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In vitro efficacy of some fungicides against *Fusarium oxysporum* f. sp. *pisi* a causal agent of wilt disease of Pea (*Pisum sativum* L.)

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Abstract
In the present study, some fungicides were tested for their ability to inhabit or cidal effect against a plant pathogen *Fusarium oxysporum* f. sp. *pisi* causes wilt disease of Pea (*Pisum sativum* L.) cultivated during the cool season in subtropical and temperate countries. The experiment was carried out by poisoned food technique in which different doses of fungicides were mixed in Potato Dextrose Medium (PDA) and growth inhibition of fungal pathogen was observed. The fungicides tested were Carbendazim 50% WP, Tebuconazole 10% + Sulphur 65% WG, Propineb 70% WP, Metiram 70% WG at four different concentrations (25ppm, 50ppm, 100ppm and 200ppm). The treatments were arranged in completely Randomized Design was followed (CRD) with three replications of each treatment and plate having no treatments served as control. Growth was observed after 3 days, 6 days and 9 days intervals after the inoculation. Maximum inhibition of growth of *F. oxysporum* f.sp. *pisi* was reported with the all doses used of fungicide combination of Tebuconazole10% + Sulphur 65%WG and then followed by higher dose (200ppm) of Carbendazim 50%WP. The fungicides Propined 70%WP and Metiram70%WG were found very less effective against fungal pathogen. The higher concentration these two fungicides (200ppm) used, inhibit 33.33% and 25.25.19%, respectively. The study revealed that combination of Tebuconazole 10% + Sulphur 65%WG can be explored to control the wilt disease of pea crops in the field.

Keywords: Fungicides, mycelium growth, percent inhibition and poison food technique

Introduction
Pea (*Pisum sativum* L.) is a winter season important annual autogamy (2n=14) pulse crop belongs to legume family (McKay *et al*., 2003). Pea improves the soil fertility by (nitrogen fixation by *Rhizobium leguminosarum*) present in root nodules and thus reduced quantity of fertilizer required. It is cultivated in wide soil kind starting from light-weight sandy loams to serious clayey with optimum pH scale 5.5 to 6.5 (FAO, 2012; CSA, 2015-16). Pea grains are rich in vitamins, dietary fibers, minerals, fats 1.4%, Proteins 27.8% and carbohydrates up to 42.65% (Tzitzikas *et al*., 2006) [9].

World pea production was over ten million in tones in 2009. Major producers of peas are Russian Federation, Canada, Asian nation, China and USA. India is that the second largest producer of pea within the world and Pea occupies 459-thousand-hectare space in Asian nation and shares 21% production of the world. Uttarakhand state shares 1.72% and production is 93.40 tones (According to National Horticulture Board (NHB) 2017-2018 data) it's utilized in contemporary state additionally as processed form. In fresh state, primarily snow pea and sugar snap are exported because of their high demand in international market. Pea are grown on large areas in India but Uttarakhand production or yield is less as compared to other states. One of the main reasons is diseases and insect pests. Some destructive fungi are associated with pea like *Ascochta pisi, Cladosporium piscicola, Sclerotina sclerotiorum* and *Fusarium oxysporum*. Among them, *F. oxysporum* is considered most destructive soil borne pathogen. Pea wilt caused by *Fusarium oxysporum* f. sp. *pisi*. In past few years, many manmade chemicals were introduced against Phytopathogen. The mishandling not solely crystal rectifier to the event of chemical resistance in pathogens however also established unsafe to each animals and human health additionally as impart negative impact on plant growth (Okigbo *et al*., 2004) [7].

In recent years, Wilt of Pea has become serious in several pea growing areas in India, up to now not a lot of analysis has been done.

Therefore, in this experimental work, an investigation was taken with to evaluate the efficacy of some fungicides in *in vitro* condition against *F. oxysporum* f. sp. *pisi* a causal agent of Wilt of Pea at School of Agriculture, Uttaranchal University, Dehradun.

Materials and Methods

All *in vitro* experiments were planned and carried out at the laboratory of Plant Pathology, School of Agriculture, Uttaranchal University, Dehradun.

Isolation and Purification of pathogen

The plant showing typical characteristic symptoms of Wilt disease of pea collected from Pea crop field and brought to the laboratory. Then the fungal pathogen isolated on Potato Dextrose Agar (PDA) and incubated at 25±1ºC. The resulting fungal culture was purified in aseptic condition by hypha tip method. The pure culture obtained was used for testing the efficacy of fungicides against *Fusarium oxysporum* f. sp. *pisi in vitro* condition.

In vitro efficacy of fungicides against *Fusarium oxysporum* f. sp. *pisi*

Four fungicide formulations viz. Carbendazim 50% WP, Tebuconazole 10% + Sulphur 65% WG, Propineb 70% WP, Metiram 70% WG were tested by using Poisoned Food Technique to check their efficacy against fungal pathogens. Purified plate of 7-10 days old culture taken and 8mm pieces of culture was cut out with the help of Cork borer. Media was poisoned with treatments at different concentrations of fungicides (25ppm, 50ppm, 100ppm, and 200ppm,) respectively and placed inside incubator at 25±2ºC. Completely Randomized Design was followed (CRD) with three replications of each treatment and plate having no treatments served as control. Growth of fungal pathogen was observed regularly at 3 days after inoculation (DAI), 6 DAI and 9 DAI, respectively. The different conservations of fungicides used in Poison food technique (Akhilesh *et al.*, 2015) were prepared by formula given below:

$C_1V_1 = C_2V_2$

Where,
C1 = Concentration of stock solution,
V1=Desired concentration (µg/ml) of fungicides
C_2 = Volume (ml) of the stock solution of fungicide
V_2 = Desired Volume (ml) of growth media
Zone of Inhibition calculated by using formula (Mc Kinney 1923):

Inhibition Zone Percentage = C-T/C X 100

Where,
I = % of inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Table 1: List of fungicides used against *Fusarium oxysporum* f. sp. *pisi*

Treatment	Common Name	Trade Name
T_1	Carbendazim 50% WP	Mavestin
T_2	Tebuconazole10% + Sulphur 65% WG	Haru
T_3	Propineb70% WP	Antracol
T_4	Metiram70% WG	Polyram
T_5	Control (media without any fungicide)	

Results and discussion

Efficacy of fungicides against *Fusarium oxysporum* f. sp. *pisi*

Radial mycelia growth

The different concentration (25, 50, 100 and 200 ppm) of fungicide (systemic + non – systemic + combine fungicides) belong to different groups were tested against *F. oxysporum* f. sp. *pisi* by Poisoned food technique. The result shows that a different range of radial growth of *Fusarium oxysporum* f. sp. *pisi* was tested for fungicides. Mycelia growth has been shown to decrease with an increase in the concentration of fungicides.

At 25ppm, mycelia growth was recorded in the range of (0.0 mm) to (34.67mm) at 3 DAI, (17.33mm) to (68.33mm) at 6 DAI, (19.33mm) to (76.67mm) at 9 DAI. At 3DAI the highest mycelia growth was reported with T3(34.67mm) followed T4(34.33mm) than less effective with T1 and T2(0.00mm), At 6 DAI the highest mycelia growth was reported with T3(68.33mm) followed T4 (64mm), T1 (19.33mm) than less effective with T1(0.00mm) and at 9 DAI. The highest mycelia growth was reported with T4 (76.67mm) followed T3 (73.00mm), T1 (40.67mm) than less effective with T2 (19.33mm) compared with grow in untreated control.

At 50ppm, all the fungicides were tested same way of mycelia growth as that of 25pp in the range of (0.0 mm) to (33.33mm) at 3 DAI, (16.00mm) to (66.33mm) at 6 DAI, (17.67mm) to (72.67mm) at 9 DAI. At 3DAI the highest mycelia growth was reported with T4(33.33mm) followed T3(32.00mm) than less effective with T1 and T2(0.00mm), At 6 DAI the highest mycelia growth was reported with T3(66.33mm) followed T4(61.00mm), T1(19.00mm) than less effective with T2(17.33mm) and at 9 DAI the highest mycelia growth was reported with T4(72.67mm) followed T3(70.67mm), T1(36.33mm) than less effective with T2(17.67mm) compared with grow in untreated control.

At 100ppm, all the fungicides were tested same way of mycelia growth as that of 25ppm,50ppm were in between from (0.0 mm) to (32.00mm) at 3 DAI, (14.67mm) to (65.67mm) at 6 DAI, (16.33mm) to (69.67mm) at 9 DAI. At 3DAI the highest mycelia growth was reported with T3(32.00mm) followed T4(30.33mm) than less effective with T1 and T2(0.00mm), At 6 DAI the highest mycelia growth was reported with T3(65.67mm) followed T4(58.67mm),T1(15.67mm) than less effective with T2(14.67mm) and at 9 DAI the highest mycelia growth was reported with T4(69.67mm) followed T3(68.00mm), T1(30.33mm) than less effective with T2(16.33mm) compared with grow in untreated control.

At 200ppm, all the fungicides were tested same way of mycelia growth as that of 25ppm,50ppm were in between from (0.0mm) to (26.67mm) at 3 DAI, (11.33mm) to (58.00mm) at 6 DAI, (14.67mm) to (67.33mm) at 9 DAI. At 3DAI the highest mycelia growth was reported with T3(26.67mm) followed T4(26.33mm) than less effective with T1 and T2(0.00mm), At 6 DAI the highest mycelia growth was reported with T3(58.00mm) followed T4(55.67mm),T1(14.17mm) than less effective with T2(11.33mm) and at 9 DAI the highest mycelia growth was reported with T4(67.33mm) followed T3(60.00mm), T1(26.33mm) than less effective with T2(14.67mm) compared with grow in untreated control.

Average mycelium growth was reported with the evaluation of fungicides at (25,50,100,and 200ppm) ranged between

T2-Tebuconazole 10% + Sulphur 65%WG(10.61mm) to T3-Propineb 70%WP(54.61mm). Maximum average radial mycelia growth was recorded with the treatment, T3-Propineb 70% WP (54.61mm), T4-Metiram 70% WG (54.17mm), T1-Carbendazim 50% WP (16.82) and T2-ebuconazole 10% + Sulphur 65% WG (10.61mm) was minimum to be less mycelia growth of pathogen over under fully grown untreated control. Effectiveness of Phytochemicals are also proved by the earlier worker in past (Bana *et al.*, 2017*;* Waqar *et al.*, 2020) [3, 10].

Table 2: Efficacy of fungicides against *Fusarium oxysporum* f. sp. *pisi* (Mycelia Growth Colony Diameter in mm)*

Treatment	3 DAI				6 DAI				9 DAI			
	Radical growth (mm)				Radical growth (mm)				Radical growth (mm)			
	25 ppm	50 ppm	100 ppm	200 ppm	25 ppm	50 ppm	100 ppm	200 ppm	25 ppm	50 ppm	100 ppm	200 ppm
T1	0	0	0	0	19.33	19	15.67	14.17	40.67	36.33	30.33	26.33
T2	0	0	0	0	17.33	16	14.67	11.33	19.33	17.67	16.33	14.67
T3	34.67	32	32	26.67	68.33	66.33	65.67	58	73	70.67	68	60
T4	34.33	33.33	30.33	26.33	64	61	58.67	55.67	76.67	72.67	69.67	67.33
T5(control)	38	38	38	38	70	70	70	70	90	90	90	90
C.D.at 5%	0.05	0.09	0.08	0.12	0.08	0.1	0.19	0.07	0.17	0.1	0.08	0.04
S.E.(m)	0.33	0.54	0.47	0.76	0.52	0.6	1.16	0.47	1.1	0.65	0.52	0.26

* Mean of Three Replications; C.D = Critical difference; S.E (m) = Standard Error Mean

3.1.2 Mycelia inhibition

As a result, (Table 3 and Plate 1) fungicides was tested at (25ppm, 50ppm, 100ppm, 200ppm each) inhibited mycelia of *Fusarium oxysporum* f. sp. *pisi* over untreated control. Further the percent mycelia inhibition of pathogen was increased with the increase in the concentration of the fungicides. At 25ppm, mycelia inhibition percentage was recorded in the range of (0.0 %) to (34.67%) at 3 DAI, (17.33%) to (68.33%) at 6 DAI, (19.33%) to (76.67%) at 9DAI. At 3DAI the highest mycelia inhibition was reported with T3(34.67%) followed T4(34.33%) than less effective with T1 and T2(0.00%), At 6 DAI the highest mycelia inhibition was reported with T3(68.33%) followed T4(64%),T1(19.33%) than less effective with T1(0.00%) and at 9 DAI the highest mycelia inhibition was reported with T4(76.67%) followed T3(73.00%), T1(40.67%) than less effective with T2(19.33%) compared with inhibit in untreated control. At 50ppm, all the fungicides were tested same way of mycelia inhibition as that of 25pp in the range of (0.0 %) to (33.33%) at 3DAI, (16.00%) to (66.33%) at 6DAI, (17.67%) to (72.67%) at 9 DAI. At 3 DAI the highest mycelia inhibition was reported with T4(33.33%) followed T3(32.00%) than less effective with T1 and T2(0.00%), At 6 DAI the highest mycelia inhibition was reported with T3(66.33%) followed T4(61.00%),T1(19.00%) than less effective with T2(17.33%) and at 9 DAI the highest mycelia inhibition was reported with T4(72.67%) followed T3(70.67%), T1(36.33%) than less effective with T2(17.67%) compared with inhibit in untreated control.

At 100ppm, all the fungicides were tested same way of mycelia growth as that of 25ppm,50ppm were in between from (0.0 %) to (32.00%) at 3 DAI, (14.67%) to (65.67%) at 6 DAI, (16.33%) to (69.67%) at 9 DAI. At 3DAI the highest mycelia inhibition was reported with T3(32.00%) followed T4(30.33%) than less effective with T1 and T2(0.00%), At 6 DAI highest mycelia inhibition was reported with T3(65.67%) followed T4 (58.67%), T1 (15.67%) than less effective with T2(14.67%) and at 9 DAI the highest mycelia inhibition was reported with T4(69.67%) followed T3(68.00%), T1(30.33%) than less effective with T2(16.33%) compared with inhibit in untreated control. At 200ppm, all the fungicides were tested same way of mycelia inhibition as that of 25ppm,50ppm were in between from (0.0%) to (26.67%) at 3 DAI, (11.33%) to (58.00%) at 6 DAI, (14.67%) to (67.33%) at 9 DAI. At 3DAI the highest mycelia inhibition was reported with T3(26.67%) followed T4(26.33%) than less effective with T1 and T2(0.00%), At 6 DAI the highest mycelia inhibition was reported with T3(58.00%) followed T4(55.67%),T1(14.17%) than less effective with T2(11.33%) and at 9 DAI the highest mycelia inhibition was reported with T4(67.33%) followed T3(60.00%), T1(26.33%) than less effective with T2(14.67%) compared with inhibit in untreated control.

Average mycelium inhibition was reported with the evaluation of fungicides at 25, 50, 100, and 200ppm ranged between T2-Tebuconazole 10% + Sulphur 65%WG(10.61%) to T3-Propineb 70%WP(54.61%). Maximum average inhibition percent was recorded with the treatment, T3-Propineb 70% WP(54.61%), T4-Metiram 70% WG(54.17%), T-Carbendazim 50% WP(16.82) and T2-Tebuconazole 10% + Sulphur 65% WG(10.61%) was minimum to be less mycelia inhibition of pathogen over under fully grown untreated control. Thus, all fungicide against *Fusarium oxysporum* f. sp. *pisi* and inhibited its mycelia over untreated control. Fungicides found most effective in the order of less T2-Tebuconazole 10% + Sulphur 65%WG followed T1-Carbendazim 50%WP,T4-Metiram 70%WGand T3-Propineb 70%WP.

Table 3: Mycelial per cent inhibition of *Fusarium oxysporum* f. sp. *pisi*

Treatment	3 DAI				6 DAI				9 DAI			
	% of inhibition				% of inhibition				% of inhibition			
	25 ppm	50 ppm	100 ppm	200 ppm	25 ppm	50 ppm	100 ppm	200 ppm	25 ppm	50 ppm	100 ppm	200 ppm
T1	100	100	100	100	72.38	72.86	77.62	79.76	54.82	59.63	66.30	70.74
T2	100	100	100	100	75.24	77.14	79.05	83.81	78.52	80.37	81.85	83.70
T3	8.77	15.79	15.79	29.83	2.38	5.24	6.19	17.14	18.89	21.48	24.44	33.33
T4	9.65	12.28	20.18	30.70	8.57	12.86	16.19	20.48	14.82	19.26	22.59	25.19
T5(control)	0	0	0	0	0	0	0	0	0	0	0	0
C.D.at 5%	0.14	0.23	0.20	0.32	0.12	0.14	0.27	0.11	0.19	0.12	0.09	0.05
S.E.(m)	0.88	1.41	1.24	2.00	0.74	0.85	1.66	0.67	1.22	0.72	0.57	0.29

C.D = Critical difference; S.E (m) = Standard Error Mean

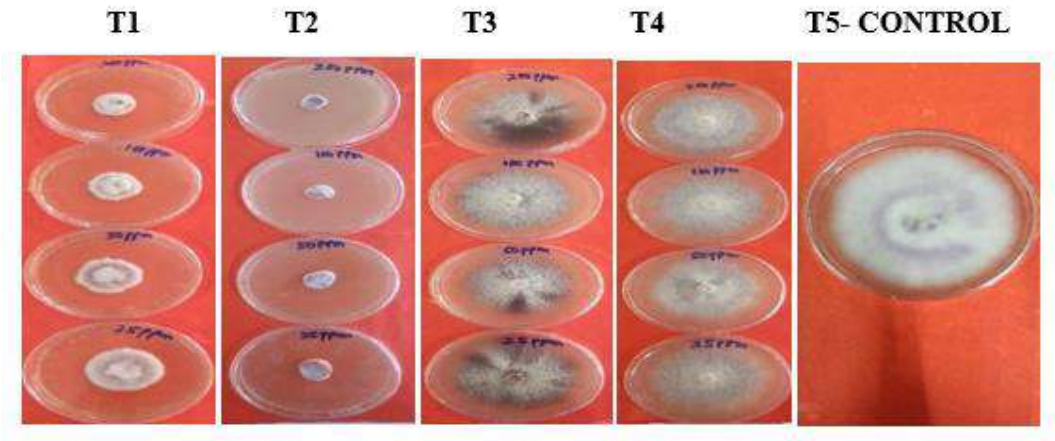

Plate 1. Inhibitory effect on Radial growth of *Fusarium oxysporum* f. sp. *pisi*

4. Conclusion

In this study we revealed that Tebuconazole 10% + Sulphur 65%WG fungicide gave best effective against mycelia growth and percent of inhibition than Carbendazim 50%WPof *Fusarium oxysporum* f. sp. *pisi* Therefore, the fungicide can be used as an alternative drug for controlling diseases and Disease Management in pea crop.

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