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Identification of Mancozeb-Tolerant and Phosphate-Solubilizing Fungal Bioagents for the Management of Fusarium Wilt of Chilli.

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Abstract: Fusarium wilt, caused by *Fusarium solani*, is a destructive soil-borne disease of chilli (*Cap-sicum annuum* L.) that causes yield losses ranging from 10 to 80% under favorable conditions. The present study, conducted during 2020–2022, aimed to isolate mancozeb-tolerant fungal bioagents and evaluate their efficacy for the integrated management of Fusarium wilt of chilli. Agricultural soil patches were pre-treated with mancozeb to facilitate the isolation of fungicide-tolerant antagonists using *Trichoderma*-selective medium. A total of 104 fungal isolates were recovered and screened for mancozeb tolerance, antagonistic activity, and phosphate-solubilizing ability. Among them, isolate SHO-13 exhibited the highest radial growth (35 mm) on mancozeb-amended medium, followed by WG-4 (33 mm), indicating high fungicide tolerance. In dual culture assays, SHO-13 showed the greatest inhibition of *F. solani* mycelial growth (84.97%), closely followed by WG-4 (84.34%). Both isolates also demonstrated efficient phosphate-solubilizing activity. Under in vivo conditions, seedling treatment with SHO-13 in combination with mancozeb significantly reduced Fusarium wilt incidence (5.50%) and markedly improved plant growth parameters, including plant height, biomass, and fruit yield, compared with untreated and fungicide-alone treatments. Based on morphological characteristics and ITS rDNA sequence analysis, isolate SHO-13 was identified as *Trichoderma virens*, and its ITS sequence was deposited in the NCBI GenBank database under accession number ON740648. The results demonstrate that the integration of mancozeb with the fungicide-tolerant, phosphate-solubilizing bioagent *T. virens* (SHO-13) provides an effective and environmentally sustainable strategy for the management of Fusarium wilt while promoting plant growth and soil health in chilli cultivation.

Keywords: Bioagents, chilli, mancozeb, *Fusarium solani*, wilt

1. Introduction

Chilli (*Capsicum annuum*) is an important vegetable and spice crop belonging to the family Solanaceae. It is cultivated worldwide and adds aroma, flavour, and colour to a wide range of cuisines. Chilli is also used in preservatives, medicines, fragrances, cosmetics, and religious ceremonies [10, 13]. During the last decade, Fusarium wilt, caused by *Fusarium* species, has emerged as a serious concern for chilli production [1]. The disease has spread across almost all chilli-growing regions of India, posing a severe threat in states such as Andhra Pradesh, Telangana, Karnataka, West Bengal, Jammu and Kashmir, Haryana, and Punjab [7, 16]. Chilli wilt causes 10–50 per cent crop losses worldwide, with losses ranging from 10 to 80 per cent in India, depending on disease severity (Loganathan *et al.*, 2013). *Fusarium oxysporum* and *Fusarium solani* have been reported to cause 56 per cent and 36 per cent seedling mortality, respectively [8].

The disease is characterized by symptoms such as vein clearing, leaf epinasty, wilting, chlorosis, and premature leaf abscission. The initial symptoms of vascular wilt include mild withering and yellowing of the lower leaves. Brown vascular discoloration is a characteristic symptom of the disease, followed by upward and inward rolling of the upper leaves and, eventually, wilting of the plants [9, 14]. The disease can be managed through a combination of cultural, chemical, and biological approaches, along with the adoption of resistant cultivars. Broad-spectrum fungicides are widely used for the management of chilli wilt; however, their excessive use can pollute the environment and adversely affect human health [6]. Furthermore, the development of fungicide resistance in pathogenic fungi is becoming a major concern. Fungicides alone do not provide long-term control and often require repeated applications. Biological management of plant diseases could therefore provide a viable alternative to the heavy reliance of modern agriculture on expensive chemical fungicides, which not only pollute the environment but may also contribute to the development of resistant strains [3, 5, 17].

The compatibility of potential bioagents with fungicides is critical for developing an efficient disease management strategy. Combining antagonists with synthetic chemicals can reduce the risk of resistance

development while lowering the quantity of fungicide required [17]. Mancozeb is a non-systemic dithiocarbamate fungicide with multisite, contact-based protective activity. It is a combination of two dithiocarbamates, namely maneb and zineb [10]. The fungicide has been shown to protect plants against a wide range of fungal diseases affecting field crops, fruits, nuts, vegetables, and ornamentals [11]. Considering the importance of bioagents and mancozeb in disease management, the present investigation was undertaken to isolate, screen, and identify potential bioagents resistant to mancozeb and to evaluate their antagonistic potential for the effective management of Fusarium wilt of chilli.

2. Materials and Methods

2.1 Isolation of pathogen

The tissue bit transfer technique was used to isolate the causal organism (Sicard *et al.*, 1997). Symptomatic stems and roots were cut into small pieces (2–3 mm) using a sterilized sharp blade, ensuring that healthy tissue was included along with the diseased portion of the plant. The pieces were surface sterilized for 30 seconds in a 0.1 per cent mercuric chloride solution. The pieces were then rinsed three times with sterile distilled water to remove all traces of the mercuric chloride solution. The tissue pieces were dried on sterilized blotter paper and then aseptically placed on Potato Dextrose Agar (PDA) medium containing potato 200 g, dextrose 20 g, agar 15 g, and distilled water 1000 mL in Petri plates (9 cm diameter). The plates were incubated at 25°C for seven days. The fungal colonies emerging from the tissue pieces were transferred to fresh medium in Petri plates and slants. Mycelial characteristics, colony colour, shape, size, and spore septation were recorded.

2.2 Pathogenicity test

The pathogenicity of the isolated and purified fungus obtained from symptomatic stems was evaluated to determine its ability to cause Fusarium wilt in chilli plants. The sick-soil method was used to prepare the inoculum for the pathogenicity test [6]. Sand–maize medium (3:1) was prepared in 250 mL flasks and autoclaved at 15 psi for 20 minutes on three consecutive days. Each sterilized flask was inoculated

with a 7 mm disc of actively growing fungal culture and incubated at $25 \pm 1^\circ\text{C}$ for 15 days for mass multiplication of the inoculum.

Plastic pots were thoroughly washed with sterilized distilled water, dried, and sprayed with spirit. Five days before sowing, the plastic pots (15 cm) were filled with sterilized soil (2 kg pot^{-1}) and inoculated with sand–maize medium containing the fungal culture at 10 g kg^{-1} soil. One pot containing the same quantity of sterilized soil without inoculation was maintained as a control. Chilli seedlings were sown in each pot after surface sterilization with 1 per cent sodium hypochlorite for two minutes, followed by three rinses with sterile distilled water. The plants were watered regularly as required. Both inoculated and non-inoculated pots were maintained under open conditions to observe the development of disease symptoms.

2.3 Isolation of mancozeb-resistant bioagents

Mancozeb-resistant biocontrol agents were isolated from soil treated with 2500 ppm mancozeb fungicide collected from various locations across the Kashmir Valley, including cultivated, uncultivated, and forest areas. An area of 2.5 square feet was treated with 2500 ppm mancozeb dissolved in water, and soil samples were collected from the treated patches after 24 hours. The samples were placed in polythene bags labelled with the respective locations and transported to the laboratory.

Serial dilution and plating techniques were used to isolate the surviving bioagents from these samples. For the selective isolation of fungal biocontrol agents, Trichoderma-specific medium (TSM), consisting of magnesium sulphate heptahydrate 0.200 g, dipotassium hydrogen phosphate 0.900 g, ammonium nitrate 1.0 g, potassium chloride 0.159 g, dextrose 3 g, Rose Bengal 0.150 g, agar 20 g, and distilled water 1000 mL, was used as the plating medium.

Each sample was subjected to six-fold serial dilution. Three grams of pre-treated soil were weighed and transferred to a test tube containing 9 mL of sterilized distilled water. The mixture was thoroughly mixed. Subsequently, 1 mL of the stock suspension was transferred to a second test tube containing 9 mL of sterilized distilled water. Serial dilution was continued by transferring 1 mL of suspension into 9 mL of sterilized distilled water in successive test tubes until a dilution factor of 10^{-6} was achieved. Aseptically, 0.5 mL of the diluted suspension was spread onto sterilized Petri plates containing

Trichoderma-specific medium (TSM). The plates were incubated at $25 \pm 1^\circ\text{C}$ for 96 hours. Morphologically distinct colonies appearing on the plates were aseptically transferred to Potato Dextrose Agar (PDA) for purification. The purified isolates were stored at 4°C and used throughout the study.

2.4 Compatibility of biocontrol agents with mancozeb fungicide *in vitro*

To confirm the resistance of the isolated biocontrol agents to mancozeb fungicide, the standard poisoned food technique [12] was employed. Potato Dextrose Agar (PDA) medium was prepared and sterilized at 121°C for 20 minutes. Mancozeb was added to the PDA medium at a concentration of 2500 ppm (2.5 g L^{-1} of medium). Eighteen millilitres of the amended medium were aseptically poured into sterilized Petri plates (9 cm diameter).

The plates were inoculated with a 5 mm disc of 7-day-old biocontrol agent culture using a sterile 5 mm cork borer. In separate plates, a 5 mm disc of the 7-day-old *Fusarium* test pathogen was inoculated. Each biocontrol isolate and the test pathogen were replicated three times, and the inoculated plates were incubated in a BOD incubator at $25 \pm 1^\circ\text{C}$ for 14 days. Radial growth of the biocontrol isolates and the test pathogen was measured daily from the fifth day after inoculation until 14 days. The experiment was repeated twice. The experiment was conducted using a Completely Randomized Design (CRD). Fravel *et al.* (2005) [4] used this method to successfully evaluate the compatibility of biocontrol fungi with various fungicides.

2.5 *In vitro* screening of isolated biocontrol agents against *Fusarium solani*

The dual culture technique [18] was used to evaluate the mancozeb-resistant antagonists against *Fusarium*. Twenty millilitres of sterilized molten Potato Dextrose Agar (PDA) medium were aseptically poured into sterilized Petri plates and allowed to solidify. Six-millimetre discs of the test fungus and biocontrol isolates were cut using a sterilized cork borer and placed at opposite ends of 9 cm PDA plates. The plates were incubated in a BOD incubator at $25 \pm 1^\circ\text{C}$ for 96 hours. Three replications were maintained for each treatment.

The colony diameter of the biocontrol isolates and the test fungus was measured. Inhibition of mycelial growth of the test fungus over the control was calculated using the formula given by Vincent [15]:

136 **Per cent inhibition (I) = [(C – T) / C] × 100**

137 where,

138 **I** = Per cent inhibition

139 **C** = Colony diameter (mm) in the control

140 **T** = Colony diameter (mm) in the treatment

141 **2.6 Phosphorus solubilization capability of effective biocontrol agents**

142 Nine mancozeb-resistant biocontrol agents exhibiting the highest antagonistic activity against the
143 pathogen *in vitro* were selected to evaluate their phosphorus-solubilizing activity using a phosphorus
144 solubilization test. Five-millimetre discs of the bioagents were inoculated at the centre of sterilized Petri
145 plates containing Pikovskaya's Agar (PKA) medium. The medium consisted of glucose 10 g, tricalcium
146 phosphate (TCP) 5 g, yeast extract 0.5 g, ammonium sulphate 0.5 g, potassium chloride 0.2 g, sodium
147 chloride 0.2 g, magnesium sulphate 0.1 g, ferrous sulphate (trace), magnesium sulphate (trace), agar 15
148 g, and distilled water 1 L.

149 The plates were incubated in a BOD incubator at $28 \pm 2^\circ\text{C}$ for 24 hours. The ability of the bioagents to
150 solubilize tricalcium phosphate (TCP) on Pikovskaya's agar medium was determined in terms of the
151 solubilization index (SI). The phosphate solubilization index was calculated by measuring the colony
152 diameter (mm) and halo-zone diameter (mm), using the formula given in [3]:

153 **Phosphate solubilization index (SI) = (Colony diameter + Halo-zone diameter) / Colony diameter**

154 **2.7 Mycoparasitism**

155 The mycoparasitic activity of the two highly antagonistic, mancozeb-resistant bioagents was studied
156 using the agar plate-assisted slide culture technique [2].

157 **2.8 Identification of potential biocontrol agents**

158 Out of 104 biocontrol isolates, two mancozeb-resistant isolates that proved most effective in the *in vitro*
159 experiments were selected and identified based on cultural and morphological characteristics, including
160 colony characteristics, mycelial growth, colour, size, and sporulation, using a compound microscope.

2.9 Mass culture and preparation of formulations of two highly effective mancozeb-resistant bioagents for *in vivo* evaluation

Cultures of the highly effective bioagents were multiplied in 1000 mL flasks containing 500 mL Potato Dextrose Broth for seven days. After one week of incubation, the fungal biomass was harvested and mixed for 30 seconds in 500 mL of distilled water. The stock suspension was serially diluted to obtain a concentration of 2×10^8 spores mL⁻¹. The fungal biocontrol agent was then mixed with sterile kaolinite and wood charcoal powder (1:1, w/w) at a ratio of 1:3 (v/w). The resultant formulation was stored at 4°C for further use.

2.10 Evaluation of potential mancozeb-resistant bioagents against Fusarium wilt of chilli in polybags

A pot experiment was conducted during *kharif* 2022 at the Faculty of Agriculture, SKUAST-K, using a Completely Randomized Design (CRD). Six treatments, including an untreated control, were evaluated with three replications per treatment. Seedling treatments with the potential biocontrol agents WG-4 and SHO-13 and mancozeb fungicide, individually and in combination, were evaluated as follows:

1. **WG-4 + mancozeb:** Chilli seedlings were treated with a paste/slurry of WG-4 formulation prepared by mixing the WG-4 formulation with water in a 1:1 ratio and mancozeb at 2.5 g kg⁻¹ of water. The seedlings were uniformly dipped in the slurry for 30 minutes and dried in the shade for one hour before sowing.
2. **SHO-13 + mancozeb:** Chilli seedlings were treated with a paste/slurry of SHO-13 formulation prepared by mixing the SHO-13 formulation with water in a 1:1 ratio and mancozeb at 2.5 g kg⁻¹ of water.
3. **WG-4 alone:** Only the WG-4 slurry formulation was used.
4. **SHO-13 alone:** Only the SHO-13 slurry formulation was used.
5. **Mancozeb alone:** Seedlings were treated with mancozeb at 2.5 g kg⁻¹.
6. **Untreated control:** Seedlings were left untreated and maintained as the control.

Seedlings in each treatment were slightly moistened with sterile distilled water to ensure that a thin film of the treatment was uniformly deposited on the seedlings. Three seedlings from each treatment were transplanted into polybags containing 3 kg of a sterilized mixture of soil, sand, and FYM in a ratio of

2:1:1. The soil in each polybag was inoculated with *Fusarium* species grown on sand–maize medium at 10 g kg⁻¹ soil. The following observations were recorded.

2.11 Disease incidence

Per cent disease incidence was calculated using the following formula:

$$\text{Disease incidence (\%)} = [\text{Number of diseased plants} / \text{Total number of plants}] \times 100$$

2.12 Plant height

The plant height of all plants in each treatment was recorded in centimetres.

2.13 Number of fruits

The total number of fruits produced per plant in each treatment was recorded.

2.14 Fruit weight

The total fruit weight per plant in each treatment was recorded in g plant⁻¹.

2.15 Plant biomass

The total biomass of each plant in all treatments was recorded in g plant⁻¹.

2.16 Statistical analysis

The data were statistically analysed using IBM SPSS Statistics Base 22.0. Critical difference values were used to compare treatment means. Data transformation using the arcsine transformation was performed wherever necessary. Analysis of variance (ANOVA) was conducted at a 5 per cent level of significance.

3. Results

3.1 Pathogenicity and morphological characterization of the isolated pathogen

Koch's postulates for the isolated fungus were confirmed using the sick-soil method (Figure 1A, B). The initiation of typical disease symptoms was observed 16–20 days after inoculation, whereas no symptoms developed in the control. The colony characteristics of the fungus were critically examined in pure culture. The fungal colony was white and cottony, with profuse, fluffy aerial mycelium after 25 days of incubation, which gradually turned creamish in colour.

Microscopic observations of the isolated fungus revealed that the mycelium was septate and hyaline, with a hyphal width ranging from 3.48 to 5.35 μm (average 4.41 μm). The fungus produced cottony and fluffy growth on PDA, which was initially white and later turned light brownish-white after 25–30 days of incubation (Figure 2A–D). In culture, the pathogen produced microconidia, macroconidia, and chlamydospores. The microconidia were hyaline, aseptate, cylindrical to falcate, and ovoid in shape. The length $\times$ breadth of the microconidia ranged from 4.65–8.90 $\times$ 2.32–3.90 μm , with an average size of 6.7 $\times$ 3.1 μm . The macroconidia were septate (3–5 septa), sickle-shaped, and possessed a slightly foot-shaped basal cell. The length $\times$ breadth of the macroconidia ranged from 4.65–8.90 $\times$ 2.32–3.90 μm , with an average size of 6.7 $\times$ 3.1 μm . Chlamydospores were produced 13–15 days after incubation. They were globose, single-celled, and aseptate, and were produced terminally or intercalarily. Their size ranged from 11.50–12.10 $\times$ 9.80–10.10 μm , with an average size of 11.8 $\times$ 9.95 μm (Figure 3A–D).



Figure 1. Pathogenicity test of *Fusarium solani* on chilli plant
(A) Inoculated (B) Uninoculated

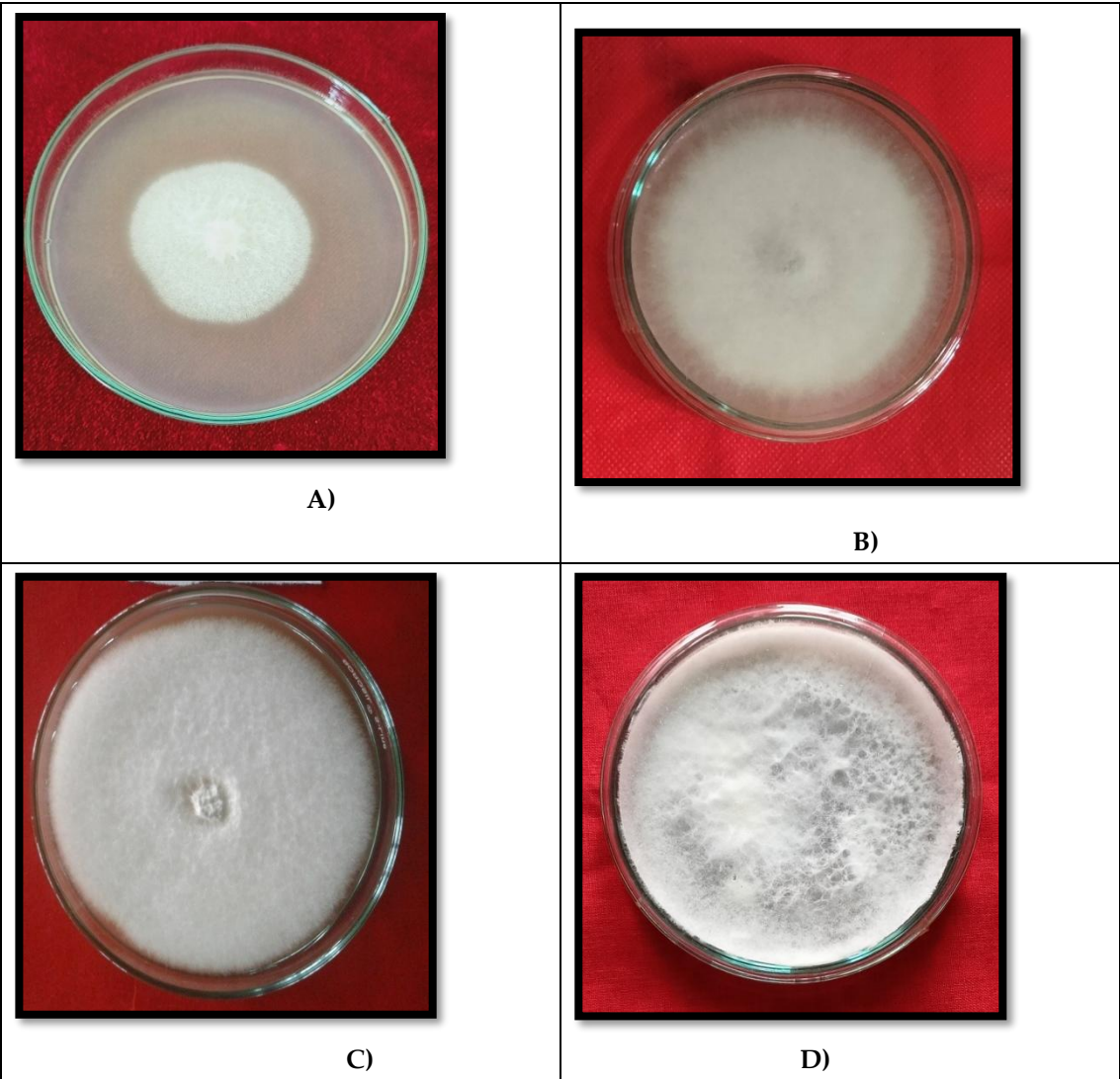
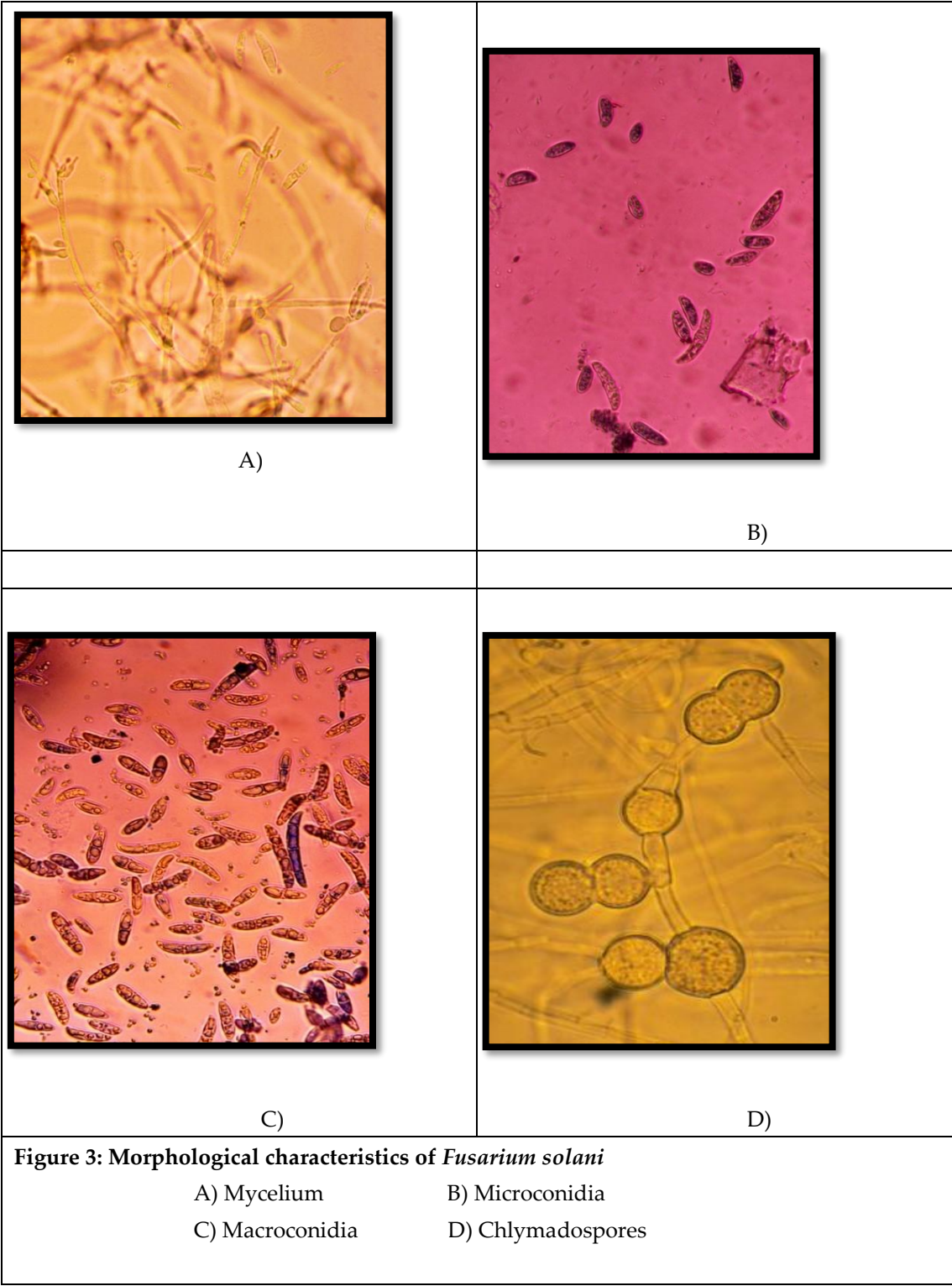


Figure 2 : Colony characteristics of *Fusarium solani*

- A) 3 days old colony B) 7 days old colony
C) 15 days old colony D) 30 days old colony



3.2 Isolation of Mancozeb-Resistant Biocontrol Agents

A total of 104 biocontrol agent isolates were obtained from five districts of the Kashmir Valley, including forest, cultivated, and uncultivated areas of Kaloosa (Bandipora district), Wadura (Baramulla district), Chadoora (Budgam district), Shalimar (Srinagar district), and Awneera (Shopian district) (Table 1).

Table 1 : Biocontrol isolates obtained from different regions of Kashmir
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S.No.	Isolate code	Place of isolation	S.No.	Isolate code	Place of isolation
Dist. Baramulla			22	WG-15	Wadura
1	Mn-17	Wadura	23	WG-13	Wadura
2	Mn-4	Wadura	24	WG-17	Wadura
3	Mn-12	Wadura	25	WG-11	Wadura
4	Mn-15	Wadura	26	WG-5	Wadura
5	Mn-6	Wadura	27	WG-10	Wadura
6	Mn-2	Wadura	28	WG-9	Wadura
7	Mn-11	Wadura	29	WG-2	Wadura
8	Mn-1	Wadura	30	WG-4	Wadura
9	Mn-30	Wadura	31	WG-7	Wadura
10	Mn-25	Wadura	32	WG-6	Wadura
11	Mn-27	Wadura	33	WG-8	Wadura
12	Mn-10	Wadura	34	WG-3	Wadura
13	Mn-13	Wadura	35	WG-1	Wadura
14	Mn-26	Wadura	Dist. Srinagar		
15	Mn-7	Wadura	36	SHA-21	Shalimar
16	Mn-29	Wadura	37	SHA-13	Shalimar
17	Mn-28	Wadura	38	SHA-27	Shalimar
18	Mn-13	Wadura	39	SHA-3	Shalimar
19	Mn-16	Wadura	40	SHA-1	Shalimar
20	Mn-27	Wadura	41	SHA-7	Shalimar
21	WG-14	Wadura	42	SHA-8	Shalimar

255

256

257

S.No	Isolate code	Place of isolation	S.No	Isolate code	Place of Isolation
43	SHA-10	Shalimar	Dist. Bandipora		
44	SHA-25	Shalimar	67	Bpr-3	Kaloosa
45	SHA-4	Shalimar	68	Bpr-8	Kaloosa
46	SHA-2	Shalimar	69	Bpr-7	Kaloosa
47	SHA-5	Shalimar	70	Bpr-10	Kaloosa
48	SHA-18	Shalimar	71	Bpr-19	Kaloosa
49	SHA-14	Shalimar	72	Bpr-12	Kaloosa
50	SHA-22	Shalimar	73	Bpr-13	Kaloosa
51	SHA-11	Shalimar	74	Bpr-21	Kaloosa
52	SHA-6	Shalimar	75	Bpr-11	Kaloosa
53	SHA-26	Shalimar	76	Bpr-22	Kaloosa
54	SHA-24	Shalimar	77	Bpr-32	Kaloosa
55	SHA-12	Shalimar	78	Bpr-27	Kaloosa
56	SHA-5	Shalimar	79	Bpr-2	Kaloosa
57	SHA-20	Shalimar	80	Bpr-9	Kaloosa
58	SHA-27	Shalimar	Dist. Shopian		
59	SHA-28	Shalimar	81	SHO-1	Awneera
60	SHA-29	Shalimar	82	SHO-2	Awneera
61	SHA-15	Shalimar	83	SHO-3	Awneera
62	SHA-30	Shalimar	84	SHO-4	Awneera
63	SHA-31	Shalimar	85	SHO-5	Awneera
64	SHA-11	Shalimar	86	SHO-6	Awneera
65	SHA-32	Shalimar	87	SHO-7	Awneera

S.No	Isolate code	Place of isolation	S.No	Isolate code	Place of isolation
89	SHO-9	Awneera	98	Bud-7	Chadoora
90	SHO-13	Awneera	99	Bud-66	Chadoora
Dist. Budgam			100	Bud-11	Chadoora
91	Bud-1	Chadoora	101	Bud-19	Chadoora
92	Bud-9	Chadoora	102	Bud-21	Chadoora
93	Bud-3	Chadoora	103	Bud-10	Chadoora
94	Bud-4	Chadoora	104	BUD-22	Chadoora
95	Bud-9	Chadoora			
96	Bud-6	Chadoora			

97	Bud-2	Chadoora			
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3.3 Compatibility of Biocontrol Agents with Fungicide

All 104 biocontrol agent isolates were evaluated on poisoned medium amended with 2500 ppm mancozeb using the poisoned food technique. The highest radial growth was recorded for isolate WG-4 (35 mm), followed by SHO-13 (33 mm), after five days of incubation on the poisoned medium. The lowest radial growth was exhibited by isolate SHALI-11 (3.03 mm).

Table 2. The radial growth of biocontrol agents on Potato Dextrose Media (PDA) amended with 2500 ppm mancozeb was recorded after 5 days of incubation (Poisoned Food Technique).

Table-2: Radial growth of biocontrol agents on Potato dextrose media (PDA) amended with 2500ppm mancozeb recorded after 5 days of incubation (Poisoned Food Technique)						
S no.	Bioagents	Mean Radial growth (mm)		S no.	Bioagents	Mean Radial growth (mm)
1	WG-4	35.00* (1.54)		34	M-30	10.03 (1.00)
2	SHO-13	33.00 (1.52)		35	M-27	9.53 (0.81)
3	SHALI-18	31.00 (1.49)		36	SHALI-8	9.53 (0.81)
4	M-29	30.00 (1.48)		37	SHALI-11	9.53 (0.81)
5	WG-13	28.11 (1.45)		38	SHO-8	9.03 (0.80)
6	M-27	27.04 (1.43)		39	M-12	9.03 (0.80)
7	WG-11	26.14 (1.41)		40	SHO-10	9.03 (0.80)
8	WG-10	22.03 (1.34)		41	M-16	8.53 (0.78)
9	SHALI-13	20.03 (1.30)		42	WG-8	8.53 (0.78)
10	WG-17	19.03 (1.28)		43	SHALI-15	8.53 (0.78)
11	M-28	18.03 (1.26)		44	SHALI-3	8.53 (0.78)
12	SHO-18	17.53 (1.24)		45	M-14	8.03 (0.77)
13	WG-6	17.53 (1.24)		46	B-16	8.03 (0.77)
14	WG-7	17.03 (1.23)		47	SHALI-14	8.03 (0.77)
15	B-3	16.53 (1.22)		48	B-20	8.03 (0.77)
16	B-19	16.03 (1.20)		49	SHALI-13	8.03 (0.77)
17	SHO-1	15.03 (1.18)		50	B-10	8.03 (0.77)
18	SHA-4	14.5 (1.16)		51	SHO-12	7.73 (0.76)
19	WG-12	13.53 (1.13)		52	SHALI-5	7.83 (0.75)

20	M-7	13.03 (1.11)		53	SHO-5	7.73 (0.75)
21	M-13	12.53 (1.10)		54	SHALI-1	7.53 (0.75)
22	B-10	12.53 (1.10)		55	M-28	7.53 (0.75)
23	WG-7	12.53 (1.10)		56	WG-11	7.53 (0.75)
24	M-12	12.53 (1.10)		57	SHALI-16	7.43 (0.74)
25	SHO-6	12.03 (1.08)		58	M-28	7.33 (0.74)
26	M-3	11.03 (1.06)		59	SHALI2	7.13 (0.73)
27	SHALI-7	11.53 (1.04)		60	M-26	7.03 (0.73)
28	SHALI-9	11.03 (1.04)		61	B-19	6.93 (0.72)
29	SHO-4	11.03 (1.04)		62	WG-11	6.83 (0.72)
30	WG-3	11.03 (1.04)		63	M-7	6.83 (0.72)
31	SHALI-13	10.53 (1.02)		64	BUD-1	6.63 (0.71)
32	M-13	10.53 (1.02)		65	BUD-3	6.53 (0.71)
33	SHALI-4	10.03 (1.00)		66	BUD-9	6.33 (0.70)

Table 2 Contd...

S no.	Bioagents	Mean Radial growth (mm)	S no.	Bioagents	Mean Radial growth (mm)
67	BUD-11	6.33 (0.70)	86	BPR-22	5.53 (0.67)
68	BUD-6	6.23 (0.70)	87	Mn-17	5.03 (0.67)
69	BUD-2	6.13 (0.69)	88	Mn-4	5.03 (0.67)
70	BUD-66	6.13 (0.69)	89	BPR-32	5.03 (0.66)
71	BUD-7	6.03 (0.69)	90	Mn-31	5.83 (0.66)
72	BUD-13	6.03 (0.69)	91	Mn-14	5.63 (0.66)
73	BUD-19	6.03 (0.69)	92	MN-15	5.63 (0.65)
74	BPR-3	6.03 (0.69)	93	BPR-27	5.53 (0.64)
75	BPR-8	6.03 (0.69)	94	BPR-2	5.53 (0.64)
76	BPR-7	6.03 (0.69)	95	BPR-9	5.23 (0.64)
77	BPR-19	5.93 (0.68)	96	WG-15	5.03 (0.64)
78	BPR-10	5.93 (0.68)	97	WG-20	5.03 (0.64)
79	BPR-19	5.93 (0.68)	98	WG-20	4.53 (0.61)
80	BPR-12	5.93 (0.68)	99	WG-6	4.33 (0.60)
81	SHALI-30	5.83 (0.68)	100	WG-5	4.03 (0.58)
82	BPR-13	5.83 (0.68)	101	SHALI-11	3.03 (0.50)
83	BPR-21	5.83 (0.68)	102	SHALI-6	3.03 (0.50)
84	SHALI-31	5.73 (0.68)	103	SHALI-24	2.03 (0.40)
85	BPR-11	5.63 (0.67)	104	SHALI-20	2.03 (0.40)

C.D (P≤0.05) = 0.30

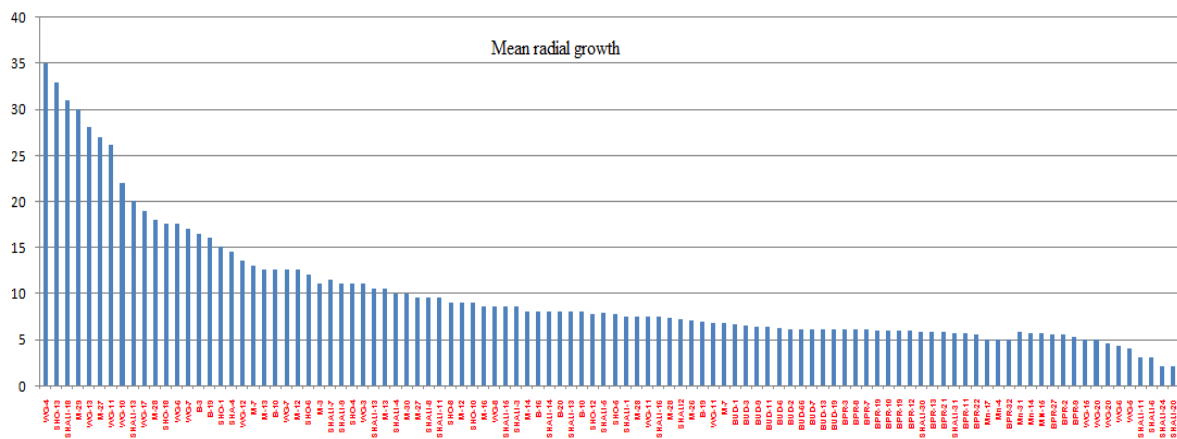
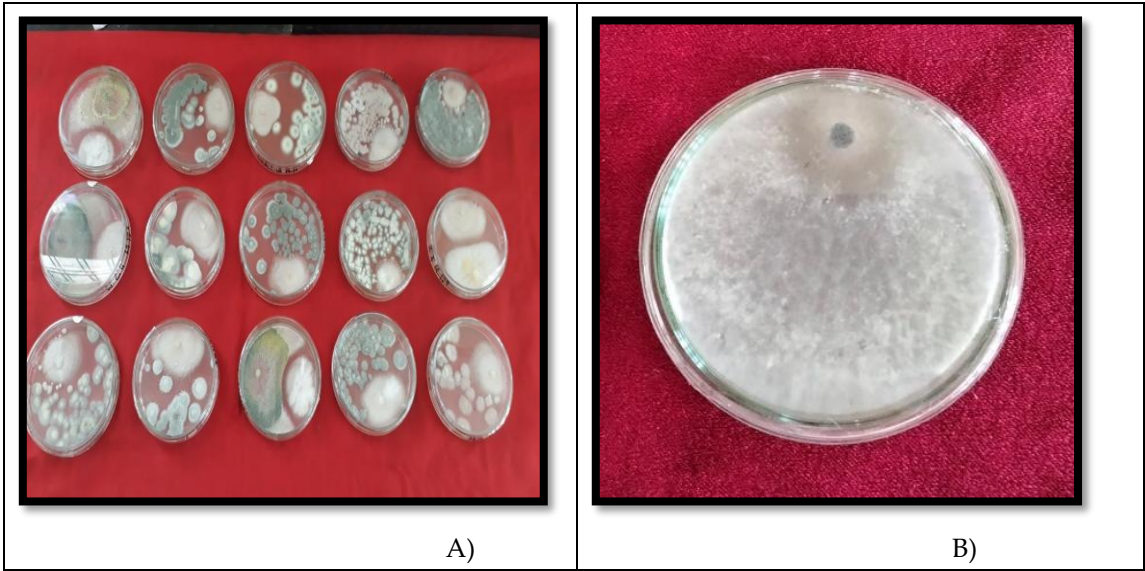


Figure 4: Radial growth (y-axis 0-40mm) of biocontrol agents (x-axis WG-4 to SHALI-20) on Potato dextrose media (PDA) amended with 2500ppm mancozeb recorded after 5 days of incubation (Poisoned Food Technique)

3.4 Dual Culture Technique

In vitro screening of 104 fungal bioagents was carried out against *Fusarium solani* to evaluate their antagonistic activity using the dual culture technique on PDA, as described in Section 3.8.1. The results presented in Table 3 and Figures 5 and 6 revealed that all the bioagents exhibited antagonistic activity against *Fusarium solani*. The minimum colony diameter of the pathogen, 12.02 and 12.52 mm, was recorded in dual culture with isolates SHO-13 and WG-04, respectively, compared with the control, where a colony diameter of 86.00 mm was recorded after seven days of incubation. Bioagent WG-4 exhibited the maximum inhibition of mycelial growth (84.97%), followed by SHO-13, which showed 84.34% inhibition.



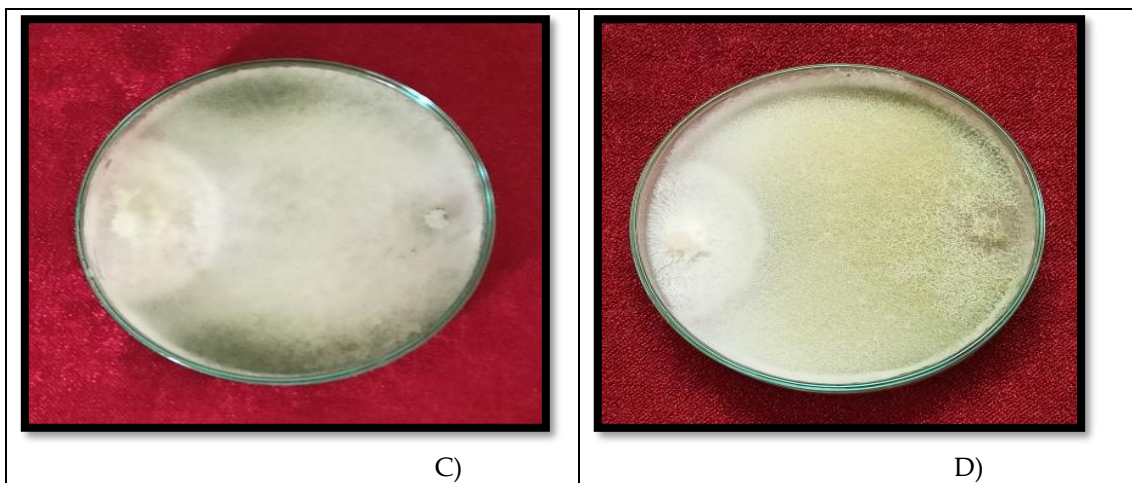


Figure 5 : Dual culture of effective biocontrol agents

- A) Dual culture between bioagents and *Fusarium solani*
 B) Control
 C) Dual culture between bioagents WG-4 and *Fusarium solani*
 D) Dual culture between bioagents SHO-13 and *Fusarium solani*

Table 3 :- *In vitro* efficacy of bioagents against mycelial growth of *Fusarium solani*

Bioagents	Mean colony diameter of pathogen (mm)	Mean percent inhibition	Bioagents	Mean colony diameter of pathogen (mm)	Mean percent inhibition
SHO-13	12.02*	84.973* (1.929)	Bpr-10	16.70	79.123 (1.899)
SHALI-18	12.75	84.060 (1.925)	SHO-6	16.99	78.757 (1.896)
M-29	12.93	83.833 (1.923)	SHALI-7	17.15	78.557 (1.895)
WG-13	13.00	83.743 (1.923)	Mn-3	18.22	77.223 (1.888)
SHO-5	13.03	83.707 (1.923)	M-28	15.02	81.223 (1.910)
Mn-7	13.33	83.333 (1.921)	SHO-14	15.34	80.823 (1.908)
SHALI-17	13.78	82.773 (1.918)	WG-6	15.52	80.595 (1.906)
WG-5	13.95	82.557 (1.917)	WG-7	15.78	80.273 (1.905)
Mn-27	14.02	82.473 (1.916)	Bpr-3	15.90	80.123 (1.904)
WG-11	14.22	82.257 (1.915)	Bpr-19	15.95	80.057 (1.903)
WG-10	14.58	81.773 (1.913)	SHO-1	16.01	79.983 (1.903)
SHALI-13	14.70	81.607 (1.912)	SHA-4	16.03	79.957 (1.903)
WG-17	14.81	81.483 (1.911)	WG-12	16.39	79.507 (1.900)
WG-4	15.01	81.233 (1.910)	Mn-13	16.56	79.293 (1.899)

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M-28	15.02	81.223 (1.910)	Bpr-10	16.70	79.123 (1.899)
SHO-14	15.34	80.823 (1.908)	Mn-12	16.91	78.857 (1.897)
WG-6	15.52	80.595 (1.906)	WG-8	25.92	67.593 (1.830)
WG-7	15.78	80.273 (1.905)	SHALI-15	26.02	67.253 (1.828)
Bpr-3	15.90	80.123 (1.904)	SHALI-3	26.55	66.797 (1.825)
Bpr-19	15.95	80.057 (1.903)	Mn-14	27.12	66.093 (1.820)
SHO-1	16.01	79.983 (1.903)	Mn-11	27.33	65.800 (1.818)
SHA-4	16.03	79.957 (1.903)	SHALI-14	27.41	65.733 (1.818)
WG-12	16.39	79.507 (1.900)	Mn-8	28.12	64.843 (1.812)
Mn-13	16.56	79.293 (1.899)	SHALI-13	28.32	64.593 (1.306)

Contd...					
Bioagents	Mean colony diameter of pathogen (mm)	Mean percent inhibition	Bioagents	Mean colony diameter of pathogen (mm)	Mean percent inhibition
Mn-25	28.65	64.183 (1.807)	BPR-12	38.10	52.373 (1.719)
SHO-12	28.92	63.843 (1.805)	BPR-13	39.18	51.023 (1.708)
SHALI-5	29.02	63.723 (1.804)	BPR-21	40.02	49.973 (1.699)
SHO-5	29.38	63.273 (1.801)	BPR-11	40.24	49.693 (1.696)
SHALI-1	30.22	62.223 (1.794)	BPR-22	40.45	49.433 (1.694)
Mn-28	30.75	61.557 (1.789)	BPR-32	40.93	48.813 (1.689)
WG-2	31.06	61.173 (1.787)	BPR-27	41.02	48.723 (1.688)
SHALI-16	32.08	59.887 (1.777)	BPR-2	41.45	48.183 (1.687)
Mn-28	32.57	59.283 (1.773)	BPR-9	41.11	48.607 (1.684)
SHALI-2	32.81	58.983 (1.771)	SHALI-30	41.37	48.283 (1.683)
Mn-26	33.02	58.723 (1.769)	SHALI-31	42.10	47.373 (1.676)
Bpr-19	33.21	58.493 (1.767)	Mn-17	43.12	46.093 (1.664)
WG-8	33.75	57.807 (1.762)	Mn-4	43.82	45.223 (1.655)
BUD-1	33.93	57.583 (1.760)	Mn-31	44.02	44.973 (1.653)
BUD-3	34.12	57.343 (1.758)	Mn-14	44.04	44.943 (1.653)
BUD-9	34.52	56.843 (1.755)	MN-15	44.47	44.407 (1.647)
BUD-11	34.73	56.583 (1.753)	WG-15	44.70	44.123 (1.645)
BUD-6	34.95	56.307 (1.751)	WG-20	44.80	43.993 (1.643)
BUD-2	35.05	56.183 (1.750)	WG-6	44.93	43.833 (1.642)
BUD-66	35.53	55.917 (1.748)	WG-5	45.02	43.723 (1.641)
BUD-7	35.75	55.307 (1.743)	SHALI-21	45.63	42.957 (1.633)
BUD-13	35.92	55.093 (1.741)	SHALI-27	45.94	42.567 (1.629)
BUD-19	36.02	54.973 (1.740)	SHALI-11	46.05	42.433 (1.628)
BPR-3	36.52	54.343 (1.735)	SHALI-25	46.70	41.623 (1.619)
BPR-8	37.22	53.473 (1.735)	SHALI-2	46.88	41.397 (1.617)

BPR-7	37.40	53.243 (1.726)		SHO-14	47.04	41.197 (1.615)
BPR-19	37.84	52.693 (1.722)		C.D(p≤0.05)= 0.13		
BPR-10	38.00	52.493 (1.720)				
* Average of three replications						
Figures in parenthesis are log transformed values						

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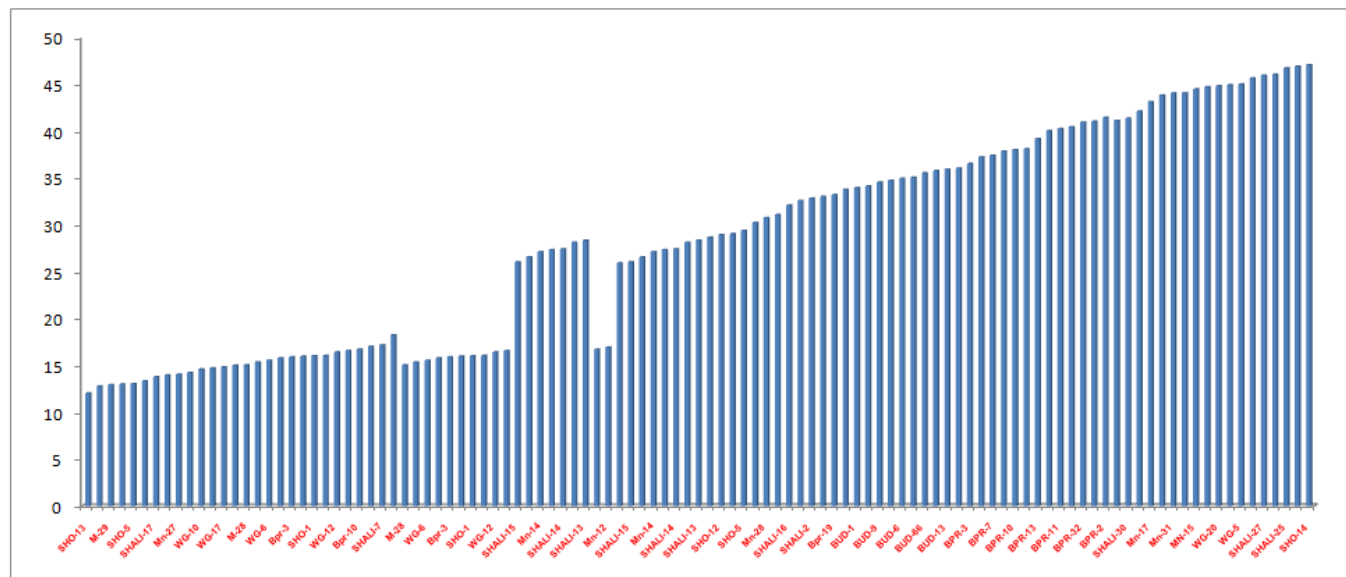


figure 6 A: Mean colony diameter (Y-axis 0-50 mm) of pathogen in dual culture against Bioagents (y-axis from SHO-13 SHO-14)

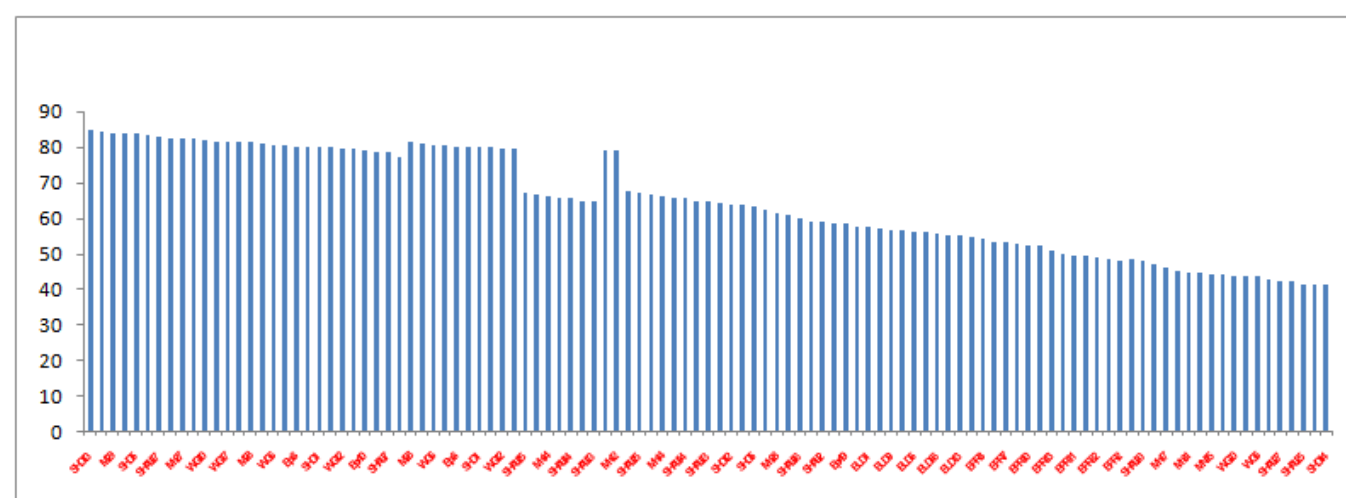


Figure 6B: Mean percent inhibition (y-axis 0 to 90 %) of pathogen by bioagents (x-axis isolate name SHO-13 to SHO-4)

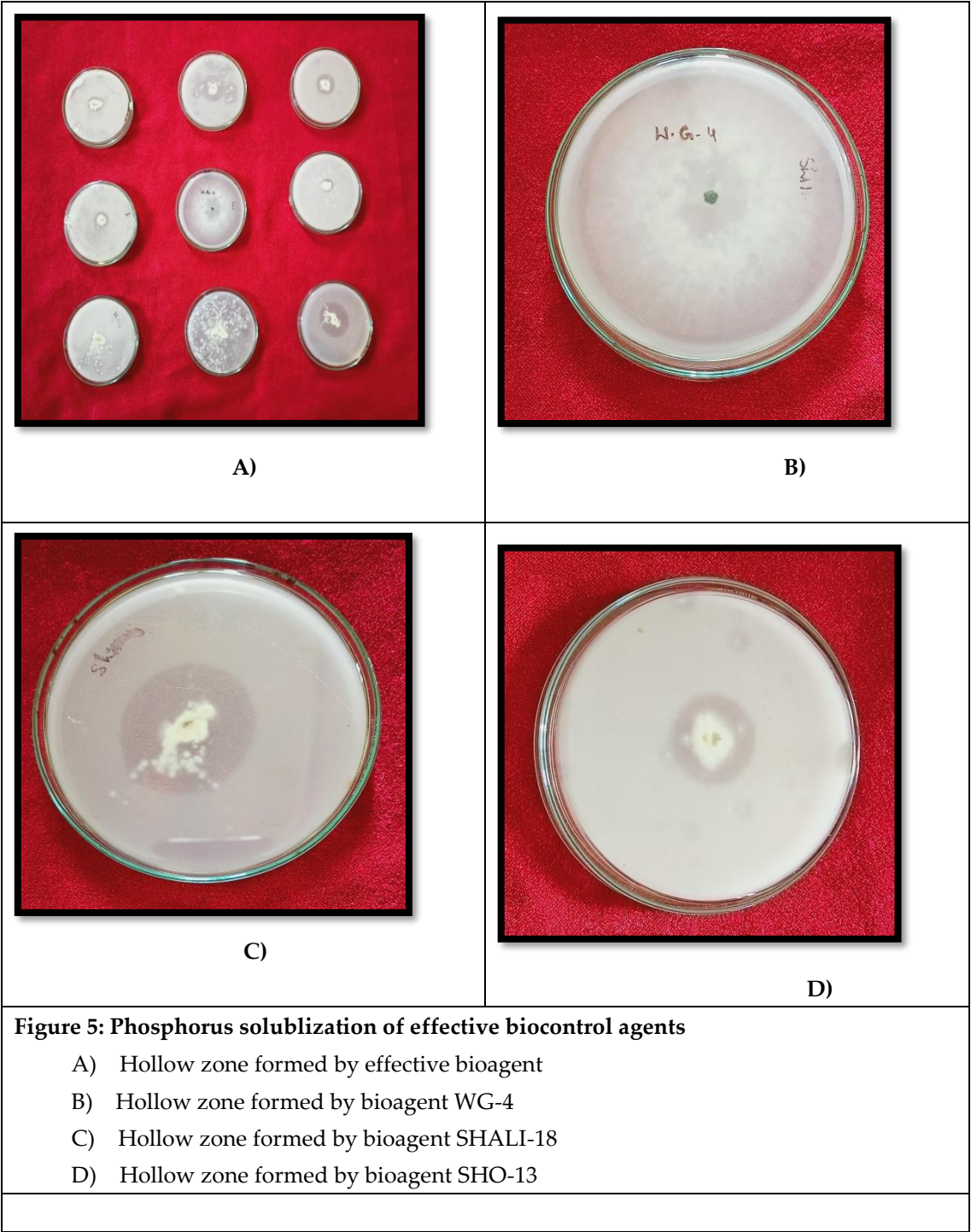
3.5 Phosphorus Solubilization Test of Effective Biocontrol Agents

Of the 104 biocontrol agent isolates, the nine most antagonistic isolates identified through the dual culture assay were selected for *in vitro* evaluation of their phosphorus-solubilizing activity. The data presented in Table 4 and Figure 5 showed that the biocontrol agents WG-4 and SHALI-18 exhibited the highest phosphorus solubilization index (PSI) on Pikovskaya's Agar medium. The PSI values of WG-4 and SHO-13 were 2.38 and 2.12, respectively.

3.6 Mycoparasitism of Effective Biocontrol Agents

One biocontrol agent, SHO-13, which exhibited high antagonistic activity in the dual culture assay, was selected for *in vitro* evaluation of mycoparasitic activity using the agar plate-assisted slide culture technique. Microscopic

344 observations (Figure 6) revealed that the bioagent employed various mechanisms to parasitize the pathogen
345 *Fusarium solani*, including coiling, attachment along the pathogen mycelium, and peg formation.
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Table 4 :-Phosphorous solubilization test of effective bioagents				
S.No	Bioagent	Colony diameter	Hollow zone diameter	Phosphorus Solubilization Index
1	WG-4	65.27	90.27	2.38 ^a
2	SHALI-18	20.2	24.27	2.20 ^b
3	M-29	30.27	35.27	2.14 ^c
4	SHO-13	39.27	44.27	2.12 ^d
5	WG-13	75.27	83.27	2.10 ^e
6	SHO-5	16.27	9.27	1.57 ^f
7	M-7	26.27	5.27	1.20 ^g
8	SHALI-17	46.27	4.27	1.09 ^h
9	WG-5	49.27	3.27	1.06 ⁱ
			C.D (p≤0.05) =0.02	

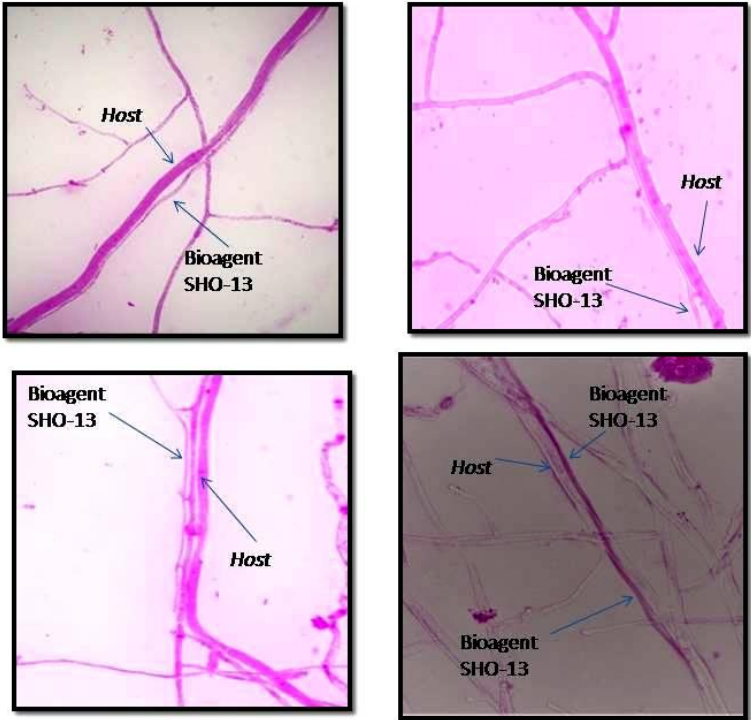
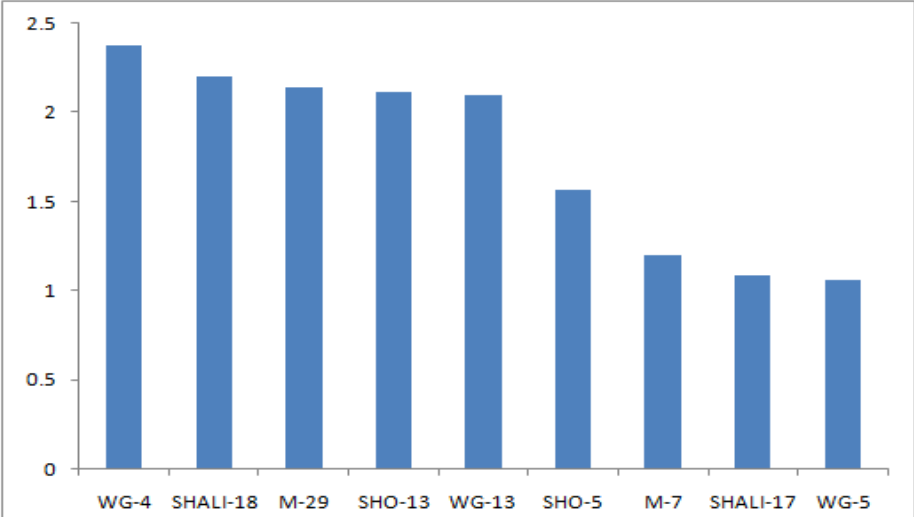


Figure 7: Mycoparasitism of Bioagent SHO-13 on host *F. solini* (400x)

3.7 Morphological Characteristics of the Effective Biocontrol Agent SHO-13

The morphological characteristics of structures produced by the biocontrol agent, including mycelium, conidia, and phialides, were studied using 5–10-day-old cultures. The pure culture was also examined for colony characteristics. The fungal colony of bioagent SHO-13 was initially light green in colour and gradually turned dark green after 10 days of incubation. The septate hyphae were hyaline, while the conidia were hyaline, globose to subglobose, and aseptate. The conidia ranged in size from $2.7\text{--}3.31 \times 2.3\text{--}2.4\text{ }\mu\text{m}$, with an average size of $3.0 \times 2.35\text{ }\mu\text{m}$. The phialides were flask-shaped and green in colour, ranging from $4.7\text{--}5.0 \times 2.7\text{--}3.6\text{ }\mu\text{m}$, with an average size of $4.9 \times 2.8\text{ }\mu\text{m}$.

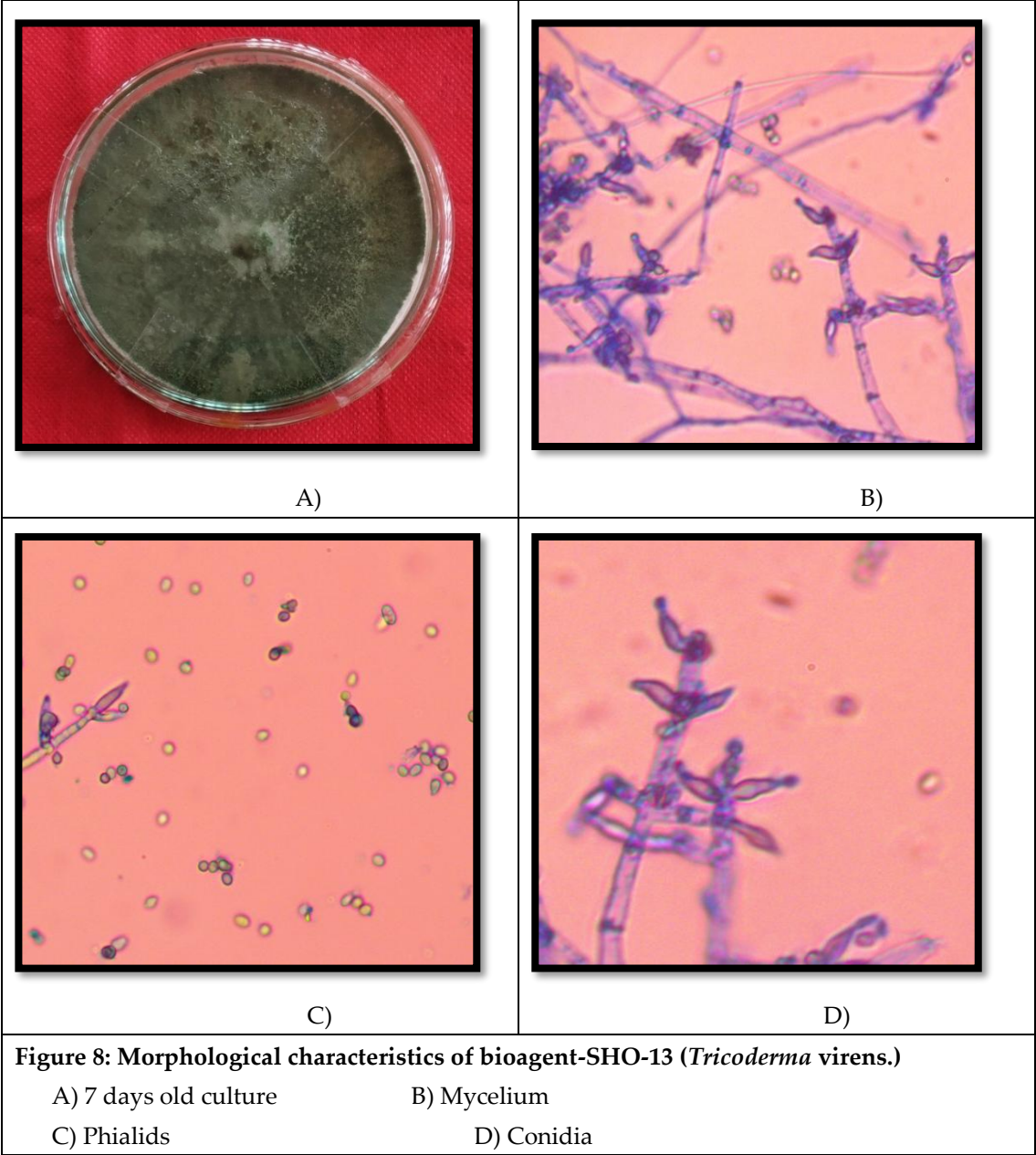
The above-mentioned morphological characteristics supported and confirmed the identification of the biocontrol agent as *Trichoderma* sp. (Figure 8).

DNA sequencing was performed to identify the effective biocontrol agents that showed promising performance in the poisoned food technique, dual culture assay, and phosphorus solubilization test. Gen(i)e Labs sequenced the amplified products obtained through PCR amplification using the ITS1 and ITS4 markers. The sequence was identified as *Trichoderma virens* isolate SKAU-TAB-3 after BLAST analysis for the isolate SHO-13 and *Penicillium goetzii* for the isolate WG-4, with sequence lengths of 573 bp. The sequence of bioagent SHO-13 was submitted to the NCBI database as *Trichoderma* sp. SKAU-TAB-3 under accession number ON740648 followed by next best isolate WG-04 as *Penicillium goetzii* under accession number OP349667.

3.8 *In vivo* Evaluation of Efficient Mancozeb-Resistant Bioagents against Wilt Disease of Chilli

An experiment was conducted using a Completely Randomized Design (CRD) during spring 2021–22 (Figure 10) to evaluate the effects of six different seedling treatments, namely mancozeb, bioagent WG-4, bioagent SHO-13, WG-4 + mancozeb, SHO-13 + mancozeb, and an untreated control. Analysis of the data presented in Table 5 revealed that the seedling treatment with SHO-13 + mancozeb resulted in the lowest disease incidence (5.50%), followed by WG-4 + mancozeb, which recorded a disease incidence of 11.10%. These treatments were followed by SHO-13, WG-4, and

mancozeb, which recorded disease incidences of 28.00, 33.00, and 39.00%, respectively. The highest disease incidence (83.00%) was recorded in the control plants.





A)



B)



C)



D)



E)

Figure 10: Effect of different seedling treatments on disease incidence (%) wilt of chilli

- A) WG-4 + Mancozeb
- B) SHO-13 + Mancozeb
- C) WG-4
- D) SHO-13
- E) Mancozeb

Table 6 : Effect of different seedling treatments on disease incidence (%) of wilt of chilli		
S.No	Seedling treatment	Mean % disease incidence
1	SHO-13 + Mancozeb	5.50 (28.77)*
2	WG-4+ Mancozeb	11.10(30.80)
3	SHO-13	28.00(36.88)
4	WG-4-13	33.00(38.91)
5	Mancozeb	39.00(40.94)
6	Control	83.00 (57.17)
C.D(p ≤0.05)	14.20	

*Figures in parenthesis are arc sine transformed values

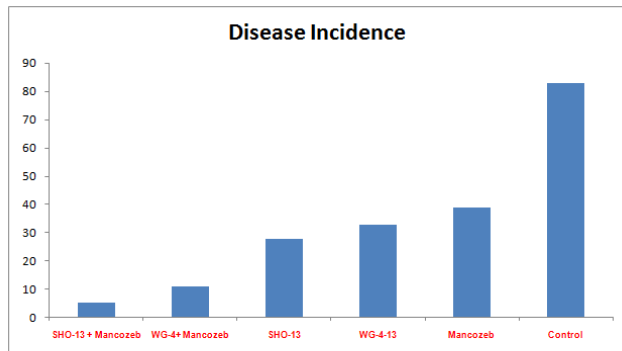


Figure 11: Effect of different seedling treatments (x-axis SHO 13=Mancozeb to Control) on disease incidence (%) (y-axis 0 to 90) of wilt of chilli

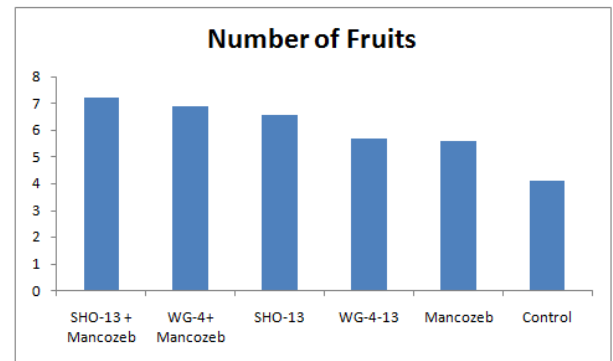


Figure 13: Effect of different seedling treatments (X-axis SHO-13 to Control) on number of fruits (y-axis 0 to 8 cms)

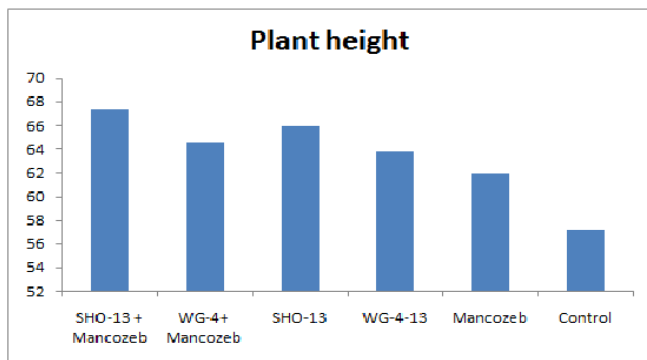


Figure 12: Effect of different seedling treatments (X-axis SHO-13 to Control) on average plant height (cms) (y-axis 52 to 70 cms)

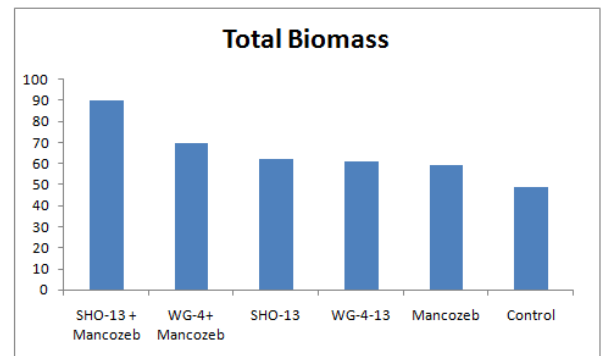


Figure 15: Effect of different seedling treatments (X-axis SHO-13 to Control) on Total biomass (grams) (y-axis 0 to 100)

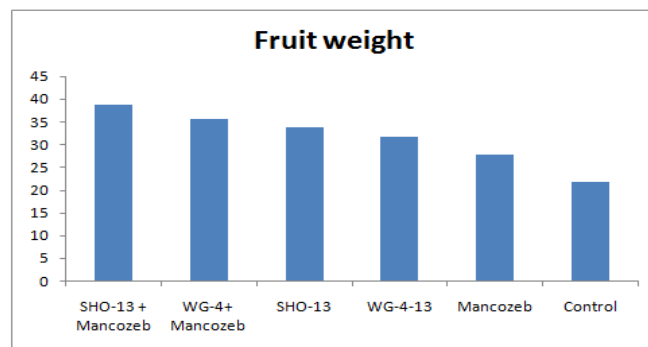


Figure 14: Effect of different seedling treatments (X-axis SHO-13 to Control) on average fruit weight (grams) (y-axis 0 to 45 cms)

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3.9 Effect of Different Seedling Treatments on Average Plant Height

The seedling treatment with SHO-13 + mancozeb resulted in the highest plant height (67.41 cm), followed by WG-4 + mancozeb (64.57 cm). This was followed by WG-4, SHO-13, and mancozeb treatments, which recorded plant heights of 65.95, 63.78, and 61.95 cm, respectively. The lowest plant height (57.15 cm) was recorded in the control plants.

3.10 Effect of Different Seedling Treatments on Number of Fruits per Plant

The seedling treatment with SHO-13 + mancozeb resulted in the highest number of fruits per plant (7.22), followed by WG-4 + mancozeb, SHO-13, WG-4, and mancozeb, which recorded 6.89, 6.56, 5.67, and 5.56 fruits per plant, respectively. The lowest number of fruits per plant (4.11) was recorded in the control.

Table 7: Effect of different seedling treatments on plant height and number of fruits per plant			
S.No.	Seedling treatment	Plant height (cm)	Number of Fruits
1	SHO-13 + Mancozeb	67.41 ^a	7.22 ^a
2	WG-4 + Mancozeb	64.57 ^a	6.89 ^{ab}
3	SHO-13	65.95 ^c	6.56 ^b
4	WG-4	63.78 ^{cd}	5.67 ^c
5	Mancozeb	61.95 ^{de}	5.56 ^{cd}
6	Control	57.15 ^f	4.11 ^e
C.D(p≤0.05)		=1.4	=1.4

3.11 Effect of Different Seedling Treatments on Fruit Weight per Plant

The seedling treatment with SHO-13 + mancozeb resulted in the highest fruit weight (38.63 g plant⁻¹), followed by WG-4 + mancozeb, WG-4, SHO-13, and mancozeb, which recorded fruit weights of 35.63, 33.63, 31.63, and 27.63 g plant⁻¹, respectively. The lowest fruit weight (21.63 g plant⁻¹) was recorded in the control plants.

3.12 Effect of Different Seedling Treatments on Total Biomass per Plant

The seedling treatment with WG-4 + mancozeb resulted in the highest biomass (90.23 g plant⁻¹), followed by SHO-13 + mancozeb, WG-4, SHO-13, and mancozeb, which recorded biomass values of 69.63, 62.14, 61.14, and 59.08 g plant⁻¹, respectively. The lowest biomass (49.04 g plant⁻¹) was recorded in the control plants.

Table 8: Effect of different seedling treatments on fruit weight and biomass of plants			
S.No.	Seedling treatment	Fruit weight (g)	Total biomass (g)
1	SHO-13 + Mancozeb	38.63 ^a	90.23 ^a
2	WG-4 + Mancozeb	35.63 ^b	69.63 ^c
3	WG-4	33.63 ^e	62.14 ^e
4	SHO-13	31.63 ^f	61.14 ^f
5	Mancozeb	27.63 ^g	59.08 ^g
6	Control	21.63 ⁱ	49.04 ⁱ
C.D(p≤0.05)		= 0.7	= 0.3

4 Discussion

The present investigation confirmed that the causal organism associated with wilt disease of chilli under the temperate conditions of the Kashmir Valley was *Fusarium solani*. Although *Fusarium oxysporum* f. sp. *capsici* has frequently been reported as the predominant causal agent of chilli wilt in many agro-climatic regions, the occurrence of *F. solani* as an important wilt pathogen has also been documented from several parts of India and abroad. The recovery of *F. solani* from diseased plants in the present study indicates that the Fusarium wilt complex in chilli may be region-specific and that different *Fusarium* species may predominate under different environmental conditions. Similar findings have been reported by Booth (1971), Leslie and Summerell (2006), Nelson *et al.* (1983), and Agrios (2005), who emphasized the wide host range and geographical variability of *Fusarium* species associated with vascular wilt diseases.

One of the significant outcomes of the present investigation was the successful isolation of mancozeb-resistant fungal bioagents from native soils. Among the isolates screened, WG-4 and SHO-13 exhibited tolerance to mancozeb at 2500 ppm, indicating their potential compatibility with chemical fungicides in integrated disease management (IDM) programmes. The isolation of fungicide-compatible antagonists is particularly important because a major limitation in integrating biological control agents with fungicides is the sensitivity of beneficial microorganisms to chemical treatments. Similar approaches have been adopted by several researchers who demonstrated that fungicide-tolerant strains of *Trichoderma* can retain their antagonistic potential after exposure to fungicides and can therefore be effectively integrated with chemical control measures (Papavizas, 1985; Harman *et al.*, 2004; Sharma *et al.*, 2012). Compatibility between *Trichoderma* spp. and protectant fungicides such as mancozeb has also been reported by Mukherjee *et al.* (2013), indicating that carefully selected fungicide-resistant strains can contribute to sustainable disease suppression.

The dual culture assay demonstrated that the resistant isolates possessed strong antagonistic activity against *F. solani*. Isolates SHO-13 and WG-4 exhibited high inhibition of the radial growth of the pathogen, with WG-4 recording the highest inhibition followed by SHO-13. The antagonistic activity observed in the present study may be attributed to several mechanisms, including rapid colonization, competition for nutrients and space, secretion of antifungal metabolites, production of volatile and non-volatile antibiotics, secretion of hydrolytic enzymes such as chitinases and β -1,3-glucanases, and direct mycoparasitism. These mechanisms have been extensively described for *Trichoderma* species

by Howell (2003), Harman *et al.* (2004), Benítez *et al.* (2004), and Vinale *et al.* (2008), who reported that multiple antagonistic mechanisms contribute collectively to the effective suppression of soil-borne pathogens.

In addition to their antagonistic ability, the selected bioagents exhibited considerable phosphorus-solubilizing activity. This finding is particularly important because phosphate-solubilizing microorganisms can contribute to both pathogen suppression and improved nutrient availability and plant growth. The solubilization of insoluble phosphates through the secretion of organic acids and phosphatases can increase phosphorus availability to plants, thereby improving root growth, nutrient uptake, and crop productivity. Similar observations have been reported by Altomare *et al.* (1999), Harman (2006), and Singh *et al.* (2011), who demonstrated that certain *Trichoderma* species possess plant growth-promoting characteristics, including phosphorus solubilization, micronutrient mobilization, and enhanced nutrient-use efficiency.

Morphological and cultural characterization, supported by molecular analysis, identified the most promising isolate SHO-13 as *Trichoderma virens*. This species is recognized as an effective biological control fungus against several soil-borne pathogens owing to its ability to produce antimicrobial metabolites and hydrolytic enzymes involved in pathogen suppression. The performance of *T. virens* observed in the present study is consistent with earlier reports in which *T. virens* effectively controlled diseases caused by *Fusarium*, *Rhizoctonia*, *Pythium*, and *Sclerotium* spp. through mechanisms involving mycoparasitism, antibiosis, and induction of systemic resistance (Howell, 1998; Harman *et al.*, 2004; Woo *et al.*, 2014).

The polybag experiment further demonstrated that seedling treatment with *T. virens* (SHO-13), particularly in combination with mancozeb, reduced wilt incidence and improved plant growth parameters, including plant height, number of fruits, fruit weight, and total biomass. The combined application of *T. virens* with mancozeb was more effective than either treatment alone. The enhanced disease suppression observed under the integrated treatment suggests a complementary interaction between the biological and chemical components. Mancozeb, being a multisite contact fungicide, may have reduced the initial pathogen inoculum during the early stages of plant establishment, thereby protecting young seedlings during the susceptible growth stage. Subsequently, the fungicide-tolerant

Trichoderma could establish in the rhizosphere, colonize root surfaces, and continue to suppress the pathogen through biological mechanisms. Such complementary activity may provide prolonged disease protection and represents a potential strategy for integrated disease management.

Similar interactions between fungicides and *Trichoderma* have been reported by Elad *et al.* (1993), Harman (2006), Sharma *et al.* (2012), and Mukherjee *et al.* (2013), who observed that compatible fungicides can suppress pathogen populations while allowing resistant antagonists to establish and contribute to disease control. This strategy may enhance disease management while reducing dependence on repeated fungicide applications, thereby minimizing environmental contamination and potentially slowing the development of fungicide resistance in pathogen populations.

The pronounced mycoparasitic behaviour exhibited by SHO-13 against *F. solani* further supports its biocontrol potential. Microscopic observations indicated active interaction between the antagonist and pathogen, involving mechanisms such as coiling around pathogen hyphae, attachment to the pathogen mycelium, and peg formation. These observations are consistent with the classical descriptions of *Trichoderma* mycoparasitism provided by Chet *et al.* (1981), Benhamou and Chet (1997), and Harman *et al.* (2004), who reported that the production of cell-wall-degrading enzymes, including chitinases, glucanases, cellulases, and proteases, facilitates the direct parasitism of pathogenic fungi by *Trichoderma*.

The substantial improvement in plant growth observed following the combined application of *Trichoderma* and mancozeb cannot be attributed solely to disease suppression. The phosphorus-solubilizing ability of SHO-13, together with the reported capacity of *Trichoderma* to produce auxins, siderophores, vitamins, and other plant growth-promoting metabolites, may have contributed to enhanced plant vigour. Previous studies have demonstrated that *Trichoderma* colonization can stimulate root development, enhance nutrient uptake, improve photosynthetic efficiency, and induce systemic resistance, resulting in improved crop growth and yield (Altomare *et al.*, 1999; Harman, 2006; Shores *et al.*, 2010; Hermosa *et al.*, 2012). Therefore, the increased fruit production and biomass recorded in the present investigation may be attributed to the combined effects of reduced pathogen pressure, improved phosphorus availability, and plant growth-promoting activities associated with *T. virens*.

The relatively lower efficacy of mancozeb when applied alone also highlights the limitations of relying exclusively on chemical fungicides for the management of soil-borne diseases. Since *Fusarium* species can survive for extended periods in soil through resistant chlamydospores and colonize the vascular tissues of plants, contact fungicides alone may not provide complete disease control. Similar observations have been reported by Agrios (2005) and in several integrated disease management studies, which recommend combining fungicides with biological control agents and cultural practices to achieve sustainable disease suppression. The present findings therefore support the concept that judicious integration of compatible fungicides with effective biological control agents can provide better disease management than individual treatments.

Overall, the present study demonstrates that the native mancozeb-resistant isolate *Trichoderma virens* SHO-13 possesses multiple beneficial attributes, including fungicide tolerance, strong antagonistic activity, phosphorus solubilization, and plant growth-promoting potential. Integration of this isolate with mancozeb resulted in effective suppression of chilli wilt caused by *Fusarium solani*, while simultaneously improving plant growth and yield-related parameters. Such a strategy represents a promising and environmentally sound component of integrated disease management. Furthermore, because *F. solani* and related soil-borne pathogens infect numerous agricultural and horticultural crops, the identified bioagent may have potential applicability against wilt and root rot diseases beyond chilli. However, further investigations under multilocation field conditions, together with molecular characterization of the antagonistic mechanisms, rhizosphere colonization dynamics, and biochemical basis of plant growth promotion, are required to validate its efficacy and facilitate its potential development as a commercial bioinoculant for sustainable crop production.

5. Conclusions

In this work, soil patches were treated with mancozeb fungicide at various places of Kashmir valley so as to apply selection pressure on fungi with biocontrol proficiency. One hundred and four biocontrol isolates were obtained from the treated soil using TSM (Tricoderma specific medium). The compatibility of all 104 isolates was cross checked by growing them on medium amended with mancozeb fungicide. *In vitro* testing of all 104 isolates was done to evaluate the antagonistic efficacy against *Fusarium solani*, *in vitro* Phosphorus solubilization and *in vitro* mycoparasitism evaluation was done. Based on morpho-cultural identification isolate SHO-13 was identified as *Tricoderma virens*. In the polybags experiment, both biocontrol agents SHO-13 and Wh-4

surpassed the fungicide mancozeb alone and combined with fungicide mancozeb. The use of potential biocontrol agents in combination with mancozeb is beneficial for better and long lasting control and helps in managing pathogen resistance in several differ ways. A unique feature of present study adds value to microorganisms under investigation as potential P-solublizers making these bioagents as biofertilizers simultaneously.

The screened bioagents SHO-13 as well as WG-4 can further be validated against the other soil borne pathogens like root rot and wilt of beans, root rot of pea, wilt of chickpea, corm rot of saffron, root rot of apple and other soil borne prevalent in the region.

Declarations

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B. Author Contributions

Conceptualization, K.A.B.; methodology, K.A.B.; software, A.A. and P.T.; validation and formal analysis, A.A. and P.T.; investigation, A.A. and K.A.B.; resources and data curation, K.A.B., P.A.S., and P.A.; writing—original draft preparation, H.S.V.; visualization and supervision, K.A.B. and P.A.S.; project administration, K.A.B.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

C. Clinical Trial Number

Not applicable.

D. Consent to Participate

Not applicable.

E. Consent to Publish

Not applicable.

F. Conflicts of Interest

The authors declare that they have no relevant financial or non-financial interests to disclose. The authors further declare that there are no financial, professional, or personal competing interests

among the authors or with any third party that could have influenced the research, analysis, interpretation, or publication of this manuscript.

G. Ethical Approval

Not applicable.

H. Data Availability Statement

The ITS sequences generated in this study have been deposited in the NCBI GenBank database and are publicly available under the following accession numbers: **OP349667** (*Penicillium goetzii*, isolate WG-04) and **ON740648** (*Trichoderma virens*, isolate SHO-13). The sequence data can be accessed through the NCBI GenBank database using the respective accession numbers.

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References

1. Agrios, G.N. (2005). *Plant Pathology*. 5th Ed. Elsevier Academic Press.
2. Altomare, C., Norvell, W.A., Björkman, T. & Harman, G.E. (1999). Solubilization of phosphates and micronutrients by *Trichoderma harzianum*. *Applied and Environmental Microbiology*, 65: 2926–2933.
3. Anonymous. Annual report of network project on wilt of chilli. ICAR, India, New Delhi, pp.1-7. (2005)
4. Benhamou, N. & Chet, I. (1997). Cellular and molecular mechanisms involved in the interaction between *Trichoderma harzianum* and *Rhizoctonia solani*. *Applied and Environmental Microbiology*, 63: 2095–2099.
5. Benítez, T., Rincón, A.M., Limón, M.C. & Codón, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*, 7: 249–260.
6. Bhat, K. A.: A New Agar Plate Assisted Slide Culture Technique to Study Mycoparasitism of *Trichoderma* sp. on *Rhizoctonia solani* and *Fusarium oxysporum*. *International Journal of Current Microbiology and Applied Science* 6(8),3176-3180 (2017)
7. Booth, C. (1971). *The Genus Fusarium*. Commonwealth Mycological Institute.
8. Chet, I., Harman, G.E. & Baker, R. (1981). *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani*. *Phytopathology*, 71: 286–290.
9. Edi-Permono., Moawad, A. M. and Vleck, P. L. G.: Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Crop Science* 11, 13-23(1996)
10. Elad, Y., Chet, I. & Henis, Y. (1993). Biological control of plant diseases using *Trichoderma* spp.
11. Fravel, D. R.: Commercialization and implementation of biocontrol. *Annual Review Phytopathology* 43,337-359 (2005)
12. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M.: *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature reviews microbiology*, 2(1), 43-56 (2004)
13. Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, 96: 190–194.
14. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. & Lorito, M. (2004). *Trichoderma* species—Opportunistic avirulent plant symbionts. *Nature Reviews Microbiology*, 2: 43–56.
15. Hassan, S. A., Gowen, S. R. and Pembroke, P.: Use of *Trichoderma hamatum* for biocontrol of lentil vascular wilt disease: Efficacy, mechanisms of interaction and future prospects. *Journal of Plant Protection Research* 53, 12-26 (2013)
16. Hermosa, R., Viterbo, A., Chet, I. & Monte, E. (2012). Plant-beneficial effects of *Trichoderma*. *Microbiology*, 158: 17–25.
17. Howell, C.R. (1998). The role of antibiosis in biocontrol by *Trichoderma*. In: *Trichoderma and Gliocladium*.
18. Howell, C.R. (2003). Mechanisms employed by *Trichoderma* species in biological control. *Plant Disease*, 87: 4–10.
19. Joshi, M., Srivastava, R., Sharma, A.K. and Prakash, A.: Screening of resistant varieties and antagonistic *Fusarium oxysporum* for bio control of *Fusarium* wilt of chilli. *Journal of Plant Pathology and Microbiology* 3, 134-136 (2012)
20. Leslie, J.F. & Summerell, B.A. (2006). *The Fusarium Laboratory Manual*. Blackwell Publishing.
21. Liang, L. Z.: Seed-borne *Fusarium* of chilli and their pathogenic significance. *Acta Phytopathologica Sinica* 20, 117-121 (1990)
22. MacHardy, W.E. and Beckman, C. H.: *Fusarium: Diseases, Biology and Taxonomy* In: Vascular wilt Fusaria: Infections and Pathogenesis, (Eds. P. E. Nelson, T. A. Toussoun and R. J. Cook). The Pennsylvania State University Press, USA. pp. 365-390 (1981)

23. Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M. & Kenerley, C.M. (2013). *Trichoderma* research in the genome era. *Annual Review of Phytopathology*, 51: 105–129.
24. Najar, A. G., Ahmad, M., Ganie, S. A. and Anjum, Q.: Evaluation of some soil amendments and fungicides on wilt incidence and yield of chilli. *International Journal of Plant Protection* 5 , 245-247 (2012)
25. Nazia. B and Sharda, M. S.: Inhibitory effects of Mancozeb on growth and stimulation of resistance against *Fusarium* wilt of brinjal. *Supplement on Agronomy* 13(1), 285-290 (2018)
26. Nelson, P.E., Toussoun, T.A. & Marasas, W.F.O. (1983). *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University Press.
27. Nene, Y. and Thapliyal. P.: *Fungicides in Plant Disease Control*, Oxford & IBH Publishing Company, New Delhi, India. pp. 691 (2000)
28. Parray, M. N., and Razdan, V. K. and Sofi, T. A.: Comparative study of different fungi associated with fruit rot of chilli and screening of germplasm against *Collectotrichum capsici*. *International Journal of Agricultural and Crop Sciences* 5, 272-279(2013)
29. Rivelli, V.: A wilt of pepper incited by *Fusarium oxysporum* f.sp. *capsici*. M.Sc. thesis submitted to Louisiana State University, Baton Rouge, pp. 72 (1989)
30. Sharma, P., et al. (2012). Biological control of plant diseases using *Trichoderma*: A review. *Journal of Plant Pathology*, 94: 559–569.
31. Shores, M., Harman, G.E. & Mastouri, F. (2010). Induced systemic resistance and plant responses to *Trichoderma*. *Plant Signaling & Behavior*, 5: 661–664.
32. Singh, V., Singh, U.S. & Singh, K.P. (2011). Phosphate-solubilizing microorganisms and plant growth promotion.
33. Vinale, F., Sivasithamparan, K., Ghisalberti, E.L., et al. (2008). *Trichoderma*-plant-pathogen interactions. *Soil Biology and Biochemistry*, 40: 1–10.
34. Vincent, J. M.: Distortion of Fungal Hyphae in Presence of Certain Inhibitors. *Nature* 154 , 850 (1947)
35. Wadejo, B.: Compatibility studies of fungicides with combination of *Trichoderma* species under *In vitro* conditions. *Virology and Mycology* 4, 2-249 (2015)
36. Wani, S A., Mohiddin, F. A., Hamid, B., Rizvi, G., Bhat, K A., Hamid, A., Alam, A., Baba, Z A Padder, S. A. and Bhat, M A.: Incidence of *Fusarium* wilt of chill (*Capsicum annum* L.) in Kashmir valley and its management by *Trichoderma* spp. *Mycopathology* (2014)
37. Woo, S.L., Ruocco, M., Vinale, F., et al. (2014). *Trichoderma*-based products and their agricultural applications. *Microbial Biotechnology*, 7: 477–486.
38. Zaidi, N. W. and Singh, U. S.: An improved method for *In vitro* testing of antagonism, In *National Symposium on Bio-control agents for Sustainable Management of Pests*. Society of Plant Protection Science, New Delhi. pp. 81-82 (2003),