelial growth of *F. oxysporum*f.sp. *pisi*.Score was found highly effective at 5ppm and 10ppm. Raxil Ultra produced best inhibition at 100ppm followed by Topsin-M. Raxil Ultra and Topsin-M were then tested *in vivo* as a seed treatment. Raxil Ultra produced highest seed germination rate followed by Topsin-M compared to control. Score, Topsin-M and Raxil Ultra can be recommended for control of this disease.

Keywords: Fungicides, Fusarium wilt, *Pisumsativum* L., screening, seed germination.

INTRODUCTION

Pea (*Pisum sativum* L.) is the fourth important leguminous crop in the world (Hulse, 1994). Peas are cultivated as a winter annual crop because they require cool and humid climate with an average temperature of 7 ºC to 30 ºC (Duke, 1981; Davies, 1985). Pea crop has high nutritional value because it contains 15.5 to 39.7% protein contents (Davies *et al.,*1985).In Pakistan pea production is facing many biotic and abiotic threats among them biotic diseases constitute the most important factor that reduceaverage yield by direct attack on the grains of the crop. Fusarium wilt is the most important fungal disease which can attack numerous crops ultimately leading to complete yield loss (Basu *et al.,* 1973).It is one of the most common fungal threat to pea production in Pakistan having significant economic consideration. *Fusarium oxysporum* f. sp. *lycopersici* is a soil borne pathogen that isattributed to huge yield loss worldwide. The pathogen has the ability to survive in soil for an indefinite period of time, during which it undergoes different biological phenomenoni.e., competition and environmental stress that leadto continuous development of new physiological races of the pathogen (Jones *et al.,* 1991). Currently, there are no effective and economical methods for management of this disease. The quickest and effective method to control Fusarium wilt diseases is with fungicides (Moosa *et al.,* 2016).Chemical management by employing the potential of fungicides have been practiced for many year to control several fungal diseases (Bharat *et al.,* 2006).Soil borne nature of the pathogen has made it difficult to control this disease. Therefore, resistant germplasm depending upon its availability has proved to be a reliable approach to manage this disease (Nelson, 1981).It is always desirable tointegrate different management strategies to reduceyield loss.

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The present project was undertaken to evaluate the response of new pea germplasm against *F. oxysporum* f.sp. *pisiin vivo* and to check the efficacy of fungicides against the pathogen *in vitro* and *in vivo*.

MATERIAL AND METHODS

Pathogen culture:*F. oxysporum* f. sp. *pisi* was isolated from infected pea plants collected form Plant Pathology Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan exhibiting typical wilt symptom. Infected samples were surface sterilized and placed on potato dextrose agar (PDA) medium followed by incubation at 25± 1 ºC. Pathogen was identified on the basis of microscopic features described in keys and literatureunder high magnification 100X.Pathogen was purified and incubated at 25± 1 ºC for further use.

Pathogenicity test:Pathogenicity test was conducted to satisfy Koch's postulates. Pea seeds cv. Samrinazard were washed thrice with water, surface sterilized with sodium hypochlorite and sown in field area of AARI. Inoculum of the pathogen was prepared by multiplying the culture on PDA medium and scraping the colony growth of the pathogen in sterilized distilled water with a scraping needle. Spore density of the inoculum suspension was adjusted to 1×10^6 spores/mL using a hemocytometer (Omar *et al.,* 2006). Root zone of 5-week-old seedlings was inoculated with inoculum suspension following drench method (Rehman *et al.,* 2014). Plants were observed daily for the development of infection. After successful establishment of infection pathogen was re-isolated from infected plants and identified under high magnification 100X.

Screening of Germplasm: Pea germplasm was collected from Vegetable Research Centre, AARI, Faisalabad, Pakistan. *In vivo* screening ofpea germplasm was conducted at field area of AARI. Sick plot was infested with the pathogen was prepared. Thirty two different varieties were sown in sick plots of size 3.35× 0.61m,with row to row distance 0.3m and variety to variety distance0.61m in an augmented design under natural field conditions. The most susceptible variety cv. Samrinazard previously tested waskept as check. Seedlings were irrigated regularly at 2 days interval using a hand sprayer. Pea plants were regularly monitored for the development of symptoms and severity of infection. The disease rating scale:0-1% = highly resistant (HR); 1-10% = resistant (R); 10.1-30% = moderately resistant (MR); 30.1-50 = moderately susceptible (MS); 50.1-70% = susceptible (S), 70-100% =highly susceptible (HS) given by (Mayee and Datar, 1986) was used to record disease response of 10 replicates from each variety.Disease incidence was calculated by the following formula.

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of Plants}} \times 100$$

In vitro effect of fungicides on pathogen:Six different fungicides used for *in vitro* assay were as follows: 1) Raxil Ultra (Tebuconazole), 2) Topsin-M (Thiophanate Methyl), 3) Score (Difenconazole), 4) Derosil (Carbendazim), 5)Hombre (Imidacloprid+Tebuconazole) and 6) Divident Star (MetalaxylM+Difenconazole). Stock solution of allfungicides was prepared by adding 0.1g active ingredient in sterilized water (Borum and Sinclair, 1968). Antifungal effect of fungicides on colony growth of the pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 2000)at six different concentrations 5,10,20,25, 50 and 100ppm added to molten PDA medium and poured into sterilized petri plates. Poisoned medium was inoculated with 5 mm dia culture blocks from 7-day-old culture of the pathogen and incubated at 25±1 ºC. Each treatment was replicated five times and one was kept as control. Observations on growthrate of the pathogen were taken for 7 days.Growth inhibition of the pathogen was calculated by the formula given by Sunder *et al.*(1995).

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Here, X = Colony growth in control plates; Y= Colony growth in fungicide treated plates

In vivo effect of fungicides on seed germination:Effect of four different fungicides as follows: 1) Raxil Ultra (Tebuconazole), 2) Divident Star (Metalaxyl M + Difenconazole), 3) Topsin-M (Thiophanate Methyl) and 4) Hombre (Imidacloprid + Tebuconazole) on seed germination rate was evaluated *in vivo*. Fourteen sterilized pea seeds cv. SamrinaZard were pre-treated with 400ppm concentration stock solution of each fungicide and sown in a row as one replication. Each treatment was replicated four times and one was kept as check. Observations were taken daily and germination rate was recorded. Percent germination was calculated by fraction comparison between total no. of seeds germinated with total no. of seed planted.

Statistical analysis:Data was subjected to statistical analysis using M-Stat (Ver. 2.3, Faisalabad, Pakistan). Least significant difference test was used to separate treatment means.

RESULTS

Out of 32 varieties 25 were moderately susceptible, 6 were susceptible and one variety was highly susceptible against the pathogen (Table 1). Not, even a single variety was found to be resistant against the disease in present investigation. *Invitro*testing of fungicides revealed that all fungicides had significant inhibitory effect on colony growth of the pathogen. Score produced best inhibition at 5ppm and 10ppm concentrations compared to other fungicides but it was not more effective at higher concentrations. While, Topsin-M and Raxil Ultra produced maximum inhibition at 100ppm concentration (Table 2).Raxil Ultra was found most effective when tested*in vivo*for its effect on seed germination with maximum germination rate followed by Topsin-M compared to control (Table 3). Seed germination was significantly increased in fungicide treated seed compared to untreated control.

Table 1.Response of pea germplasm against *F. oxysporum* f.sp.*pisi*

Sr. No.	Varieties	Response	Disease Incidence	Sr. No.	Varieties	Response	Disease Incidence
1	92007	MS[a]	47.05efg[b]	17	0093	MS[a]	35.52opq
2	01006	MS	43.43ghijk	18	08001	MS	45.83fghi
3	0505	MS	50.56de	19	m-0911	MS	45.58fghij
4	00567	MS	54.92c	20	900156	MS	41.86jkl
5	9807	MS	45.56fghij	21	06640	MS	35.55opq
6	03008	MS	32.29q	22	05001	S	55.38c
7	05030	MS	42.16ijkl	23	V400	HS	41.23klm
8	9327	MS	43.18hijkl	24	SamrinaZard	MS	83.33a
9	06001	MS	37.64mnop	25	92001	MS	46.16fgh
10	05014	MS	35.78opq	26	98010	S	39.36lmno
11	8823	MS	41.79jkl	27	9805	MS	52.80cd
12	7022	MS	43.33ghijk	28	0909	MS	40.57klmn
13	01678	S	52.17cd	29	2001-20	MS	40.69klm
14	01432	S	59.74b	30	Meteor	MS	36.78nop
15	c-233	MS	48.97def	31	Olympia	MS	46.98efgh
16	09045	MS	35.06pq	32	2001-40	MS	51.06d

[a]0-1% = highly resistant (HR); 1-10% = resistant (R); 10.1-30% = moderately resistant (MR); 30.1-50 = moderately susceptible (MS); 50.1-70% = susceptible (S), 70-100% = highly susceptible (HS)

[b]Values in the column followed by same letter are not significantly different from each other at P <0.05,analyzed using LSD test, values are average of 10 replicates.

Table 2.Effect of fungicides on colony growth of *F. oxysporum* f.sp.*pisi*

Treatment	Growth Inhibition (%) at different concentrations (ppm)					
	5ppm	10ppm	20ppm	25ppm	50ppm	100ppm
Raxil Ultra	36.72e	49.58X	61.38R	68.33N	86.11F	95.55A
Divident Star	31.17g	42.89c	54.44	62.22Q	80.28I	91.94C
Topsin-M	46.91a	56.54V	66.67O	72.77L	85.96G	93.88B
Derosal	34.56f	4.08b	61.11S	70.00M	83.61H	91.11D
Score	47.53Z	57.38T	63.33P	61.11S	79.16J	89.72E
Hombre	26.54h	39.38d	50.00W	56.94U	76.66K	91.11D
Control	0i	0i	0i	0i	0i	0i

[a] Mean values in the column followed by same letter in the column are not significantly different from each other at *P*< 0.05, analyzed using LSD test, Values are average of 3 replicates.

Table 3. Effect of fungicides on seed germination

Treatments	Active ingredient	Concentration (ppm)	Germination rate (%)
Raxil Ultra	Tebucanazole	400	89.26a[a]
Score	Difenocanazole	400	64.28c
Topsin- M	Thiophanate-Methyl	400	80.20b
Hombre	Imidacloprid +tebucanozole	400	57.14d
Control			28.57e

[a] Mean values in the column followed by same letter in the column are not significantly different from each other at $P< 0.05$, analyzed using LSD test, Values are average of 4 replicates.

DISCUSSION

Fusarium wilt of pea is a major constraint to crop yield. It is always difficult to manage due to soil borne nature of the pathogen. Resistant germplasm developed through conventional breeding process can be used as management strategy to overcome this problem. Therefore, present investigation was aimed to find resistant germplasm through screening against the pathogen and to test the effect of fungicides on the colony growth of the pathogen *in vitro* and on seed germination rate *in vivo*. The germplasm evaluated was not effective against the disease. All varieties were found to be susceptible or moderately susceptible, not even a single variety was found to be resistant. Therefore, these varieties cannot be recommended for commercial breeding purpose against Fusarium wilt of pea. Rehman *et al.*(2014) also tested pea germplasm and reported variable response of different varieties. *In vitro* testing of fungicides revealed that Score, Topsin-M and Raxil ultra produced best inhibition of the pathogen. Harpal and Singh (2001) tested several fungicides to control Fusarium wilt of pea and found effective results. Present investigations are supported by Rehman *et al.* (2014) where they tested several fungicides *in vitro*with significant inhibitory effect on growth of the pathogen. They stated that Topsin-M was the best fungicide among all tested chemicals due to its systemic mode of action. Overman and Jones (1984) concluded that different soil fumigants with broad spectrum action havesignificantly reduced the incidence of wilt disease and increased total yield of the crop. Fuches *et al. (*1970) recommended the Topsin-M because of its systemic activity against *F. oxysporum* f. sp. *Lycopersici* and *pisi.*Nel *et al.* (2007) also reported effectiveness of chemicals to control Fusarium wilt.Moreover, effect of fungicides on seed germination was also assessed that showed significant uplift in germination rate of fungicide treated seed compared to control. Present investigation reports that almost all fungicides had somewhat inhibitory effect on the colony growth of the pathogen. These chemicals can be integrated with different management strategies or can be used for management of Fusarium wilt of pea on susceptible germplasm.Integration of chemicals and tolerant germplasm can might contribute to enhance effectiveness of disease management practices.

CONCLUSION

Based on present investigation it can be concluded that Score, Topsin-M and Raxil Ultra should be used for control of Fusarium wilt of pea. These fungicides can be recommended to local growers of Pakistan. However, the germplasm being tested in this study was not found effective against this disease. Hence, it may not be used alone either it should be integrated with other management practices or chemicals being tested in this study. The fungicides being tested should be further investigated in combination with susceptible germplasm.

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