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SURVEY, MEDIA REQUIREMENT AND MANAGEMENT OF FUSARIUM WILT OF PEA

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* Schl. f. sp. *pisi* Snyd & Hans, is a fungal disease that affects peas and is important worldwide. A survey was carried out for assessing the disease outbreak in pea growing areas of 10 blocks of district Kanpur Nagar in Uttar Pradesh. The highest average percentage of wilt incidences was recorded in Bhitargaon block (15%) while minimum percentage was recorded in Sarsol block (9%). Out of seven solid and liquid media, potato dextrose agar medium/potato dextrose medium was found best and supported maximum fungal growth (7.70 cm) and maximum dry mycelial weight (440.18 mg). Same composition of media was also found best for sporulation. Eight bioagents were evaluated against *Fusarium oxysporum* f. sp. *pisi* and showed varying degrees of antagonism. Maximum inhibition percentage was observed with *Aspergillus niger* (62.77%) and lowest was with *Chaetomium globosum* (39.70%). Out of 23 accessions none was found immune/ resistant and 15 were moderately resistant, 2 were moderately susceptible, 3 accessions were found susceptible and remaining were found highly susceptible. Present study reported suitable media for growth and sporulation of *Fusarium oxysporum* f.sp. *pisi* and two management strategies viz., bioagents and resistant sources. Both management strategies could be used for integrated management of this disease in eco-friendly way.

INTRODUCTION

Pea (*Pisum sativum*) is a major annual pulse crop of temperate region of the world and was originally cultivated in the Mediterranean basin. It is one of the most important multipurpose pulse crops. Pea is the second most important food legume in the world after pigeon pea. Pea crop is prone to a number of diseases such as rhizoctonia seedling blight, bacterial blight, ascochyta foot rot, downy mildew, powdery mildew, pythium blight, aphanomyces root rot, wilt and root rot diseases (Sharma, 2011) . Wilt of pea caused by *Fusarium oxysporum* f.sp. *pisi* (linford) Snyder and Hansen, is one of the most important disease. The Fusarium wilt of pea was first recognized during 1918 by Bisby in Minnesota (Chupps and Sherf, 1960). In India, first report of the occurrence of Pea wilt organism was made available by Patel *et al.* (1949) from Bombay. Maheshawari *et al.* (1983) made a survey of wilt and root rot complex of pea in the various pea growing areas in Northern India and reported 13.9 to 95% loss in Hosiarpur district of Punjab where disease intensity was 25-100%. The disease is often severe where short rotation with other crops is practiced. Under these conditions, when the pathogen has built up sufficient inoculums numbers and a susceptible cultivar is planted, severe crop losses result (Kraft, 1994). Generally, fungal pathogens need different types of media for their growth and sporulation. Several culture media showed differential effects on the growth and cultural characteristics of different fungal pathogen on various host plants (Ratnoo and Bhatnagar, 1991; Singh and Kaiser, 1994). For the growth and sporulation of *Fusarium oxysporum* f.sp. *pisi,* is less in formations are available regarding different media. Management of Fusarium wilt of pea is difficult to achieve and no single control measure is fully effective. Considering the nature of damage and survival ability of the fungus, the use of biocontrol agents and resistance pea accessions were considered is good option. There is a pressure from environmentalist that reducing the use of fungicides due to its negative effect in plant ecosystem. The most effective and practical method of management worldwide is to use resistant cultivars (Subhani *et al.*, 2011). Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should includes minimum and efficient use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculums, modification of cultural practices and use of resistant varieties. Thus, there is a need to explore resistant cultivars and the efficacy of different bio- agents to manage this disease below economic threshold level in the absence of other management method. The present investigation was undertaken that survey, media study,

biological control and host resistance for the management of Fusarium wilt disease of pea.

MATERIALS AND METHODS

A survey for the occurrence and severity of pea wilt disease was made during the Rabi season of 2009-2010 in pea growing areas of 10 blocks district of Kanpur Nagar. At each block minimum ten farmers field were visited and mean value of severity was represented for that block. The cultural studies of the pathogen were made on solid as well as in liquid media to study the growth and reproduction of the fungus to find out suited media for growth and sporulation. Following seven media are used as liquid as well as solid media:

Natural Media	Synthetic media	
Potato Dextrose Agar Medium: Peeled potatoes -200.00 g Dextrose- 20.00 g Agar-agar 20.00 g Distilled Water -1000.00ml	Czapek (Dox) Medium: Magnesium sulphate - 50.00 g Potassium di hydrogen phosphate - 1.00g Potassium chloride - 0.50g Ferrous sulphat - 0.01g Sodium nitra - 2.00g Sucrose - 30.00g Distilled water - 1000.00ml	Richard's Medium: Potassium nitrate-10.00g Potassium di hydrogen phosphate5.00g Magnesium sulphate-2.50g Ferric chloride-0.20g Sucrose-50.00g Distilled water-1000.00ml
	Brown's Starch Medium: Tripotassium phosphate-1.25g Magnesium sulphate-0.75g Asparagine-2.00g Glucose-2.00g Distilled water 1000.00ml	Oat meal agar medium: Oat meal -50.0 g Distilled water -1000 ml
	Asthana and Hawker's Medium: Potassium nitrate-3.50g Magnesium sulphate-0.75g Potassium di hydrogen phosphate-1.75g Glucose-5.00g Distilled water-1000.00ml	Sabouraud's agar medium: Glucose- 40.0 g Peptone- 10.0 g Distilled Water- 1000 ml

Dominant rhizosphere fungi viz. *Trichoderma harzianum, T. viride,T. atroviride, T. longibrachiatum, T. hamatum, T. koningii, Chaetomium globosum* and *Aspergillus niger* were isolated using serial dilution method (Harris and Sommers,1868) from pea grown field affected by the wilt disease in the research farms of C.S Azad Agriculture University of technology Kanpur India. The pure culture of the bioagents was maintained in Potato Dextrose Agar Medium slants at 4ºC for further use. *F. oxysporum* f. sp *pisi* was isolated following standard method on PDA Medium. Five mm disc of 10 days old culture of *F. oxysporum* f. sp *pisi* and the antagonists were inoculated separately plates and incubate at 27 ± 1ºC. Radial growth of colony was recorded after seven days of incubation on to PDA and the per cent inhibition was calculated (Vincent, 1947). Screening of pea accessions was carried out under artificial conditions to find out the source of resistance to wilt of pea caused by *F. oxysporum* f.sp. *pisi*. In Rabi season during 2009- 2010, accessions of pea were grown in glass house compound at CSA University Kanpur. Each accession under test was sown in pots with three replications. In order to ensure epidemics of the disease, the inoculums of the test fungus was added to the soil (spore suspension 100mL/ 1kg sterile soil) in pot before sowing. Fifteen days after sowing of the total number of plants in each pot was counted and wilting was recorded with an interval of a month. To avoid error in counting, the counted wilted plants were uprooted. For categorizing pea varieties/cultures, 0-5 disease scale used (Singh and Srivastava, 1987).

RESULTS AND DISCUSSION

Survey

A survey for the occurrence and severity of pea wilt disease was made during the Rabi season of 2009 - 2010 in different blocks of district of Kanpur Nagar. The results of survey revealed that overall average percentage of wilt incidence was 12.10%. The wilt incidence was highest in Bhitargaon block (15.00%), followed by Ghatampur block (14.00%) and minimum percentage of wilt incidence was found in Sarsol block (9.0%) Fig. 1. Maheshawari *et al.* (1983) also reported severity ranged 25-100% in Punjab due to outbreak of wilt and root rot complex of pea.

Media study

The *Fusarium oxysporum* f. sp. *pisi* was grown on the solid and liquid states of media for 10 days at 25 ±1ºC. Results revealed, on solid media in Table 1 that potato dextrose agar

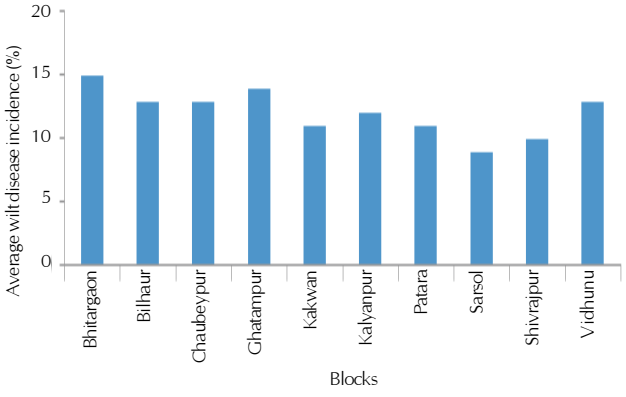

Figure 1: Average wilt incidence in different blocks of district Kanpur Nagar

Table 1: Average diameter of mycelial colony and sporulation of *Fusarium oxysporum* f. sp. *pisi* on different media at 25 ± 1°C

Solid Media	Average diameter (cm)	sporulation	Liquid Media	Average dry mycelial weight (mg)	Sporulation
Potato dextrose agar medium	7.70	Excellent	Potato dextrose medium	440.18	Excellent
Richard's agar medium	6.80	Good	Oat meal medium	382.53	Good
Czapek's (dox) agar medium	7.16	Good	Czapek's (Dox) medium	237.63	Good
Oat meal agar medium	6.80	Good	Sabouraud's medium	136.16	Fair
Sabouraud's agar medium	5.26	Fair	Asthana and Hawker's medium	134.30	Fair
Brown's starch agar medium	4.60	Fair	Richard's medium	123.26	Fair
Asthana & Hawker's agar medium	3.50	Poor	Brown's starch medium	75.32	Poor
C.D. at 5%	0.371			3.283	

Table 2: Inhibitory effect of different bio agents on the growth of *Fusarium oxysporum* f. sp. *pisi in vitro*

Bio-agents	*Average diameter of mycelium growth (cm)	Percent inhibition over control (%)
Aspergillus niger	3.00	62.77
Trichoderma viride	3.23	59.92
Trichoderma koningii	3.46	57.07
Trichoderma harzianum	3.50	56.57
Trichoderma atroviride	3.66	54.59
Trichoderma longibrachiatum	3.96	50.86
Trichoderma hamatum	4.46	44.66
Chaetomium globosum	4.86	39.70
Control	8.06	
CD (P = 0.05)	0.449	

*Means of three replications

(7.70 cm) medium supported the best growth of the fungus followed by Richard's, Czapek's (Dox) agar medium and Richard's agar medium. Asthana and Hawker's agar medium (3.50 cm) supported the lowest growth of the fungus. Fungus sporulation was excellent on PDA medium and poor on Asthana and Hawker's agar medium (Table 1). In case of liquid media, the best medium for the vegetative and reproductive growth of fungus was the PDA medium followed by Oat meal medium and Czapek's (Dox) medium. PDA medium was excellent for sporulation of *Fusarium oxysporum* f. sp. *pisi* while poor on Brown's starch medium. Pradeep *et al.* (2013) also reported similar lined of finding in their study that Potato Dextrose Agar and Potato Dextrose Broth medium are suitable for the vegetative and reproductive growth of *Fusarium moniliforme*. Tandel *et al.* (2012) reported that PDA medium was best supported for growth of *Macrophomina phaseolina*.

Biological control

Among tested bio-agents (Table 2), maximum suppression of the growth of pathogen was observed with *Aspergillus niger* (62.77%) followed by *Trichoderma viride* (59.92%), *T. koningii* (57.07%) and *T. harzianum* (56.57%) were statistically at par to each other. The least effective bioagents were found *T. hamatum* (44.66%) and *Chaetomium globosum* (39.70%). Rajput *et al.* (2013) reported that *Trichoderma viride* was found effective against *Alternaria alternata in vitro* whereas *T. harzianum* was performed better percent inhibition against *Rhizoctonia solani* and *Sclerotium rolfsii* (Kumar *et al.,* 2012; Singh *et al.,* 2013). Present study was corroborated by Shalini *et al.* (2005) that *T. viride* and *T. harzianum* inhibits maximum antagonistic activity against *Fusarium oxysporum* f.sp. *pisi* in dual culture technique. Sharma (2012) reported that *Aspergillus niger* have long been known as effective antagonists against *F. oxysporum* f. sp. *cumini* and also mentioned that antagonism behave as mycoparasite. There was evidence that the mechanism of antagonism employed by *Aspergillus niger* were competition, lysis, penetration and hyperparasitism. *Trichoderma* sp. was found effective against the chickpea vascular wilt pathogen, *Fusarium oxysporum* f. sp. *ciceri* (Hesamedin, 2009). Charoenporn *et al.*, 2010 also reported that the highest inhibition of conidial production of *F. oxysporum* f. sp. *lycopersici* causing tomato wilt from the metabolites (Chaetoglobosin-C) extracted from *C. globosum*.

Host resistance

It is evident from the Table 3, out of 23 accessions of pea tested under artificial conditions 15 accessions were found moderately resistant, 2 were found moderately susceptible, 3 were found susceptible and 3 accessions were found highly susceptible. None of these accessions were free and resistant from infection. Results have clearly indicated that out of which 23 accessions of screened under artificial conditions only 15 accessions possess moderately resistant and none of them was free from infection. Kumar and Kohli (2001) and Svabova *et al.* (1998, 2011) also reported various degree of resistance and susceptibility in pea germplasms.

Table 3: Screening of varieties/cultures against *Fusarium oxysporum* f. sp. *pisi* under pot culture experiment

Grade	Reaction	Varieties/cultures
0	Free (Immune)	Nil
1	Resistant (1-5 % wilted plants)	Nil
2	Moderately resistant (5-10% wilted plants)	KPMR-839, KPMR-811, KPMR-870, Swati, KPMR-898, KPMR-817, KPMR-906, P-1, P-3, KPMR-871, Arkel, KPMR-815, Rachna, KPMR-874, KPMR-912
3	Moderately susceptible (10-25% wilted plants)	KPMR-902, KPMR-899
4	Susceptible (25-50% wilted plants)	KPMR-907, KPMR-905, KPMR-908
5	Highly susceptible (more than 50% wilted plants)	KPMR-745, KPMR-857, Shikha

Our investigations provides information on status of wilt of pea in district Kanpur Nagar, suitable media for growth and development of pathogen and two eco-friendly approaches for management of this disease. In future, promising antagonistic fungi and accessions could be incorporated to manage the disease in integrated manner.

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REFERENCES

Charoenporn, C., Kanokmedhakul, S., Lin, F.C., Poeaim, S. and Soytong, K. 2010. Evaluation of bio-agent formulations to control Fusarium wilt of tomato. *Afr. J. Biotech.* **9(36):** 5836-5844.

Chupp, C. and Sherf, A. F. 1960. *Vegetable disease and their control.* The Ronald Press Company, New York, p. 668.

Harris, R. F. and Sommers, L. E. 1968. Plate-dilution frequency technique for assay of microbial ecology. *Appl. Microbiol.***16:** 330-334.

Hesamedin, R. 2009. Efficacy of some fungal and bacterial bioagents against *Fusarium oxysporum* f.sp. *ciceri* on chickpea. *Plant Prot J.* **1(1):** 108-113.

Kraft, J. M. 1994. Fusarium wilt of pea (a review). *Agronomie*. **14:** 561-567.

Kumar, A. and Kohli, U. K. 2001. Evaluation of garden pea genotypes for horticultural traits and resistance against Fusarium wilt. *Haryana J. Horticultural Sciences*. **30:** 217-219.

Kumar, R., Maurya, S., Kumari, A., Choudhary, J., Das, B., Naik, S. K. and Kumar, S. 2012. Biocontrol potentials of *Trichoderma harzianum* against sclerotial fungi. *The Bioscan.* **7(3):** 521-525.

Maheshwari, S. K.,Jhooty, U. S. and Gupta, J. S. 1983. Survey of wilt and root rot complex of pea in northern India and assessment of losses. *Agric. Sci. Dig. India*. **3:** 139-141.

Pradeep, F. S., Begam, M. S., Palaniswamy, M. and Pradeep, B. V. 2013. Influence of Culture Media on Growth and Pigment Production by *Fusarium moniliforme* KUMBF1201 Isolated from Paddy Field Soil. *World Appl. Sci. J.* **22(1):** 70-77.

Rajput, R. B., Solanky, K. U., Prajapati, V. P., Pawar, D. M. and Suradkar, S. R. 2013. Effect of fungal and bacterial bioagents against *Alternaria alternata* (fr.) Keissler *in vitro* condition. *The Bioscan.* **8(2):** 627-629.

Ratnoo, R. S. and Bhatnagar, M. K. 1991. Effect of temperature and pH on growth and sclerotia formation of *M. phaseolina*. *Indian J.Mycol. Pl. Pathol.* **21(3):** 279-280.

Shalini, V. and Dohroo, N. P. 2005. Biocontrol of Fusarium with of pea caused by *Fusarium oxysporum* f.sp *pisi*. *Ind. J. Plant Pathol.* **23(192):** 58-62.

Sharma, P. 2011. Alarming occurrence of Fusarium wilt disease in pea (*Pisum sativum* L.) cultivations of Jabalpur district in Central India revealed by an array of pathogencity tests. *Agric. Biol. J. N. Am.* **2(6):** 981-994.

Sharma, S. 2012. Antagonistic interaction between *Fusarium oxysporum* f.sp.*cumini* and *Aspergillus niger*. *J. Res. Bio.* **1:** 032-034.

Singh, R. D. N. and Kaiser, S. A. K. M. 1994. Effect of different culture media and pH levels on growth and cultural characteristics of charcoal root pathogen (*Macrophomina phaseolina*) infecting maize. *Crop Res. Hissar.* **7(2):** 282-287.

Singh, S. and Srivastava, M. P. 1987. A method of grading for resistance to Myrothecium leaf spot of mungbean (*Vigna radiata* (L.) Wilzeck) and screening for resistance. *Indian J. Pl. Pathol.* **5:** 139-141.

Singh, S. P., Singh, H. B. and Singh, D. K. 2013. *Trichoderma harzianum* and *Pseudomonas* sp. mediated management of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum* mill.). *The Bioscan.* **8(3):** 801-804.

Subhani, M. N., Sahi, S. T., Hussain, S., Ali, A., Iqbal, J. and Hameed, K. 2011. Evaluation of various fungicides for the control of gram wilt caused by *Fusarium oxysporium* f.sp. *ciceris*. *Afr. J. Agric. Res.* **6(19):** 4555-4559.

Svabova, L., Lebeda, A. and Griga, M. 1998. Comparison of *in vitro* testing methods for screening of resistant peas to *Fusarium* spp. *Acta Phytopathol. Entomol. Hung.* **33:** 275-284.

Tandel, D. H., Sabalpara, A. N. and Patel, R. C. 2012. Evaluation of different solid and liquid media for the growth and sclerotial formation *of Macrophomina phaseolina* Goid *in vitro.The Bioscan.* **7(4):** 743-745.

Vincent, J. M. 1947. Distoration of fungal hyphae in the presence of certain inhibitors. *Nature*. **159:** 850.