

Eco-friendly Management of Fusarium wilt (*Fusarium oxysporum* f. sp. *pisi*) of Field Pea (*Pisum sativum* L.)

Abstract

Pea is one of the most important *Rabi* pulse crop of India. Fusarium wilt which is caused by *Fusarium oxysporum* f. sp. *pisi* is considered to be the most devastating disease of this crop. The general inadequacy of chemical fungicides to tackle Fusarium wilt in field pea has lead to the search for ecofriendly management to these diseases. Therefore, present study “Eco-friendly management of wilt of field pea (*Pisum sativum* L.) caused by *Fusarium oxysporum* f. sp. *pisi*” was carried out in *Rabi* season in the year 2023-24 under field conditions. Effects of treatments were evaluated on disease incidence and different growth and yield parameters of field pea under field conditions. Among the treatments the disease incidence (%) at 30, 60 and 90 DAS was significantly decreased in treatment T₄ – Neem cake (S.A.) + *Trichoderma viride* (S.T.) (12.03%), (17.59%) and (24.07%). The plant height (cm) at 30, 60 and 90 DAS was significantly increased in T₄ – Neem cake (S.A.) + *Trichoderma viride* (S.T.) (23.36cm), (36.76cm) and (52.04cm) respectively. The yield (q/ha) was significantly increased in T₄ – Neem cake (S.A.) + *Trichoderma viride* (S.T.) (10.7q/ha) respectively as compared to control T₀.

Key words: field pea, *Fusarium oxysporum* f. sp. *pisi*, organic amendments and *Trichoderma viride*.

1. Introduction

Pea is a legume crop and is native to South Europe and grown as garden or field crop throughout the temperate regions of the world and was originally cultivated in the Mediterranean countries. In India, pea is one of the most important *Rabi* pulse crop, in which the crop is grown on a field scale for its dry seeds and smaller scale for green peas. It is also a good source of carbohydrates, vitamins A and C, calcium, phosphorus and has a small quantity of iron [13].

Fresh field pea seeds contain 17 to 22 g carbohydrates, 20 to 50 g starch, 14 to 26 g dietary fibre, 6.2 to 6.5 g protein, 0.4 g fat, 1.0 g ash per 100 g with 9 to 10 mg calcium, 3 to 5mg sodium, 97 to 99 mg potassium per 100 g and vitamin contents are 0.7 mg riboflavin, 5 to 6 mg thiamine and folate 0.54 mg per kg [20].

Pea is attacked by a number of diseases viz., powdery mildew caused by *Erysiphe polygoni*, downy mildew by *Pernospora viciae*, rust by *Uromyces viciae*, Fusarium wilt by *Fusarium oxysporum* f. sp. *pisi*, Aschochyta blight by *Aschochyta pisi*, bacterial blight by *Pseudomonas syringae* pv. *pisi* and pea mosaic by Pea mosaic virus [35].

Wilt caused by *Fusarium oxysporum* f. sp. *pisi* is one of the most destructive disease of field pea and occurs as an epiphyte almost every year. The disease is essentially soil borne and poses a greater problem in management by using fungicides which are uneconomical and their frequent and indiscriminate use often leads to atmospheric pollution and development of resistance in the pathogens. In this context, biological control is an alternative strategy of disease management which is eco-friendly [16].

Several organisms have been successfully used as biocontrol agents, oil cake and organic manure such as: Neem cake have a positive effect on controlling wilt disease in pea plants. The antifungal properties of neem cake can help suppress the growth and activity of these pathogens, reducing the incidence and severity of wilt disease. Mustard cake is a rich source of organic matter that improves soil fertility and structure. Healthy soil conditions support robust plant growth and can contribute to better disease resistance in pea plants, including resistance against wilt. Castor cake contains various bioactive compounds, including ricin oleic acid, which has fungicidal properties. These compounds can inhibit the growth and activity offungal pathogens that cause wilt disease, such as *Fusarium* and *Verticillium* species. By reducing the fungal population, castor cake may help alleviate the incidence and severity of wilt in pea plants. Well- nourished plants are generally more resilient and better able to resist diseases, including wilt [5].

Wilt disease is a common and destructive fungal disease that affects pea plants. It is primarily caused by *Fusarium oxysporum* f. sp. *pisi*. These pathogens invade the plant's vascular system, disrupting water and nutrient transport and causing wilting symptoms. Wilt disease is favoured by warm temperatures, high soil moisture, and poor drainage. Overwatering or excessive rainfall can promote pathogen growth and facilitate the entry and spread of the fungi in the plant. Stress factors such as nutrient deficiencies, improper irrigation, or mechanical damage toroots can also make plants more susceptible to wilt disease. Spraying of various fungicides has been recommended for control of wilt of pea in past. Control achieved by these chemicals is inadequate and harmful. Not much light has been shed on organic amendments and biological control which are effective against the pathogen.

2. Materials and Method

2.1 Experimental site:

The present investigation was carried out in *Rabi* season in the year 2023-24 in the Central Research Farm, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences (SHUATS), Prayagraj.

2.2 Symptomology:

The specific symptoms may vary depending on the causal agent involved. However, here are some common symptoms associated with *Fusarium* wilt of pea:

Wilting and stunting: The most apparent symptom of *Fusarium* wilt is the wilting and drooping of the affected pea plants. The wilting may be gradual or sudden, and it typically starts with lower leaves. The entire plant may become stunted and fail to grow properly

Yellowing and browning: The foliage of *Fusarium* wilt-affected pea plants often shows yellowing or browning of leaves. The discoloration may begin at the tips or margins of the leaves and gradually spread throughout the plant. In severe cases, the leaves may eventually turn brown, dry out and become necrotic.

Vascular discoloration: Some *Fusarium* wilt-causing pathogens (*Fusarium oxysporum*) can cause discoloration of the vascular tissues, which can be observed when the stem is cut open. The vascular tissues may turn brown or black due to the colonization and damage caused by the pathogens

Root rot: In many cases, *Fusarium* wilt is associated with root rot. The root system of affected pea plants may show symptoms such as rotting, browning, or a foul odor. The roots may become

discolored and show signs of decay, leading to impaired nutrient uptake and water absorption.

Reduced pod production: Fusarium Wilt-infected pea plants often exhibit a decrease in pod production. The number of pods formed may be significantly reduced, and the pods that do develop may be smaller in size or show abnormal shapes.

Plant death: In severe cases, the Fusarium wilt disease can lead to the death of the entire plant. The affected plants may wither and die prematurely, resulting in a complete loss of yield [18].



Plate 1. Symptoms of field pea of wilt

2.3 Isolation:

The infected parts were surface sterilized with 1:1000 Sodium hypochlorite solution for 60 seconds and washed separately in sterilized distilled water to remove the traces of chemical if any and then transferred to sterilized petri-plates containing potato dextrose agar (PDA). The petri plates were incubated at room temperature ($27\pm 1^{\circ}\text{C}$) and observed periodically for the growth of pure colonies [11]. The pure colonies which developed from the bits were transferred to PDA slants and incubated at $27\pm 1^{\circ}\text{C}$ for 5 - 7 days.

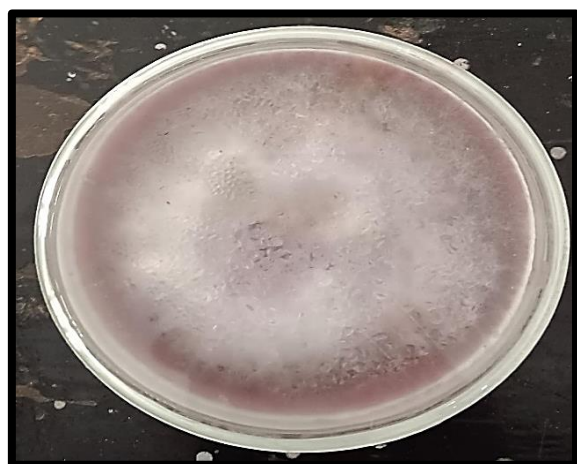
2.4 Morphology:

Colony colour of *Fusarium* spp. varies from white to pink. *Fusarium oxysporum* appear in purple, red, bluish grey, brown or black colour stroma. Aerial mycelium can be also produced in this fungus. These mycelia show little to no pigmentation and maximum growth on growth media. Best media is PDA but sometimes micro conidia will not appear on PDA [21]. The hyphae of *Fusarium* spp. are branched and septate. Three types of spores are formed in *Fusarium* species: micro conidia and chlamydospores. The micro conidia and macro conidia are formed on hyphae like conidiophore, which categorizes *Fusarium* as a Hyphomycete [32]. Micro conidia tend to be uninucleate and appear on sporodochia and monophialides. Micro conidia are cylindrical, slightly curved, hyaline and produced on short unbranched monophialides [13]. These conidia are scattered from mycelium and produce in less numbers, macro conidia are multinucleate and are produced in large numbers. Hardy spores appear in the form of chlamydospores which later modify in conidial or vegetative growth [36].

Fusarium oxysporum f. sp. *pisi* of pea on culture media consists of cottony white colonies which grows rapidly with aerial mycelium becoming purple to orange or dark blue to dark purple when sporodochia are formed. Macro conidia are 23-54 3-4.5 μm in size mostly with three septate and are fusiform in shape and slightly curved with pointed end at the tip and basal cells are pedicellate. Micro conidia are ellipsoidal to cylindrical (straight or often

curved). 5-12 2.3-3.5 μm and are mostly non-septate. Smooth to rough-walled chlamydospores of 5-13 μm in size are terminal or intercalary borne [34].

Pure cultures of *Fusarium oxysporum* of pea produced white colonies at the beginning which later turned to peach brown colour at agar base and attained a growth of 90 mm in 10 days of incubation at $25\pm 1^\circ\text{C}$. Mycelium was recorded to be smooth, cylindrical, septate and branched with 3.00- 4.80 μm in width. Conidiophores were measured to be 87.40-112.50 2.50- 5.00 μm in size Micro conidia were ellipsoidal to cylindrical. straight or curved in shape, born on short phialides, hyaline, 0-1 septate and measuring 6.80-16.00 \times 3.50-4.00 μm in size. Macroconidia were fusiform, pointed at ends, pedicellate basal cells, hyaline, 2-4 septate and measuring 32.50-44.00 4.00-5:50 μm in size. Chlamydospores were produced terminally, singly or in chains, nearly spherical, hyaline and measured 5.00-11.00 μm in diameter [12].



A



B

Plate 2A. Pure culture of *Fusarium oxysporum* f. sp. pisi

Plate 2B. Macro and Micro conidia of *Fusarium oxysporum* f. sp. pisi

2.5: Procedure for mass multiplication of *Fusarium oxysporum*

The fungus was mass cultured on sorghum seeds. The seeds were soaked overnight in a 5% sucrose and 30 mg/l chloramphenicol solution. The soaked seeds were transferred to 500 ml conical flasks and autoclaved twice at 15Kg cm^3 , 121°C , for 15-20 minutes. Thereafter, the flasks were inoculated with the pure culture of *F. oxysporum* f. sp. *ciceri* and incubated at $27\pm 2^\circ\text{C}$ for 8-10 days in an incubator. For soil inoculation, fungus colonised seeds (532 g) were ground in a mixer-grinder and suspended in 10 litre tap water. The suspension was spread uniformly on micro plots of $2\times 1\text{ m}^2$ to achieve an inoculum level of 1.5 g colonised seeds/kg soil. Soil inoculation was done two days before seed was sown [19] [22].

Table 1. Detail of treatment

Treatment No.	Treatment Detail
T ₀	Control (untreated check)
T ₁	Neem cake @ 400gm/plot (S.A.)
T ₂	Mustard cake @ 400gm/plot (S.A.)

T₃	Castor cake @ 400gm/plot (S.A.)
T₄	Neem cake @ 400gm/plot (S.A.) + <i>Trichoderma viride</i> @4g (S.T.)
T₅	Mustard cake @ 400gm/plot (S.A.) + <i>Trichoderma viride</i> @4g (S.T.)
T₆	Castor cake @ 400gm/plot (S.A.) + <i>Trichoderma viride</i> @4g (S.T.)
T₇	Carbendazim @ 0.2% (S.T.)

2.6: Disease incidence (%) at 30,60 and 90 DAS

The sequential development of symptoms of the disease was studied on naturally infected plants in the experimental plots in field at Central Research Farm (CRF) of Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj during the *Rabi* season of 2023- 24. Plants were observed over time to investigate the wilt severity under natural conditions. Data were recorded on the basis of symptoms and subsequent observation by given formula [4].

$$\text{Disease Incidence (\%)} = \frac{\text{Number of wilted plants/plot}}{\text{Total number of plants/plot}} \times 100$$

2.7: Cost Benefit Ratio:

Benefit cost ratio is the ratio of gross return to cost of cultivation, which can also be expressed as return per rupee invested. This index provides an estimate of the benefit a farmer derives from the expenditure he incurs in adopting a particular cropping system. Any value above 2.0 is considered safe as the farmer gets Rs. 2 for every rupee invested. The benefit cost ratio was calculated using the formula [26].

$$\text{CBR} = \frac{\text{Gross return (Rs/ha)}}{\text{Total cost of cultivation (Rs/ha)}}$$

3. Results and Discussion

3.1 Effect of Organic amendments and bio agent on wilt Disease Incidence (%) of field pea

At 30 DAS

Data recorded in **Table 2** showed that, among all treatments, minimum per cent disease incidence (%) at 30 DAS was recorded with Neem cake + *Trichoderma viride* (12.03%) followed by Mustard cake + *Trichoderma viride* (13.8%), Castor cake + *Trichoderma viride* (14.8%), Neem cake (17.5%), Mustard cake (19.44%), Castor cake (20.37%), and maximum per cent disease incidence was recorded in control (23.140%).

The minimum per cent disease incidence at 30 DAS was recorded with neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₄, and T₇) were significant to each other as well as (T₂ and T₃) and (T₅ and T₆) were found

not significant to each other.

At 60 DAS

Data recorded in **Table 2** showed that, among all treatments, minimum per cent disease incidence (%) at 60 DAS was recorded with Neem cake + *Trichoderma viride* (17.59%) followed by Mustard cake + *Trichoderma viride* (19.5%), Castor cake + *Trichoderma viride* (20.3%), Neem cake (24.07%), Mustard cake (25.9%), Castor cake (27.7%), and maximum per cent disease incidence was recorded in control (30.7%).

The minimum per cent disease incidence at 60 DAS was recorded with Neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₂, T₃, T₄ and T₇) were significant to each other as well as (T₅ and T₆) were found not significant to each other.

At 90 DAS

Data recorded in **Table 2** showed that, among all treatments, minimum per cent disease incidence (%) at 90 DAS was recorded with Neem cake + *Trichoderma viride* (24.07%) followed by Mustard cake + *Trichoderma viride* (28.17%), Castor cake + *Trichoderma viride* (28.7%), Neem cake (34.25%), Mustard cake (36.1%), Castor cake (39.8%), and maximum per cent disease incidence was recorded in control (41.67%).

The minimum per cent disease incidence at 90 DAS was recorded with Neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₂, T₃, T₄ and T₇) were significant to each other as well as (T₅ and T₆) were found not significant to each other.

The probable reasons for such findings may be because of inhibitory effect of Neem cake as soil amendment combination with *Trichoderma viride* as seed treatment which can lowering the disease incidence of Fusarium wilt by inhibiting the pathogen as compared to the other treatments by **Kala et al. [14]**. The soil application of neem cake to other significantly increased the plant height by reducing the disease incidence may be due to the presence of active compounds such as azadirachtin, nimbin, nimbinene and azadirone in neem which has shown strong fungitoxicity against fusarium wilt by **Sharma et al. [27]**; **Animisha et al. [3]** *Trichoderma viride* are significant role in biocontrol activity like cell wall degradation, hyphal growth, antagonistic activity against fusarium wilt. Similar findings have been reported by **Kapoor et al. [15]**.

Table 2. Effect of treatment on Per cent Disease Incidence (%) of field pea at 30, 60 and 90 DAS

Treatment No.	Treatment detail	Per cent Disease Incidence (%)		
		30 DAS	60 DAS	90 DAS
T ₀	Control	23.14	30.71	41.66

T₁	Neem cake	17.59	24.07	34.25
T₂	Mustard cake	19.44 ^a	25.92	36.11
T₃	Castor cake	20.37 ^a	27.77	39.81
T₄	Neem cake + <i>Trichoderma viride</i>	12.03	17.59	24.07
T₅	Mustard cake + <i>Trichoderma viride</i>	13.88 ^b	19.50 ^a	28.17 ^a
T₆	Castor cake + <i>Trichoderma viride</i>	14.81 ^b	20.37 ^a	28.70 ^a
T₇	Carbendazim	9.25	12.94	18.51
CD(p=0.05)		1.330	0.867	0.615
S. Ed±		0.527	0.325	0.283

*Data followed by same alphabets in a column are non-significant to each other at 5% level

3.2 Effect of selected treatments on the Plant height (cm) of field pea

At 30 DAS

Data recorded in **Table 3** showed that, among all treatments, maximum plant height (cm) at 30 DAS was recorded with Neem cake + *Trichoderma viride* (23.36cm) followed by Mustard cake + *Trichoderma viride* (22.9cm), Castor cake + *Trichoderma viride* (22.2cm), Neem cake (20.8cm), Mustard cake (19.9cm), Castor cake (19.2cm), and minimum plant height was recorded in control (17.2cm).

The highest plant height at 30 DAS was recorded with Neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₂, T₃, and T₇) were significant to each other as well as (T₄ and T₅) and (T₅ and T₆) were found not significant to each other

At 60 DAS

Data recorded in **Table 3** showed that, among all treatments, maximum plant height (cm) at 60 DAS was recorded with Neem cake + *Trichoderma viride* (36.7cm) followed by Mustard cake + *Trichoderma viride* (33.9cm), Castor cake + *Trichoderma viride* (32.8cm), Neem cake (32.7cm), Mustard cake (32.02cm), Castor cake (31.6cm), and minimum plant height was recorded in control (28.8cm).

The highest plant height at 60 DAS was recorded with Neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₄, T₅, and T₇) were significant to each other as well as (T₂ and T₃) and (T₁ and T₆) were found not significant to each other.

At 90 DAS

Data recorded in **Table 3** showed that, among all treatments, maximum plant height (cm) at 90 DAS was recorded with Neem cake + *Trichoderma viride* (53.6cm) followed by Mustard cake

+ *Trichoderma viride* (52.04cm), Castor cake + *Trichoderma viride* (51.3cm), Neem cake (50.5cm), Mustard cake (48.4cm), Castor cake (46.8cm), and minimum plant height was recorded in control (44.1cm).

The highest plant height at 90 DAS was recorded with Neem cake + *Trichoderma viride* followed by Mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₁, T₂, T₃, T₄ and T₇) were significant to each other as well as (T₅ and T₆) were found not significant to each other.

The probable reasons for such findings may be due to the presence of antifungal compounds such as alkaloids, flavonoids, azadirachtin, nimbin, nimbinene and azadirone in Neem cake by Pandey *et al.* [23]; Suresh *et al.* [33]. Which has shown strong fungicidal effect against the pathogen by reducing the disease incidence and it enhanced the plant growth by producing phyto hormones by Rafi *et al.* [24]. *Trichoderma viride* suppressed other pathogen growth and increased resistance that enhance the plant growth and yield. Similar findings have been reported by Raghu *et al.* [25].

Table 3. Effect of treatment on Plant height (cm) of field pea at 30, 60 and 90 DAS

Treatments No.	Treatment detail	30 DAS	60DAS	90DAS
T ₀	Control	17.23	28.83	44.11
T ₁	Neem cake	20.84	32.76 ^b	50.53
T ₂	Mustard cake	19.93	32.02 ^a	48.45
T ₃	Castor cake	19.23	31.7 ^a	46.89
T ₄	Neem cake + <i>Trichoderma viride</i>	23.36 ^a	36.76	53.65
T ₅	Mustard cake + <i>Trichoderma viride</i>	22.91 ^{ab}	33.90	52.04 ^a
T ₆	Castor cake + <i>Trichoderma viride</i>	22.21 ^b	32.80 ^b	51.38 ^a
T ₇	Carbendazim	24.85	37.70	55.04
CD(p=0.05)		0.515	0.559	0.654
S. Ed±		0.24	0.26	0.30

*Data followed by same alphabets in a column are non-significant to each other at 5% level

3.3 Effect of treatment on yield (q/ha) of field pea

Data recorded in Table 4 showed that, among all treatments, maximum yield (q/ha) was recorded Neem cake + *Trichoderma viride* (10.7q/ha) followed by Mustard cake + *Trichoderma viride* (10q/ha), Castor cake + *Trichoderma viride* (9.9q/ha), Mustard cake (9.9q/ha), Neem cake (9.86q/ha), Castor cake (9.63q/ha) and minimum yield was recorded in control (8.7q/ha).

The highest yield (t/ha) was recorded in Neem cake + *Trichoderma viride* followed by

mustard cake + *Trichoderma viride* as compared to carbendazim (treated check) and control.

All the treatments were found statistically significant from T₀ control. Among the treatments (T₄ and T₇) were significant to each other as well as treatments (T₅, T₆, T₂ and T₁) (T₆, T₂, T₁ and T₃) were found not significant to each other.

The probable reasons for such findings may be that Neem cake has been found to have antifungal properties and can be used as a natural remedy against fusarium wilt disease in plant by **Kapoor et al. [15]**. The active compounds present in neem cake, such as azadirachtin, limonoids and other bio active compounds have been shown to exhibit antifungal activity by **Sharma et al. [27]**. It reducing environmental impacts and promoting sustainable agriculture practices. neem cake combination with *Trichoderma viride* most effective soil amendment and seed treatment for controlling the wilt disease as well as increased for the plant growth and yield quality. Similar findings have been reported by **Animisha et al. [3]**; **Singh et al. [30]**.

Table 4. Effect of various treatments on Yield (q/ha)

Treatments No.	Treatment detail	Yield (q/ha)
T ₀	Control	8.7
T ₁	Neem cake	9.86 ^{ab}
T ₂	Mustard cake	9.9 ^{ab}
T ₃	Castor cake	9.63 ^b
T ₄	Neem cake + <i>Trichoderma viride</i>	10.73
T ₅	Mustard cake + <i>Trichoderma viride</i>	10 ^a
T ₆	Castor cake + <i>Trichoderma viride</i>	9.9 ^{ab}
T ₇	Carbendazim	12.56
	CD(p=0.05)	0.308
	S. Ed (±)	0.14

*Data followed by same alphabets in a column are non-significant to each other at 5% level

Table 5. Cost benefit ratio

Tr. No.	Yield q/ha	Cost of Yield/q	Total cost of yield (₹)	Common cost(₹)	Treatment cost(₹)	Total cost of cultivation (₹)	C:B ratio
T ₀	8.7	5100	44,370	42,975	-----	42,975	1:1.03
T ₁	9.86	5100	50,286	42,975	2,700	45,675	1:1.10

T₂	9.9	5100	50,490	42,975	3,200	46,175	1:1.09
T₃	9.63	5100	49,113	42,975	2,300	45,275	1:1.08
T₄	10.73	5100	54,723	42,975	5,050	48,025	1:1.13
T₅	10	5100	51,000	42,975	5,050	48,025	1:1.06
T₆	9.9	5100	50,490	42,975	4,650	47,625	1:1.06
T₇	12.56	5100	63,750	42,975	446	43,421	1:1.46

4. Conclusion

Based on the result obtained from present investigations it was found that Neem cake @ 400gm/plot as soil amendment with *Trichoderma viride* @ 4g/kg as seed treatment was most effective and recorded minimum disease incidence (%) of wilt of pea crop and maximum plant height (cm), overall yield (q/ha) and cost benefit ratio under field conditions, therefore it may be recommended for the better management of wilt of pea. Results of the present study were limited to one crop season (Dec 2023-March 2024) and found to be significantly effective under Prayagraj agro-climatic conditions. It may vary with region and climatic conditions, therefore, for validation of the results more such trials should be carried out in future along with *in-vitro* testing will be helpful for more effective strategy development in eco-friendly management of the disease.

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