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In vitro Bioefficacy of bioagents Against *Fusarium oxysporum* f. sp *pisi*, causing *Fusarium* Wilt Disease in Pea

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ABSTRACT: Stresses both biotic and abiotic are considered as major constraints in the production of pea. Among biotic stresses apart from bacterial and viral diseases, many fungal diseases are of economic importance. *Fusarium* Wilt seems to beone of the most important and devastating disease of pea. Wilt caused by *Fusarium oxysporum* f. sp *pisi*, being soil borne is the major threat to successful cultivation and growing of pea. Therefore, various potential biocontrol agents were evaluated *in vitro* against the pathogen *F. oxysporum* f. sp. *pisi*, at the Department of Plant Pathology, VNMKV, Parbhani. Though all test bioagents were found antagonistic to the test pathogen, but most efficient were, *T. harzianum*, which resulted with significant highest mycelial growth inhibition (100.00 %), of the test pathogen, followed by *Aspergillus niger* (64.03 %), *T. asperellum* (55.16 %) and *Metarhizhium anisopliae* (49.60 %).

Keywords: Bioagents, *Fusarium oxysporum*, *Fusarium* wilt, *In vitro*, Inhibition.

INTRODUCTION

Pea botanically known as *Pisum sativum* L, belongs to Fabaceae/Leguminosae family. It is also known as plant species on which Gregor Mendel worked out Mendel's laws of heredity. It also stands as an eco-friendly crop, as it belongs to nitrogen fixing crops category (Graham and Vance 2000; Anglade *et al*., 2015).

Pea is one of the important legume crops of India, it is an annual herbaceous legume vegetable crop adapted in cool and humid conditions. Pea is cultivated in winter season (*Rabi*) preferably from the beginning of October (15) to November. (Shubha *et al*., 2016). In India pea is grown in about 0.37 million ha area with annual production of 3.57 million tonnes. It is one of the major commercial vegetable crops of many states across India during winter season (Kharte *et al*., 2022).

In Maharashtra, the area under maize crop was 0.93 million hectares with 1.77 million tonnes of production and productivity of 1.90 tonnes, during 2020-2021 (Anonymous, 2021).

Pea (*Pisum sativum* L.) production is facing many biotic and abiotic threats among them, biotic diseases constitute the most important factor in reducing the average yield (Chanu *et al*., 2020).

Among a number of fungal, bacterial and viral diseases, *Fusarium* Wilt caused by *F. oxysporum* f. sp *pisi* is one of the most destructive disease and commonly prevailing in almost all pea growing pockets of India. *Fusarium* wilt affected winter crops resulted in 100% yield losses as it was difficult to control through cultural practices due to its aggressive nature (Sharma *et al*., 2010).Whatever, Pea varieties, cultivars under cultivation are more or less prone to wilt. Hence, employing biocontrol agents to manage the diseases seems to be eco-friendly, cost-effective and promising option, over chemical disease management. Therefore, present study was undertaken to evaluate *in vitro* efficacy of efficient biocontrol agents against *F. oxysporum* f. sp *pisi*, causing Wilt disease of pea.

MATERIALS AND METHODS

A total of seven biocontrol agents were evaluated *in vitro* against *F. oxysporum* f. sp *pisi*, applying Dual Culture Technique (Dennis and Webster 1971). Seven days old cultures of the test bioagents and test pathogen were grown on PDA media and used in present study. One each culture disc (5 mm) of the test pathogen and the test fungal bioagent (cut using sterilized cork borer) were placed at equidistance and exactly opposite to each other, on autoclaved and solidified PDA medium in sterilized glass Petri plates (90 mm). For each test bioagent, three PDA plates were inoculated and all the treatments replicated thrice. The PDA plates inoculated (in the centre) alone with pure culture disc of the test pathogen were maintained as untreated control. The experimental details were as given below.

Experimental Details:

Design	:	Completely Randomized design (CRD)
Replications	:	Three
Treatments	:	Eight

Treatment details:

Tr. No.	Treatments
T_1	*Trichoderma harzianum*
T_2	*T. asperellum*
T_3	*Aspergillus niger*
T_4	*Metarhizhium anisopliae*
T_5	*Verticillium lecanii*
T_6	*Nomuraea rileyi*
T_7	*Beauveria bassiana*
T_8	Control (untreated)

Observations on linear colony growth/diameter (mm) of the test pathogen and the test bioagent were recorded at an interval of 24 hrs of incubation and continued up to seven days or till the untreated control plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhay 1978). The data was statistically analysed at 1 per cent C.D.

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

RESULTS AND DISCUSSION

The results obtained on mycelial growth and inhibition of *F. oxysporum* f. sp *pisi* with seven fungal antagonists is presented in Table 1.

Results (Plate 1, Table 1 and Fig. 1 revealed that among the seven bioagents tested, *Trichoderma harzianum* with highest mycelial growth (90.00 mm) was found most effective in controlling the mycelial growth of *F. oxysporum* f. sp. *pisi* (00.00 mm), *Aspergillus niger* was the next best bioagent restricting mycelial growth of the *F. oxysporum* f. sp. *pisi* (32.00 mm), followed by *T. asperellum* (40.00 mm), *Metarhizhium anisopliae* (45.33 mm). *Beauveria bassiana* (50.00 mm) and *Verticillium lecanii* (54.00 mm) were found statically at par with each other and followed the trend. Whereas *Nomuraea rileyi* (65.00 mm) was found least effective in controlling the mycelial growth of *F. oxysporum* f. sp. *pisi.*

Thus, the bioagents *viz.*, *T. harzianum*, *Aspergillus. niger, Metarhizhium anisopliae and Trichoderma asperellum*, were found most potential antagonists against *F. oxysporum* f. sp. *pisi.*

These results of the present study are in consonance with the reports of several earlier workers. Similar results were reported by Verma and Dohroo (2005); Dakika *et al.* (2007); Mohamedy and Mohmoud (2008); Hamid *et al.* (2012); Subhani *et al.* (2013); Mudasser Ahmed Khan and Yella Goud (2022) they reported highest effectivity of *Trichoderma harzianum* against *F. oxysporum* f. sp. *pisi.*

Table 1: *In vitro* bioefficacy of the bioagents against *F. oxysporum* f. sp. *pisi*, causing pea wilt.

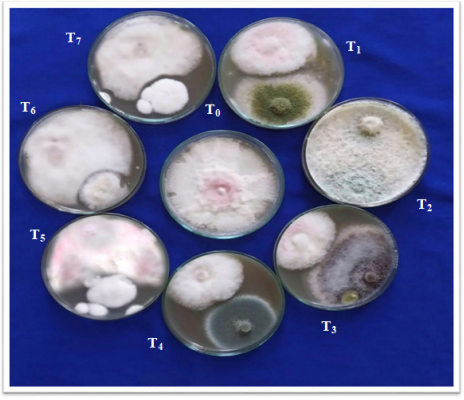
Tr. No.	Bioagents	Mean colony Diameter (mm)	Per cent inhibition (%)
T_1	*Trichoderma asperellum*	40.00	55.16 (47.96)*
T_2	*T. harzianum*	00.00	100.00 (90.00)
T_3	*Aspergillus niger*	32.00	64.03 (53.19)
T_4	*Metarhizhium anisopliae*	45.33	49.60 (44.75)
T_5	*Verticillium lecanii*	54.00	39.20 (38.74)
T_6	*Nomuraea rileyi*	65.00	27.33 (31.50)
T_7	*Beauveria bassiana*	50.00	43.66 (41.34)
T_0	Control (Untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**1.64**	**1.15**
C.D. at 1%		**4.96**	**3.49**

*Mean of three replications. Figure in parenthesis are arc sine transformed values.

The similar effectivity of *Aspergillus niger* was also reported in the study by Ali *et al.* (2014). Showing 62 % inhibition of mycelial growth of *F. oxysporum* f. sp. *pisi*.

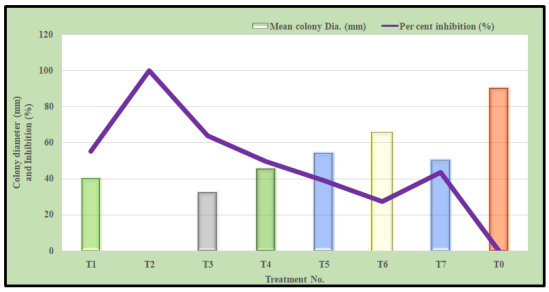
Mycelial growth inhibition of *F. oxysporum* by *Trichoderma* spp. may be attributed to the secretion of extracellular cell wall degrading enzymes such as chitinase β-1, 3-glucanase, cellulose and lectin etc., production of secondary metabolites such as glioviridin, viridian and gliotoxin, also various mechanisms such as competition, lysis, antibiosis, and production of volatile / non-volatile substances.

Thus, biocontrol agents such as *Trichoderma harzianum, A. niger* and *Tasperellum*, proved to be potential antagonist could be extensively employed to manage several plant diseases/pathogens, including *F. oxysporum* f. sp. *pisi.*



T_1: *Trichoderma asperellum* ; T_2: *T. harzianum* ; T_3: *Aspergillus niger* ; T_4: *Metarhizhium anisopliae*; T_5: *Verticillium lecanii* ; T_6: *Nomuraea rileyi* ; T_7: *Beauveria bassiana* ; T_0: Control

Plate 1. Efficacy of bioagents on *F. oxysporum* f. sp *pisi.*



T_1: *Trichoderma asperellum*; T_2: *T. harzianum* ; T_3: *Aspergillus niger* ; T_4: *Metarhizhium anisopliae* ; T_5: *Verticillium lecanii* ; T_6: *Nomuraea rileyi* ; T_7: *Beauveria bassiana* ; T_0: Control

Fig. 1. Efficacy of bioagents against *F. oxysporum* f. sp *pisi.*

CONCLUSIONS

All bioagents tested *in vitro* were found antagonistic to *F. oxysporum* f. sp. *pisi*. However, *Trichoderma harzianum* resulted with cent per cent mycelia growth inhibition (100 %), followed by *Aspergillus niger* (64.03 %).

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