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## Efficacy of Fungicides, Biocontrol Agents and Neem Cake to Suppress the Wilt of Cluster Bean caused by *Fusarium solani* (Mart.) Sacc

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### ABSTRACT

#### Keywords

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neem cake

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The present study was carried out with an aim to find out the effective fungicides and biocontrol agents under *In vitro* and *In vivo* conditions. *In vitro* evaluations revealed that Tebuconazole was found highly effective in controlling the mycelial growth of *F. solani* completely at all the concentrations (50, 100, 250 and 500ppm) among the different fungicides tested by poised food technique. The antagonism activity of *Trichoderma viride* found to be highly effective in controlling the mycelia growth (73.5%) against *F. solani* among biocontrol agents tested under dual culture method. *In vivo* study revealed that maximum germination per cent (82.78 per cent) and maximum per cent disease control (75.66% at 40days, 85.04% at 60 days) were recorded in treatment combination of Tebuconazole (ST) + *T. viride* (SA) + neem cake (SA). Thus treatment combination works better in suppressing the wilt disease of cluster bean and also helps in reducing environmental pollution which is caused by over use of chemicals.

### Introduction

Cluster bean (*Cyamopsis tetragonoloba* L.) is an annual legume and the source of guar gum. Traditionally, it is well known as 'Guar' in Rajasthan (Pathak *et al*, 2010). It is a drought hardy crop of the arid and semi-arid zones and cultivated under rain fed conditions in *kharif* season. Its seeds contain 18% protein and 32% fibre and about 30-33 % gum in the endosperm. The gum is having usages in many industries like cosmetics, pharmaceuticals, textile, mining, oil, drilling, paper, explosive industry etc (Singh, 2014).

Cluster bean is attacked by many diseases which are responsible for its poor quality and low yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange (Chand and Gandhi, 1978). Wilt and root rot caused by *Fusarium* and *Rhizoctonia* species are a major problem in cluster bean cultivated under arid zone during *rabi* season and may cause up to 21.6% plant loss at pre-emergence and post-emergence stages (Satyavir and Grewal, 1971; Lodha, 1998). Among the diverse pathogens infecting the crop *Fusarium solani* (Mart.) Sacc. is the major

fungal pathogen causing significant yield losses. The pathogen causes wilt of seedlings and the diseased plants develop rotting symptom near the soil surface which leads to wilting of host plant (Pareek and Varma, 2014). Wilt of guar was first reported by Singh (1951) from Kanpur. In view of the importance of this disease, there are several workers made attempts *In vitro* and *In vivo* using fungicides to combat this pathogen and reduce the losses in yield of the crop (Tetarwal and Trivedi 2011; Kushawah and Rakholiya 2015; Anita and Ratnoo 2015). Since, the pathogen *Fusarium solani* causing wilt in cluster bean is cosmopolitan in nature and can survive on seed and in soil in the form of its infective propagules that are difficult to control by conventional measures.

The use of IPM strategies for its management is crucial. Looking to the increasing severity of wilt of guar in Rajasthan and keeping in view the importance of the disease, the present studies were undertaken in order to reduce dependence on fungicides alone. Bio-control (*Trichoderma viride*) and Organic cakes (Neem cake) were integrated with the fungicide in various combinations for eco-friendly management of this disease.

## **Materials and Methods**

### **Isolation, purification and identification of pathogen**

Fungal isolates were isolated from fresh infected root, collar region and stem of cluster bean plant showing typical wilt symptoms which were collected during field survey made around Udaipur district. The diseased wilted parts of stem/roots were cut into smaller bits for isolating the pathogen. These bits were surface sterilized in 0.1% mercuric chloride (HgCl<sub>2</sub>) solution for 30 seconds followed by three washings with sterilized distilled water and then transferred into the

Petri plates containing PDA medium and incubated at 28±1°C. When the fungal growth occurs from the diseased tissues, it was purified aseptically using hyphal tip method on PDA slants. The pure cultures were preserved for future studies in refrigerator at 4°C. The pathogen isolates were mainly identified on the basis of cultural and morphological characters as *Fusarium solani* (Mart.) Sacc. as described by Burnett and Hunter, 2003; Leslie and Summerell, 2006. Among the isolates obtained, highly virulent fungal isolate *F. solani* (BKU Fs-03) was chosen for further management studies based up on their pathogenic virulence caused on the cluster bean cultivars (Pusa Navbahar and Swati) tested under sick soil method.

### ***In vitro* efficacy of fungicides**

Efficacy of six fungicides (three non-systemic and three systemic) viz, Mancozeb 75 WP (Stargem-45), Carbendazim 12% WP + Mancozeb 63% WP (Saaf), Copper oxychloride 50 WP (Maincop), Tebuconazole 25 EC (Folicur), Propiconazole 25 EC (Tilt), and Hexaconazole 5 SC (Mainex) against mycelial growth of *F. solani* was tested by poisoned food technique (Sinclair and Dhingra, 1985). Four different concentrations viz., 50, 100, 250 and 500 ppm of each fungicide was tested. The required quantity of each chemical at different concentration was added aseptically in 100 ml PDA in 250 ml flasks and shaken well for mixing of the chemical.

After that, pouring of medium was done and allowed to solidify. 5 mm diameter mycelial disc, cut from the periphery of 10 days old fungus cultures were inoculated into each Petri plate and incubated at 28±1°C for 7-8 days. In each treatment, five replications were maintained. Observations on linear growth were recorded when full growth of fungus observed in control Petri plate and per cent

growth inhibition was calculated by following formula (Vincent's, 1947);

$$\text{Per cent growth inhibition} = \frac{C}{C - T} \times 100$$

where,

C = Growth of the colony in control (mm)

T = Growth of colony in respective treatment (mm)

### ***In vitro* efficacy of bioagents**

*In vitro* efficacy of four bio-control agents two fungal [viz; *Trichoderma viride* (IIHR-Tv-5) and *T. harzianum* (Jh.h.89-2)] and two bacterial (viz; *Pseudomonas fluorescens* and *Bacillus subtilis*) were tested by using dual culture plate method on PDA medium (Bell *et al.*, 1982). 20 ml PDA media was poured aseptically in each Petri plates and allowed to solidify. Then, Petri plates were inoculated with 5mm mycelia disc of 7 days old culture of *F. solani* and fungal bioagents using sterilized cork borer by placing on solid media approximately 4 cm away from each other. In case of bacterial bioagents evaluation, 5 mm fungal mycelia disc was placed at the centre of each plates and bacterial bioagent of 2-3 day old culture was streaked around the fungal disc at 4 sides. Control plates were also maintained in bioagent free conditions and incubated at 28±1°C for 7 days. In each treatment five replications were maintained. Growth of test pathogen in dual culture and in control plates were recorded and Per cent growth inhibition zone of pathogen and Index of antagonism were determined in each treatment by following standard formula,

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent growth inhibition of pathogen

C = Growth of test fungus in control (mm)

T = Growth of test fungus in respective treatment (mm).

### **Integrated management of cluster bean wilt caused by *Fusarium solani* under field conditions**

Based up on the *In vitro* studies, one fungicide Tebuconazole 25 EC, two bioagents *Trichoderma viride* and *Pseudomonas fluorescens* that were found most effective was further evaluated in field condition alone as well as their integration with the neem cake for the management of cluster bean wilt. The pathogen *F. solani* isolate (BKU Fs-03) was mass multiplied using sterilized sorghum grain substrate under laboratory conditions and mixed with field soil in micro plots @ 10 g/plot before sowing. Required quantity of Tebuconazole 25 EC (0.1%), *T. viride* (2%), *P. Fluorescens* (2%) and neem cake(300gm/plot in individual or 100gm/plot in combination) were used alone or in combination of each were treated at the time of seed sowing. Cluster bean cultivar 'Pusa Navbahar' was raised in a randomized block design with three replications and eight treatments were imposed as in the table 3. Observations on the number of total germinated seedlings were recorded at 10 days and number of infected plants showing typical wilting symptoms were taken along with control at 40 and 60days. The per cent wilt incidence and Per cent wilt control were calculated to find out the best treatment for the management of cluster bean wilt by using standard formula given by Wheeler (1969);

$$\text{Per cent seed germination} = \frac{\text{Number of seeds germinated/plot}}{\text{Total number of seeds sowed/plot}} \times 100$$

$$\text{Per cent wilt incidence} = \frac{\text{Number of wilt infected plants per}}{\text{Total number of plants per plot}} \times 100$$

$$\text{Per cent wilt control} = \frac{\text{PWI}_{(C)} - \text{PWI}_{(T)}}{\text{PWI}_{(C)}} \times 100$$

Where,  $\text{PWI}_{(C)}$  – Per cent wilt incidence in control plot

$\text{PWI}_{(T)}$  – Per cent wilt incidence in treated plot

## Results and Discussion

### *In vitro* testing of fungicides against *F. solani* (BKU Fs-03)

All the tested fungicides significantly ( $P=0.05$ ) inhibited the mycelial growth of *F. solani* at all concentrations *In vitro* (Table-1). Tebuconazole was found most effective with 94.44 per cent inhibition of the mycelial growth of *F. solani* at all concentrations (50, 100, 250 and 500 ppm) followed by Propiconazole was found effective with inhibition of 66.44, 74.0, 85.78 and 94.44% at 50, 100, 250 and 500 ppm, respectively.

Hexaconazole, Saaf and Mancozeb showed 90.22%, 84.89% and 40.67% inhibition at 500 ppm, respectively. Copper oxychloride was found least effective at all concentrations against *F. solani*.

Similar results have been observed by Timbadiya (2013) reported that Tebuconazole showed complete inhibition (100%) followed by Propiconazole (92.35%) against *Fusarium solani* causing wilt in cluster bean under *In vitro*. Kushawah and Rakholiya (2015) reported that Propiconazole and Hexaconazole were found relatively effective against *F. solani* with 91.67 and 77.44 per cent growth inhibition at 250 ppm concentration.

### *In vitro* testing of biocontrol agents against *F. solani* isolate (BKU Fs-03)

All the four bioagents were significantly ( $P=0.05$ ) showed the antagonism and check /

inhibited the mycelial growth of *F. solani* isolate (BKU Fs-03) from Table-2. Severe antagonism and significant higher percent inhibition of *F. solani* growth (73.5%) was recorded by *T. viride* which was followed by *T. harzianum* and *Pseudomonas fluorescens* showed moderate antagonism with 64.0% and 59.3% growth inhibition, respectively.

The least and weak antagonism was showed by *Bacillus subtilis*. Sneha (2016) reported that maximum inhibition of *Fusarium solani* (83.88%) was recorded with the bioagent *T. viride* followed by that of *P. fluorescens* and *B. subtilis*. The reports of Kapoor *et al*, 2010; Singh *et al*, 2010; Ram and Pandey, 2011 were depicted the similar results.

### Integrated management of cluster bean wilt caused by *F. solani* under field conditions

The data presented in table 3 showed that all the treatments are significantly effective over control. Maximum seed germination (82.78%), minimum per cent wilt incidence (12.92%) with highest wilt control 75.66%, 85.04% recorded at 40 and 60 days, respectively in the treatment combination of Tebuconazole (ST) + *T. viride* (SA) + neem cake (SA) that was found most effective followed by Tebuconazole (ST) + *T. viride* (SA) had 77.22% germination, 17.39 % wilt incidence with 79.88% wilt control at 60days after sowing.

The treatment combination Tebuconazole (ST) + neem cake (SA) showed 69.70% wilt control at 60 days, while *P. fluorescens* (ST) + (neem cake + *T. viride*) (SA) recorded 61.43% wilt control at 60 days.

Among the individual treatments, Tebuconazole (ST) showed 56.67% germination with 51.21% wilt control at 60days while *T. viride* (SA) recorded 51.67% germination with 36.11 wilt control at 60days.

**Table.1** Comparative efficacy of different fungicides on the growth of *Fusarium solani* isolate (BKU Fs-03) *in vitro*

Treatments	Name of the Fungicide	Different concentrations (ppm)							
		50		100		250		500	
		Colony Diameter (mm)*	Per Cent Growth inhibition*	Colony Diameter (mm)*	Per Cent Growth inhibition*	Colony Diameter (mm)*	Per Cent Growth inhibition*	Colony Diameter (mm)*	Per Cent Growth inhibition*
<b>T<sub>1</sub></b>	Mancozeb 75 WP	80.40 (63.74)	10.67 (18.91)	71.00 (57.41)	20.44 (26.85)	62.20 (52.04)	30.89 (33.74)	53.40 (46.93)	40.67 (39.60)
<b>T<sub>2</sub></b>	Saaf 75WP	45.40 (42.34)	48.89 (44.35)	36.20 (36.97)	59.78 (50.62)	23.4 (28.90)	74.00 (59.33)	13.80 (21.78)	84.89 (67.11)
<b>T<sub>3</sub></b>	Copper oxychloride 50 WP	84.80 (67.06)	5.78 (13.72)	78.00 (62.01)	12.89 (20.99)	70.40 (57.03)	21.78 (27.78)	60.40 (50.99)	32.89 (34.97)
<b>T<sub>4</sub></b>	Tebuconazole 25 EC	5.00 (12.92)	94.44 (76.33)	5.00 (12.92)	94.44 (76.33)	5.00 (12.92)	94.44 (76.33)	5.00 (12.92)	94.44 (76.33)
<b>T<sub>5</sub></b>	Propiconazole 25 EC	30.20 (33.31)	66.44 (54.58)	23.40 (28.91)	74.00 (59.33)	12.80 (20.95)	85.78 (67.83)	5.00 (12.92)	94.44 (76.33)
<b>T<sub>6</sub></b>	Hexaconazole 5 SC	47.60 (43.61)	47.11 (43.32)	34.80 (36.13)	61.56 (51.66)	19.60 (26.26)	78.22 (62.17)	8.80 (17.23)	90.22 (71.76)
<b>T<sub>7</sub></b>	Control	90.00 (71.54)	0.00	90.00 (71.54)	0.00	90.00 (71.54)	0.00	90.00 (71.54)	0.00
<b>SEm±</b>		0.546	0.768	0.393	0.445	0.391	0.445	0.365	0.396
<b>CD (P=0.05)</b>		1.590	2.235	1.145	1.295	1.137	1.296	1.062	1.153

\* Mean of five replications; Figures in parentheses are arcsine  $\sqrt{\text{per cent}}$  angular transformed values.

**Table.2** Per cent growth inhibition of *Fusarium solani* (BKU Fs-03) isolate by different bioagents *in vitro*

Treatments	Bio-control agent and procured places	Mycelial growth (mm) *	Per Cent growth Inhibition *	Antagonism Index**
T <sub>1</sub>	<i>T. viride</i> (IIHR-TV-5)	23.8 (29.2)	73.5 (59.1)	++++
T <sub>2</sub>	<i>T. harzianum</i> (Jh.h.89-2)	32.4 (34.7)	64.0 (53.1)	+++
T <sub>3</sub>	<i>Pseudomonas fluorescens</i>	36.6 (37.2)	59.3 (50.4)	+++
T <sub>4</sub>	<i>Bacillus subtilis</i>	53.8 (47.2)	40.2 (39.3)	++
T <sub>5</sub>	Control	90.0 (71.6)	0.00	--
<b>SEm±</b>		0.576	0.628	
<b>CD (P=0.05)</b>		1.710	1.865	

\*Average of five replications. Values in the parentheses are arcsine  $\sqrt{\text{per cent angular transformed values}}$ .

\*\* Antagonism index (Bell *et al.*, 1982)

++++ = Severe antagonism

+++ = Moderate antagonism

++ = Weak antagonism

-- = No antagonism

**Table.3** Relative efficacy of promising fungicide, bioagent and neem cake for the management in wilt disease of cluster bean cultivar ‘Pusa Navbahar’ in micro plots

S.No.	Treatments	Germination per cent	Per cent wilt incidence*		Per cent wilt control*	
			Up to 40days	Up to 60days	Up to 40days	Up to 60 days
01.	Tebuconazole 25 EC @ 0.1% (ST)	56.67 (48.83)	32.35 (34.48)	42.16 (40.44)	39.05	51.21
2.	<i>Trichoderma viride</i> @ (2%) (SA)	51.67 (45.94)	43.75 (41.31)	55.21 (47.99)	17.57	36.11
3.	Neem cake (SA) @ 300gm /sqm.	54.44 (47.54)	44.08 (41.51)	60.22 (50.89)	16.95	30.32
4.	Tebuconazole 25 EC @ 0.1% (ST) + <i>Trichoderma viride</i> @ (2%)(SA)	77.22 (61.51)	15.94 (23.45)	17.39 (24.61)	69.96	79.88
5.	Tebuconazole 25 EC @ 0.1% (ST) + Neem cake (SA)	70.56 (57.19)	23.81 (29.17)	26.19 (30.75)	55.14	69.70
6.	Tebuconazole 25 EC @ 0.1% (ST) + Neemcake (SA) + <i>Trichoderma viride</i> @ (2%) (SA)	82.78 (65.65)	12.92 (20.81)	12.92 (20.81)	75.66	85.04
7.	<i>Pseudomonas fluorescens</i> @ (2%) (ST) + Neem cake (SA) + <i>Trichoderma viride</i> @ (2%) (SA)	70.56 (57.19)	22.52 (28.28)	33.33 (35.23)	57.57	61.43
8.	Control	45.00 (42.09)	53.08 (46.80)	86.42 (72.11)	0.00	0.00
	<b>SEm±</b>	1.900	2.716	3.60	--	--
	<b>CD (P=0.05)</b>	5.820	8.317	11.04	--	--

\*Mean of three replications; Figures in parentheses are arcsine  $\sqrt{\text{per cent}}$  angular transformed values.  
ST- Seed treatment and SA- Soil application

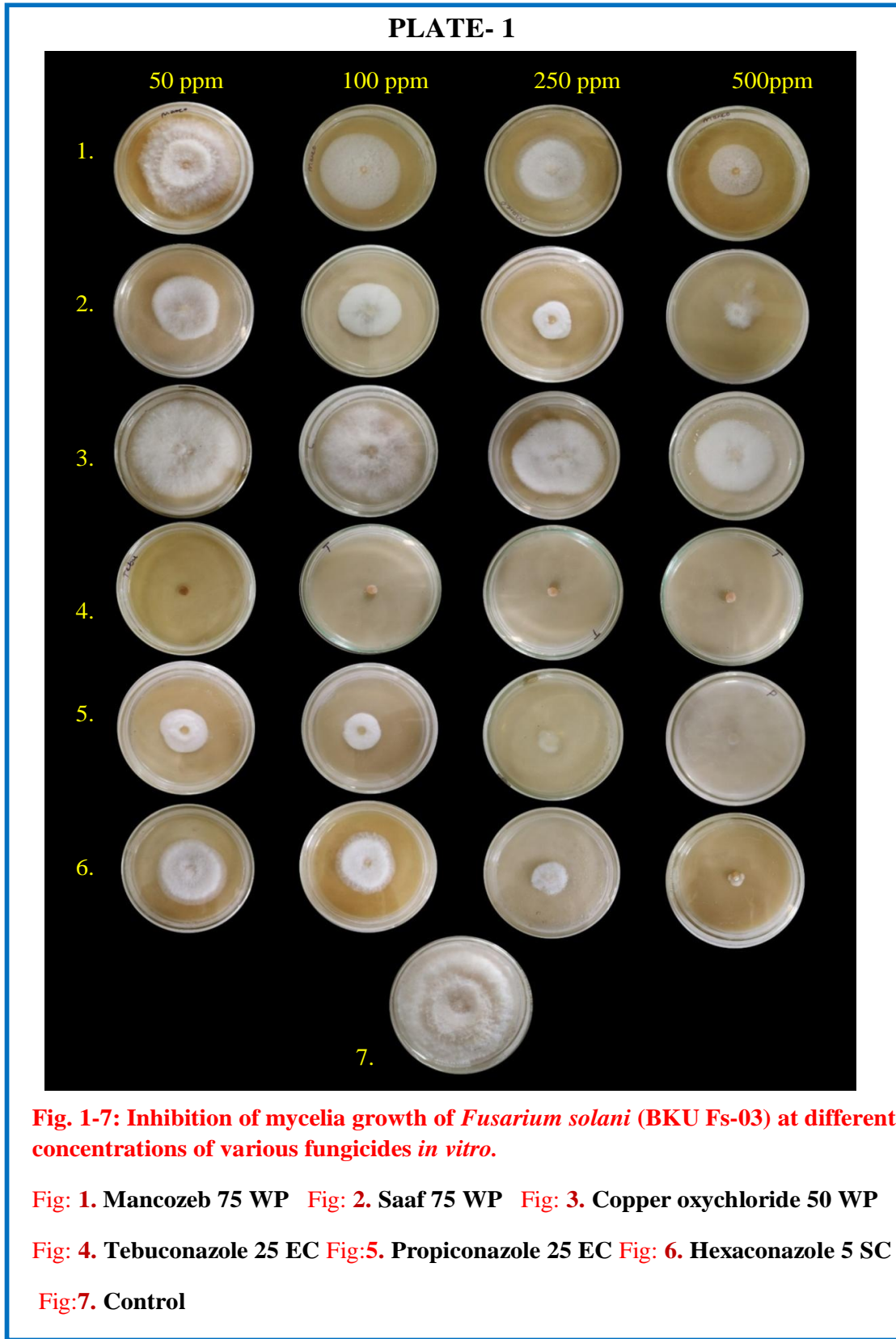
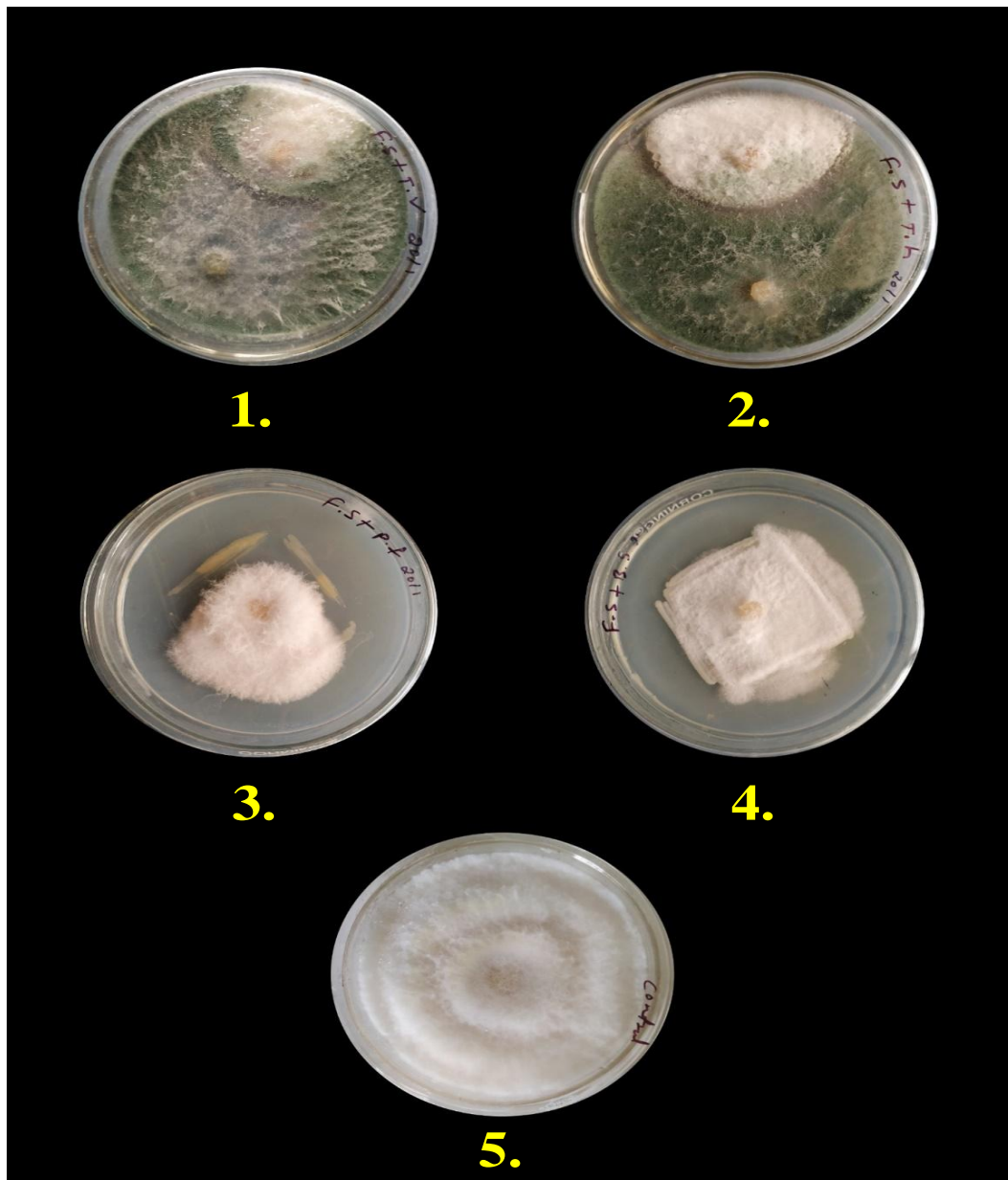


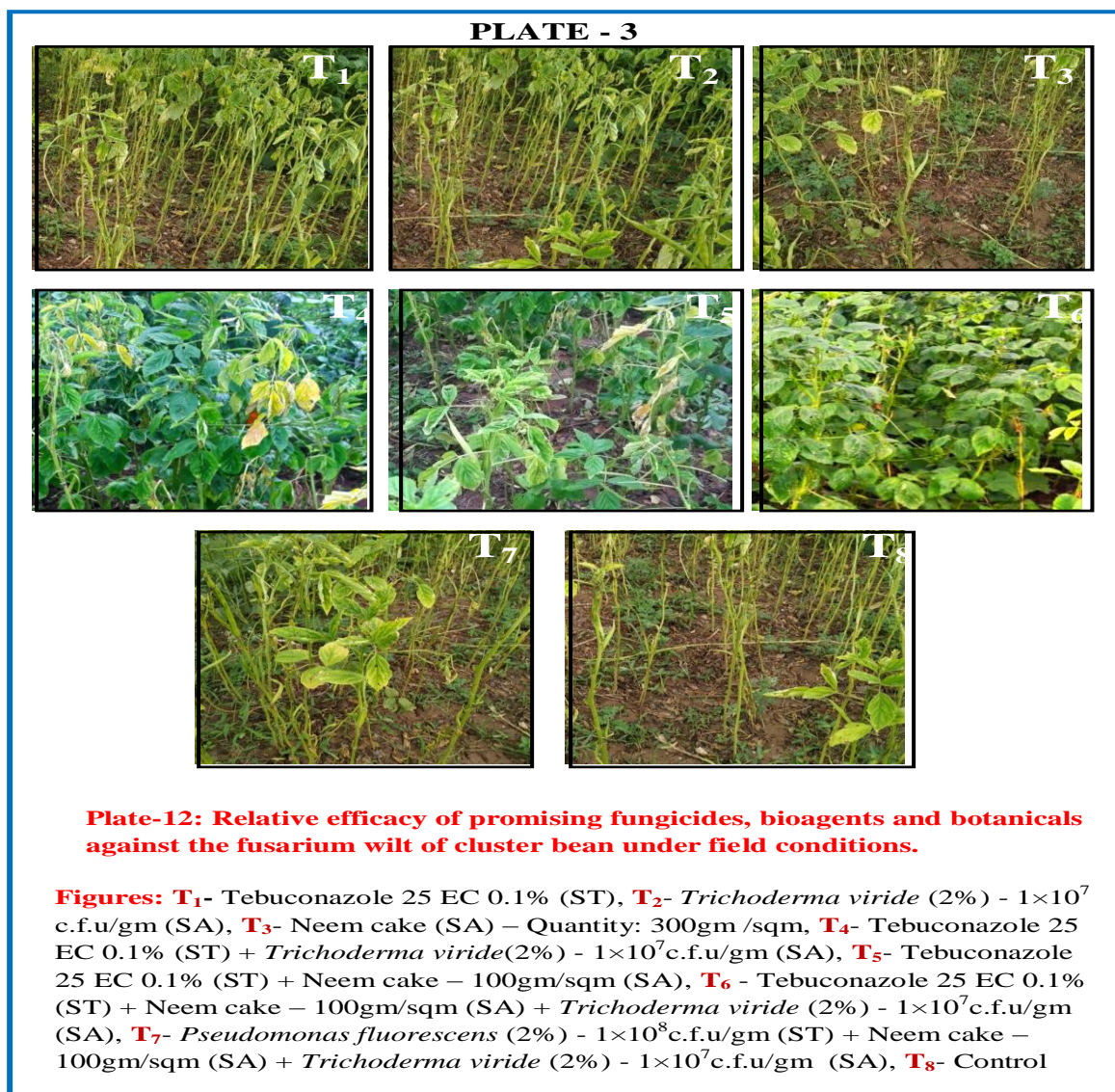


PLATE - 2



**Fig. 1-5: Efficacy of different bioagents against *Fusarium solani* (BKU Fs-03) isolate *in vitro*.**

- 1. *Trichoderma. viride* 2. *Trichoderma harzianum*  
3. *Pseudomonas fluorescens* 4. *Bacillus subtilis* 5. Control**



The treatment neem application showed lowest per cent wilt control (30.32% at 60days) as compare to control. Anita and Ratnoo (2015) reported that most effective treatment was with Bavistin (ST) + Neem oil + *T. harzianum* + neem cake (SA) to control the root rot of pea caused by *F. solani*. Kumar and Girija (2003) showed that the combined treatment of *T. viride* (ST+SA) + neem cake 150kg/ha (SA) + soil drenching of Mancozeb 0.3% significantly controlled the Fusarium wilt of cowpea with 16.66% least disease index and it increased the biomass, pod yield of the crop. Therefore, integrated

management strategy would help to work out the plans to reduce indiscriminate use of fungicides, to minimize the chance of development of resistance against fungicides and to suppress the growth of seed and soil borne pathogens at maximum level.

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