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FIELD EVALUATION OF FUNGICIDES AND BIO-PRODUCTS FOR MANAGEMENT OF *FUSARIUM* WILT IN CHILLI (*CAPSICUM ANNUM* L.)

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ABSTRACT

Chilli (*Capsicum annum* L.) is an important economic crop and mainly cultivated in tropical and sub-tropical countries of the world. The crop is rich in nutrients and has good medicinal properties. Chilli is commonly affected by many bacteria, viruses, nematodes and fungal diseases. Among these *Fusarium* wilt is caused by the fungus *Fusarium oxysporum* causes moderate to severe loss. Very little work has been done for the management of the *Fusarium* wilt of chilli in the Red and Lateritic Agro-climatic zone of West Bengal. A field study was carried out during the Rabi season 2021-22 at the agricultural farm of Palli-Siksha Bhavana (Institute of Agriculture) at Birbhum district of West Bengal. The characteristic symptom of *Fusarium* wilt includes yellowing and an inward rolling of leaves followed by progressive wilting of the whole plant which is caused by damage in the vascular system. Fungicides such as Thiophanate methyl 70% WP, Tubeconazole 50% + Trifloxystrobin 25% WG and Carbendazim 12% + Mancozeb 63% WP can be used to manage the disease very effectively. The result of field trials revealed that Thiophanate methyl 70% WP is excellent control of wilt infection as compared to other treatments. The neem oil (Botanical) and *Trichoderma viride* (Biocontrol agent) were found to be inhibitory but not as much as effective than fungicides. However, the cost of economics was also calculated and found that the highest cost benefit ratio in Thiophanate methyl 70% WP followed by Carbendazim 12% + Mancozeb 63% WP.

***Keywords* :** *Fusarium* wilt, Chilli, *Fusarium oxysporum,* fungicides, Management.

Introduction

Chilli (*Capsicum annum* L., Family: Solanaceae) is an important economic crop and mainly cultivated in tropical and sub-tropical countries of the world (Pickersgill, 1997). From the nutritional point of view, the green fruit of chilli is a good source of minerals like potassium, magnesium, calcium and iron (Serra *et al.,* 2002), besides vitamins A, E, C and P (Singh, 1989). India is the leading producer of chilli with an area of 411 thousand hectares, production of 4363 million tonnes and productivity of 10.62 MT/ha. Chilli is cultivated in all states and union territories in India. One among the state Andhra Pradesh is the leading producer contributing 44 % of total production followed by Karnataka (12%), West Bengal (8%), Madhya Pradesh (7%), Maharastra (4%) and Tamil Nadu (2%). In West Bengal, Chilli area cultivation, production and productivity are 62.39 Ha, 217.65 MT and 3.49 MT/Ha respectively (Anonymous, 2021). One of the main constraints to chilli cultivation is damage caused by pathogens including bacteria, viruses, nematodes and fungi, which cause severe losses in production. More than 40 diseases are caused by fungi, which significantly reduce the yields (Rangaswami, 1972). Common fungal diseases are damping-off (*Pythium* spp.), Rhizoctonia root rot (*Rhizoctonia* *solani*), Anthracnose/ Dieback/ Fruit rot (*Colletotrichum capsici*), Powdery mildew (*Leveillula taurica*), Black spot (*Alternaria* spp), *Fusarium* wilt (*Fusarium oxysporum* f.sp. *capsici*), Cercospora leaf spot/ Frogeye (*Cercospora capsici*) and Phytophthora blight (*Phytophthora capsici*) are commonly affected the chilli crop (Anonymous, 2005). One among the important disease is Fusarium wilt caused by *Fusarium oxysporum* f. sp *capsici* has become a major problem in chilli cultivation and causes 10-50% loss in India (Bai *et al.,* 2018). The characteristic symptom of *Fusarium* wilt includes yellowing and an inward rolling of leaves followed by progressive wilting of the whole plant which is caused due to the damage in vascular system (MacHardy and Beckman, 1981). Chemical management is an imperative means for the control of different plant diseases (Iqbal and Mukhtar, 2020; Mahr, 2021). Fungicides like Carbendazim, Benomyl, Nativo, Alliete, Topsin-M and Difenoconazole are used for the management of *Fusarium* wilt pathogen (Bashir *et al.,* 2018; Faraz *et al.,* 2020). The present investigation was carried out to evaluate the potential application of the most effective chemical treatment under field conditions. So that the disease could be effectively controlled.

Material and Methods

The Randomised Block Design (RBD) with three replications for each treatment was used for the experiment. The entire experiment was conducted in the agricultural farm of Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, Bolpur in Birbhum district, West Bengal during the Rabi season 2021-22. The experimental site is situated at 23˚40 6 ʺ N latitude, 87˚37 ʺ 57 ʹ E longitude and 58.90m above mean sea level in the lateritic belt of West Bengal, with dry sub-humid and sub-tropical climate. The long-term average maximum temperature varied from 29.46˚C to 34.3˚C and the average minimum temperature varied from 17.39˚C to 25.96˚C in this region. The soil type of the experimental site is slightly acidic (pH 5.5-5.8) with sandy loam (Ultisol) in texture. Very hot summer (34-42 ˚C) and cold winter (5-15 ˚C) are characteristics of the area. Throughout the experiment, the chilli variety 'Bullet' was used. The size of the individual plot was 2.5m x 3m. One month old seedlings were transplanted into each plot at a distance of 60 X 40 cm^2. The other cultivation techniques were used in accordance with the package practice prepared by the Department of Plant Pathology, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati.

Isolation and identification of the pathogen

Chilli plants that showed typical symptoms of wilt disease were collected from agricultural farm and then attempted for the isolation of pathogen. The diseased sample was cut into small pieces (2-3 mm size) along with a healthy portion, washed with distilled water and surface sterilized with 0.1% $HgCl_2$ for 1 min. The samples were placed on Potato Dextrose Agar (PDA) media with the help of forceps and incubated at room temperature (28 ± 1˚C) for 5-7 days and were observed on daily basis for colony growth. Identification of *Fusarium oxysporum* was done under light microscope, based on morphological characters of the pathogen like colony growth, colour (Purple and white) and conidiophores, macroconidia and microconidia (Muhammad *et al.,* 2022). The characters were compared with those described by Booth (1971).

Pathogenicity test

A pathogenicity of the isolated fungus was performed on chilli seedlings (28 days old) in earthen pots (Bashir *et al.,* 2018). The concentration of spore suspensions was calculated using haemocytometer and adjusted to 1 x10^6 spore/ml. The spore suspensions were inoculated in chilli plants by root dip method described by Okiror (1986). One month old healthy seedlings were taken from the nursery. The roots of the plants were rinsed with sterile distilled water and then soaked in prepared fungal suspension for 15 minutes. This treatment was done for 6 plants. Meanwhile, the healthy seedlings roots were soaked in sterile distilled water and used as control. Each seedling was planted in sterile soil in the earthen pot. The pots were constantly observed for development of the disease symptoms. The percent disease of each inoculated plant was recorded 20 days after inoculation. After recording the symptoms on the inoculated plant, the causal pathogen was re-isolated and resulted culture compared with original inoculants to prove Koch's postulates.

Effect of different fungicides and Bio-product against the pathogen

The field experiment was conducted to evaluate ten different treatments including untreated control viz., T1-Carbendazim 12% + Mancozeb 63% WP, T2-Tubeconazole 50% + Trifloxystrobin 25% WG, T3-Difenconazole 25% EC, T4-Propineb 70% WP, T5-Thiophanate methyl 70% WP, T6-Neem oil (Botanicals), T7-Chlorothalonil 75% WP, T8-*Trichoderma viride* (Biocontrol), T9-Fosetyl-Aluminium 80% WG, and T10-untreated control against *Fusarium* wilt pathogen of chilli. The treatments were sprayed three times at 15 days intervals starting from the first appearance of the disease in field condition. The per cent disease incidence (PDI) of *Fusarium* wilt of chilli was observed at 45 days after 1st, 2nd and 3rd spraying of different treatment under field condition. The percent disease incidence was calculated by using the formula (Mayee and Datar, 1986).

$$\text{Disease Incidence \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} X\ 100$$

On the basis data recorded in control, Percent increase in yield and the percent decreased in disease incidence were calculated by using the formula (Mathur *et al.,* 1971)

$$\text{Disease control \%} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} X\ 100$$

$$\text{Yield Increase \%} = \frac{\text{Yield in treatment} - \text{Yield in control}}{\text{Yield in control}} X\ 100$$

Economics of chilli cultivation as influenced by management practices

Economics of chilli cultivation in respect of chemical and eco-friendly approach from pooled data were worked out to evaluate the treatments for the management of wilt disease of chilli caused by *Fusarium oxysporum* (Chilli isolate) in field condition. The benefit: cost ratio (B: C) for each treatment was obtained by using the following equation.

B: C= N/T

Where, B = Benefit, C = Cost, N = Net return (Total cost of cultivation - Gross return),

T = Total cost of cultivation

Statistical Analysis

The data from each observation recorded in the preceding study were statistically analysed, and calculations were made using the test of significance for treatment means. Analysis of data was carried out using angular transformation at the 5% level of significance with the help of OPSTAT software (Sheoran, 2006).

Results and Discussion

The Pathogen confirmation

Chilli plants showing typical symptoms of wilt disease were collected from agricultural farm and attempted for the isolation of pathogen. The pathogen was isolated in Potato Dextrose Agar (PDA) medium and it was observed that the pathogen was started to grow after 48 hours of incubation. The pure culture of the *Fusarium oxysporum* was obtained on the 7th day of incubation, which had white cottony aerial mycelium on PDA (Ferniah *et al.,* 2014). On microscopy, the microconidia, macroconidia and chlamydospores were observed. The morphological features such as mycelium, conidiophore, microconidia, macroconidia and chlamydospore were observed (Table 1, Plate 1). Similar results were reported by Angel et al. (2016) who observed that the *Fusarium* isolates showed hyaline, oval-shaped microconidia without septations and curved, straight-shaped macroconidia with three to five septations. Thus, confirming the identification of the *Fusarium oxysporum* (chilli isolates).

The pathogenicity test showed that *Fusarium oxysporum* isolate caused wilting symptoms, when pure culture inoculated in the chilli seedlings. It was observed 20 days after inoculation only. Initially, the plants showed yellowing of the plants, which progressed to chlorosis and necrosis; slowly it changed to wilting and eventually the death of the whole plant (Shafique *et al.,* 2015). Hami *et al.* (2021) proved the pathogenicity of *F. oxysporum* purified from diseased plants. The pathogens were re-isolated from the inoculated seedlings and compared with their original inoculated cultures. The re-isolated pathogens completely resembled the original inoculated pathogen in their morphological, cultural and pathogenic characteristics, so satisfied Koch's postulates.

Management of *Fusarium* wilt of chilli under field condition

Different fungicides, biocontrol and botanical were tested for their efficiency against *Fusarium* wilt disease. The per cent disease incidence (PDI) and the yields of different treatments were compared. There was no significant variation among the treatments before spraying. The disease severity was significantly low in all the treated plots as compared to the untreated control. Evaluation of fungicides for the treatment of plant diseases is a major way to save a huge amount of money and the environment (Iqbal *et al.* 2010). The per cent disease incidence (PDI) was observed at 15 day intervals of 1st spraying, 2nd and 3rd spraying from the first appearance of the disease.

After 1st spraying (60 DAT), the minimum *Fusarium* wilt incidence was recorded in T5-Thiophanate methyl 70% WP (8.29 %) which was followed by T2-Tubeconazole 50%+ Trifloxystrobin 25% WG (9.61%) and T1-Carbendazim 12% + Mancozeb 63% WP (10.91%), while maximum disease incidence was observed in T10-untreated control (17.11%) followed by T7-Chlorothalonil 75% WP (15.20%). It was found that PDI of *Fusarium* wilt of chilli in the T2 (18.04) was statistically at par with T5 (16.73). After 15 days of second spraying lowest PDI was recorded in T5-Thiophanate methyl 70% WP

(10.57%) followed by T2-Tubeconazole 50% + Trifloxystrobin 25% WG (15.38%), T1-Carbendazim 12% + Mancozeb 63% WP (15.70%). At this stage, the maximum incidence was recorded in untreated control T10-untreated control (28.68%) followed by T7 Chlorothalonil 75% WP (22.65%). The mean of wilt incidence was also lowest in T5 (18.95) followed by T2 (23.07). These two treatments are statistically to control wilt disease. Chlorothalonil 75% WP (T7) and *Trichoderma viride* (T8) treatments were not so effective against this disease. After 15 days of third spraying lowest PDI was recorded T5 (13.38%) Thiophanate methyl 70% WP followed by T2 (17.67%) Tubeconazole 50%+ Trifloxystrobin 25% WG, whereas highest wilt incidence was recorded in untreated control T10 (36.85%) followed by T7 (28.55%) Chlorothalonil 75% WP (Table 2).

The most effective control (PDC- percent disease control) of wilt disease was achieved by spraying with Thiophanate methyl 70% WP (T5) @ 2g/litre water (63.69) followed by Tubeconazole 50%+ Trifloxystrobin 25% WG (T2) @ 2.5g/litre water (52.04) and Carbendazim 12% + Mancozeb 63% WP (T1) @ 2.5g/litre water (51.83) Fig.1). Thiophanate methyl 70% WP (T5) showed excellent control of wilt disease infection as compared to other treatments. Sahar et al. (2013) reported that three sprays of Topsin-M shown maximum minimization of disease incidence followed by Difenoconazole, Nativo and Alliete, whereas in present study Thiophanate methyl 70% WP gave maximum protection followed by Tubeconazole 50%+ Trifloxystrobin 25% WG and Carbendazim 12% + Mancozeb 63% WP. Likewise, Weitang et al. (2004) opined that Prochloraz and Carbendazim were the most effective fungicides to inhibit growth of *F. oxysporum*. The findings of the present investigation are quite in conformity with the reports of earlier workers.

The yield was found highest in T5-Thiophanate methyl 70% WP (107.59 q/ha) which was significant over control and the second highest yield in T1-Carbendazim 12% + Mancozeb 63% WP (96.39 q/ha) followed by T2-Tubeconazole 50%+ Trifloxystrobin 25% WG (87.06 q/ha) as presented in Fig 2.

Economics of chemical treatments

The benefit cost ratio of each treatment was worked out to evaluate the effective treatment for the management of *Fusarium* wilt disease in field conditions. Work was undertaken to calculate the common cost of cultivation. The total common cost of cultivation was Rs. 63, 867.00 (Sixty three thousand eight hundred sixty seven) in Table 3. The total cost of cultivation for each treatment was calculated and the highest cost of cultivation was recorded in the Difenconazole 25% EC (Rs. 67, 617.00) treatment. The lowest cost of cultivation was recorded in the untreated control (Table 4).

The net return was found highest in T5-Thiophanate methyl 70% WP (Rs. 1,50,876.00) treatment followed by T1-Carbendazim 12% + Mancozeb 63% WP (Rs. 1,27,663.00), T2-Tubeconazole 50%+ Trifloxystrobin 25% WG (Rs. 107,128.00). All the treatments showed positive benefits and cost ratios. However, the benefit and cost ratio was found highest in Thiophanate methyl 70% WP (3.346) treatment followed by Carbendazim 12% + Mancozeb 63% WP (2.960), Tubeconazole 50%+ Trifloxystrobin 25% WG (2.599) (Table 5). Similarly, Raghu et al. (2018) observed the highest benefit cost on chemical module (3.25) followed by biological module (2.29). The result of the present study revealed that, Thiophanate methyl 70% WP was significantly effective in controlling the disease under field condition. However, the evaluation of the economics of each treatment is essential before their recommendation for disease management to the farmers.

Conclusion

Fusarium wilt of chilli caused by *Fusarium oxysporum* is increasing gradually in the Birbhum district of West Bengal. The development of *Fusarium* wilt of chilli under field conditions was influenced by prevailing weather conditions during the Rabi season. Timely application of fungicides with proper doses can save the crop from this harmful pathogen. In field trials, foliar application of Thiophanate methyl 70% WP was excellent for controlling the wilt of chilli followed by Tubeconazole 50% + Trifloxystrobin 25% WG and Carbendazim 12% + Mancozeb 63% WP. The cost economics was also calculated and found that, highest cost benefit ratio was obtained in Thiophanate methyl 70% WP. Further studies are required to develop the integrated fusarium wilt management approach to enhance overall yield, and to decrease the economic crisis and food shortage.

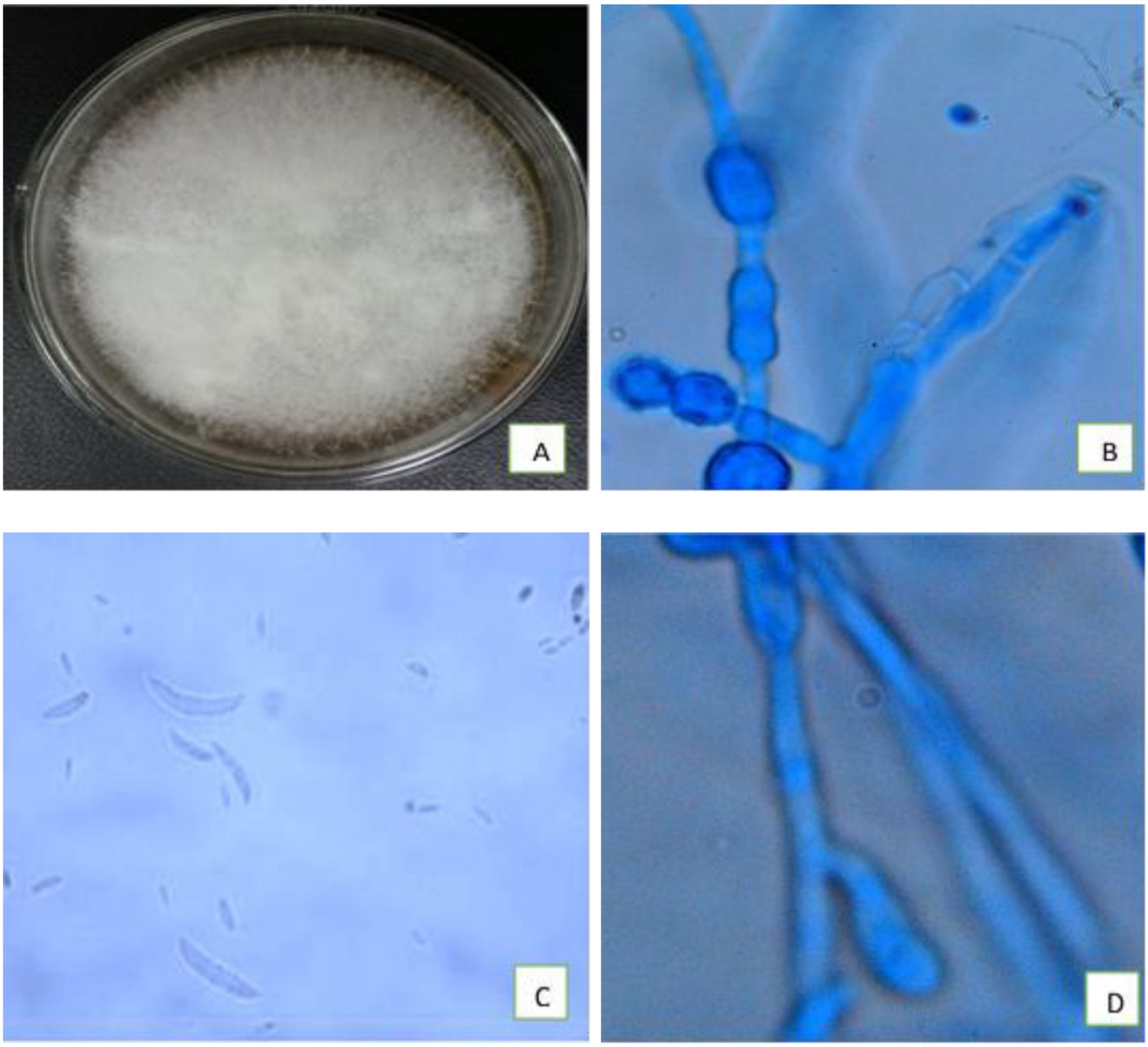

Plate 1: Morphological characteristics of *Fusarium oxysporum*
A. Pure culture, **B.** Chlamydospore, **C.** Microconidia and Macroconidia, **D.** Phialids

Table 1: Cultural and morphological characteristics of *Fusarium oxysporum*

Characteristics	Colour	Shape	Septation
Colony	Creamish white colour	Floccose, sparse aerial mycelium	-
Mycelium	Hyaline	Smooth, slendrical, branched	septate
Conidiophore	Hyaline	Slendrical, short	septate
Phialides	Hyaline	Slendrical, arising laterally	-
Macroconidia	Hyaline	Sickle/ curved shaped, slightly curved apex	Septate (3-5)
Microconidia	Hyaline	Ovoid to short cylindrical	No septation
Chlamydospore	Hyaline	Spherical, smooth, terminal and intercalary, single (or) chains	-

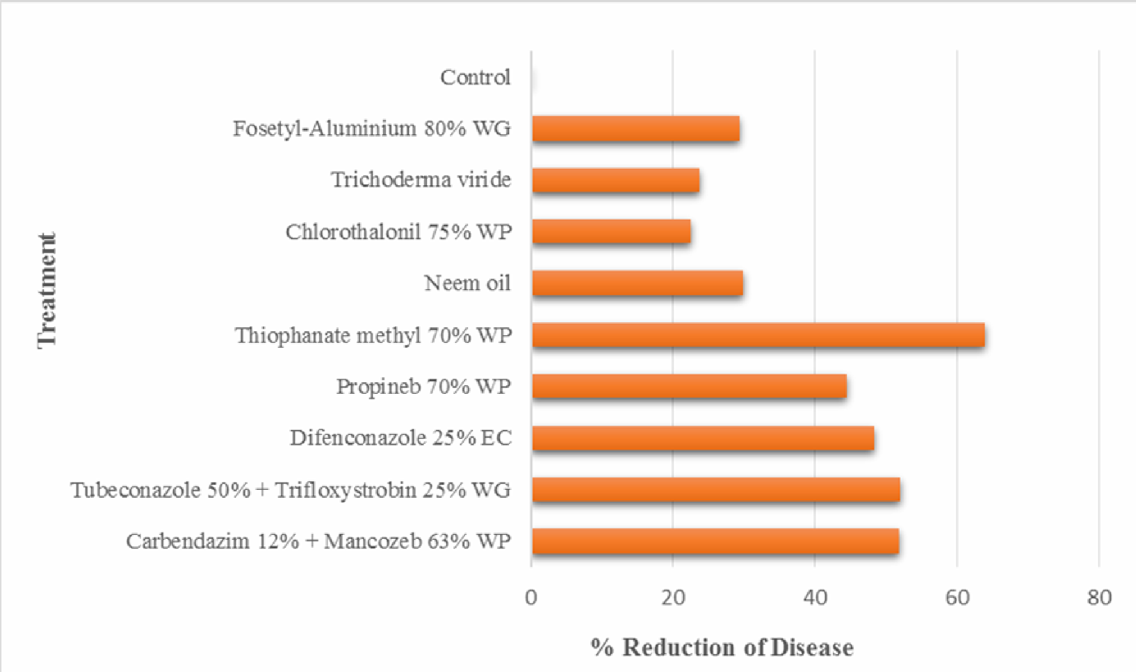

Fig. 1 : Percent reduction of disease incidence over control after 3rd spray

Table 2 : Different treatments for Management of chilli wilt disease under field conditions

Treatments	Dose (g or ml/l)	PDI at 15 days interval			PDC	Yield (q/ha)	% increase of yield over control
		1st spray	2rd spray	3rd obser			
T1: Carbendazim 12% + Mancozeb 63% WP	2.5g	10.91 (19.27)*	15.70 (23.32)*	17.75 (24.90)*	51.83	96.39	162.46
T2: Tubeconazole 50% + Trifloxystrobin 25% WG	2.5 g	9.61 (18.04)*	15.38 (23.07)*	17.67 (24.83)*	54.68	87.06	138.72
T3: Difenconazole 25% EC	1.5 ml	14.24 (22.15)*	16.48 (23.93)*	19.08 (25.88)*	48.22	71.99	96.09
T4: Propineb 70% WP	2.5 g	12.38 (20.56)*	16.20 (23.72)*	20.45 (26.87)*	44.50	81.33	121.50
T5: Thiophanate methyl 70% WP	2 g	8.29 (16.72)*	10.57 (18.95)*	13.38 (21.44)*	63.96	107.59	192.92
T6: Neem oil (Azadiractin)	2 ml	13.34 (21.40)*	20.27 (26.74)*	25.77 (30.49)*	29.93	51.99	41.69
T7: Chlorothalonil 75% WP	2 g	15.20 (22.93)*	22.65 (28.40)*	28.55 (32.28)*	22.52	75.99	106.97
T8: *Trichoderma viride*	2 g	14.41 (22.29)*	21.06 (27.30)*	28.14 (32.01)*	23.63	55.59	51.48
T9: Fosetyl - Aluminium 80% WG	1.5 g	13.09 (21.19)*	19.64 (26.28)*	26.05 (30.68)*	29.30	66.66	81.60
T10: Untreated control	-	17.11 (24.41)*	28.68 (32.36)*	36.85 (37.35)*	-	36.66	-
SE(m) ±		0.420	0.338	0.365		0.21	
CD(P=0.05)		1.259	1.012	0.912		0.60	

*Figures in parenthesis are angular transformed values, PDC= Percent reduction of the disease over control, PDI= Percent disease incidence

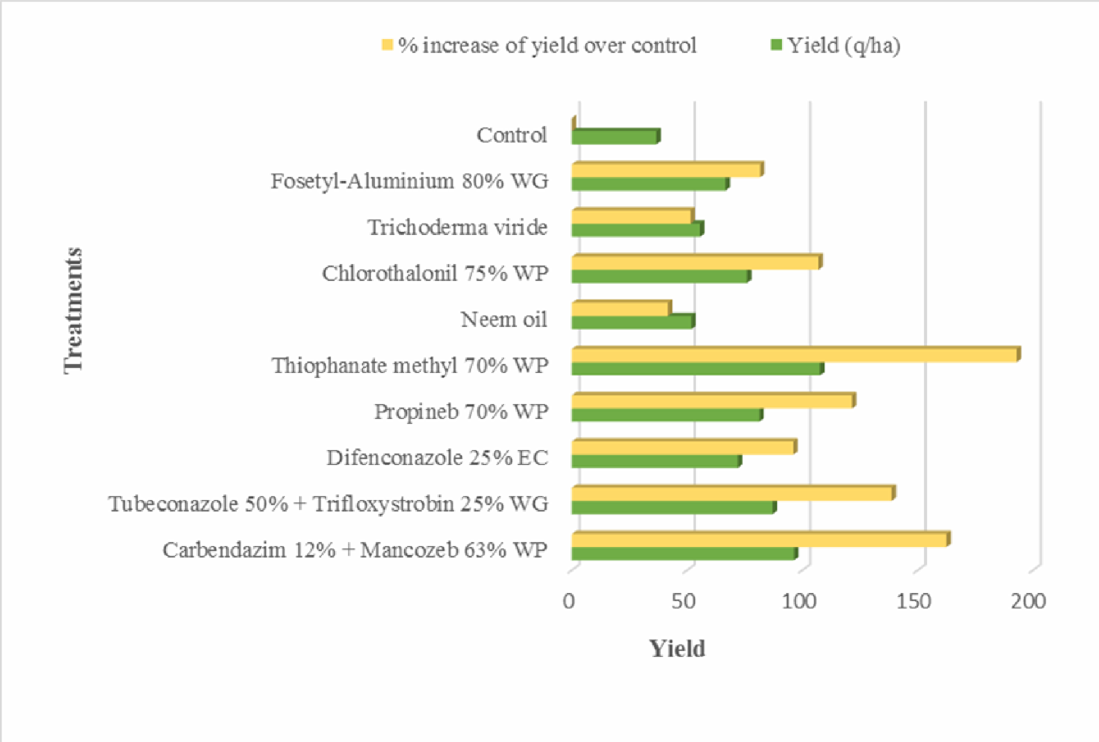

Fig 2. Effect of different treatments on yield against *Fusarium* wilt disease

Table 3: Calculation of fixed and variable cost of different fungicides in field evaluation against *Fusarium* wilt disease

(A)	Common cost of cultivation			Rupees
1.	Field preparation (4hr. @ Rs.800.00/hr.)			3,200.00
2.	Layout (35 man days @ Rs.240.00/ man day)			9,000.00
3.	Transplanting (60 man days @ Rs.240.00/ man day)			14,200.00
4.	Seed (600gm. @Rs.2000.00/kg.)			1,200.00
5.	Fertilizer (N:P:K::65.2:134:500)			
	(a) Urea 195.6 kg. @ Rs.7.3/ kg.			1,427.00
	(b) Single super phosphate 410 kg @ Rs.8/ kg.			3,280.00
	(c) Muriate of potash 120 kg. @ Rs.18/ kg.			2,160.00
6.	Interculture operation (including spraying.) 60 man days @ Rs.240.00/ man day			14,400.00
7.	Irrigation (Rs.400.00/ irrigation /ha, 7 irrigations)			2,800.00
8.	Harvesting (50 man days @ Rs.240.00/man day)			12,000.00
9.	Total common cost			63,867.00
(B)	**Cost of individual treatment**			
T1	Carbendazim 12% + Mancozeb 63% WP	2.5g/liter	1.25kg/ha	1,250.00
T2	Tubeconazole 50% + Trifloxystrobin 25% WG	2.5g/	1.25li/ha	3,125.00
T3	Difenconazole 25% EC	1.5ml/liter	750ml/ha	3,750.00
T4	Propineb 70% WP	2.5g/liter	1.25kg/ha	1,250.00
T5	Thiophanate methyl 70% WP	2.5g/liter	1.25kg/ha	437.00
T6	Neem oil	2ml/liter	1lit/ha	1,000.00
T7	Chlorothalonil 75% WP	2g/liter	1kg/ha	1,500.00
T8	*Trichoderma viride*	2.5g/liter	1.25kg/ha	1,250.00
T9	Fosetyl-Aluminum 80% WG	1.5g/liter	750g/ha	2,400.00

Table 4: Total cost of cultivation (Common cost + Variable cost) for managing the *fusarium* wilt disease

Treatments	Common cost (Rs./ha)	Cost of individuals	Total cost of cultivation
T1:Carbendazim 12% + Mancozeb 63% WP	63,867.00	1,250.00	65,117.00
T2:Tubeconazole 50% + Trifloxystrobin 25% WG	63,867.00	3,125.00	66,992.00
T3:Difenconazole 25% EC	63,867.00	3,750.00	67,617.00
T4:Propineb 70% WP	63,867.00	1,250.00	65,117.00
T5:Thiophanate methyl 70% WP	63,867.00	437.00	64,304.00
T6:Neem oil	63,867.00	1,000.00	64,867.00
T7:Chlorothalonil 75% WP	63,867.00	1,500.00	65,367.00
T8:*Trichoderma viride*	63,867.00	1,250.00	65,117.00
T9:Fosetyl-Aluminum 80% WG	63,867.00	2,400.00	66,267.00
T10:Control	63,867.00	-	63,867.00

Table 5: Calculation of Benefit-cost ratio (B: C) of different treatments on *Fusarium* wilt

Treatments	Yield (q/ha)	Value of the produce (Gross return) Rs./ha	Total cost of cultivation (Rs./ha)	Net return (Rs./ha) (Gross return- TCC)	Benefit cost ratio (B:C)
T1:Carbendazim 12% + Mancozeb 63% WP	96.39	192,780.00	65,117.00	1,27,663.00	2.960
T2:Tubeconazole 50% + Trifloxystrobin 25% WG	87.06	174,120.00	66,992.00	1,07,128.00	2.599
T3:Difenconazole 25% EC	71.99	143,980.00	67,617.00	76,363.00	2.129
T4:Propineb 70% WP	81.33	162,660.00	65,117.00	97,543.00	2.497
T5:Thiophanate methyl 70% WP	107.59	215,180.00	64,304.00	1,50,876.00	3.346
T6:Neem oil	51.99	103,980.00	64,867.00	39,113.00	1.602
T7:Chlorothalonil 75% WP	75.99	151,980.00	65,367.00	86,613.00	2.325
T8:*Trichoderma viride*	55.59	111,180.00	65,117.00	46,063.00	1.707
T9:Fosetyl-Aluminum 80% WG	66.66	133,320.00	66,267.00	67,053.00	2.011
T10:Control	36.66	73,320.00	63,867.00	9,453.00	1.148
The selling price of chilli: Rs.2000/q (i.e. Rs.20./kg)					

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Conflict of interest

The authors declare that they have no conflicts of interest.

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