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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(1): 936-944

www.thepharmajournal.com
Received: 16-11-2022
Accepted: 19-12-2022

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Molecular characterization, *in vitro* screening of fungicide, bio agents against the Fusarium wilt disease of muskmelon

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Abstract
Muskmelon (*Cucumis melo* L.) is one of India's most important and widely grown vegetable crops. This crop is affected by a number of diseases, the most destructive of which is Fusarium wilt, which causes severe yield losses. An experiment was carried out to determine the fungal pathogen associated with Fusarium wilt disease of muskmelon, which was obtained from a field in the Kolar district of Karnataka. The obtained fungus is identified using morphological characteristics such as colony features, myceli and conidia, as well as molecular characterization utilising internal transcribe spacer (ITS) region of the fungus. ITS sequence of our studied fungus *Fusarium solani* was genetically 25.9% WW maximum mean inhibition of 97.74 percent similar to sequences of *Fusarium solani* in NCBI database. To confirm the pathogen pathogenicity test was conducted twenty days after inoculation symptoms was observed. Fifteen commercial fungicides and two bacterial, three fungal bio agents were evaluated against isolated fungus under *in vitro* condition, in which the highest percent inhibition of radial growth of the fungus was observed in tebuconazole 25.9% WW maximum mean inhibition of 97.74 percent and in bio control agents *Trichoderma viride* was found as the most suitable to control the growth of *F. solani* under laboratory conditions. However, further pot and field trials needed to be confirmed the bio-control potential of it.

Keywords: Muskmelon, *Fusarium* wilt, fungicide, bio agents, ITS primers, dendrogram

Introduction
Muskmelon (*Cucumis melo* L.) is a major vegetable crop that belongs to the Cucurbitaceae family. Iran is considered as the primary muskmelon development center and secondary centers are India, Afghanistan, Persia, China and Southern Russia. It is a significant riverbed crop, accounting for roughly 80% of total muskmelon production (Pradhan, 2014) [13]. Muskmelon provides almost all the fat and water-soluble vitamins except vitamin D and E. Content of ascorbic acid is more than 40 mg/100 g of fresh weight. Additionally, muskmelon also contains vitamin B1, B3 and B6, which makes it a special fruit compared to others (Manchali and Murthy, 2020) [23]. During the summer and rainy months in India, it is primarily cultivated as a vegetable in Uttar Pradesh, Andhra Pradesh, Punjab, Madhya Pradesh and Karnataka. Meanwhile, it is grown as a "Diara" cultivation in the Ganga, Yamuna, Gomati and other tributaries of these rivers during the winter and spring months (Kumari *et al.*, 2018). Karnataka being having a productivity of 22.33 thousand metric tonnes shares about the 1.48% to the national muskmelon fruit production (Anon., 2021) [1, 2]. However there are number of constraints in muskmelon cultivation among which *Fusarium* wilt is a major prominent. The main disease symptoms are wilting, followed by chlorosis and subsequently necrosis of the leaf interveinal regions. Plants that were infected early did not set fruit, while those that were infected later produced little, deformed fruits. *Fusarium* wilt of muskmelon is the major factor in yield loss of muskmelon cultivation in India. Some of the varieties of muskmelon are susceptible to *Fusarium* wilt. So it is necessary to identify the pathogen and characterization at molecular level. It is also important to identify suitable Management practices for management of *Fusarium* wilt. In the present study, we have attempted to identify suitable chemical fungicides, bio agents and against Fusarium wilt of muskmelon under *in vitro*. The genetic relationship among the isolates through rDNA sequence analysis has also been carried out. Characterization of the population structure of fungal pathogens is important for understanding the biology of the organism and for development of disease-control strategies (Malvick and

Percich, 1998) [11] and for molecular studies among structure (Leung *et al.*, 1993). [9]

The rapid and accurate identification of pathogens is essential for efficient plant disease management. Particularly, pathogenic genetic characterization variants of plant pathogens found in a specific area are required for successful management and crop growth productivity. *Fusarium* spp. is large cosmopolitan pathogens and having many number of formae specialis. Among different methods researchers have used rDNA-IGS, rDNA-ITS regions, the large submit RNA gene, and translation elongation factor-alpha to study the phylogenetics of *F. solani* species. Therefore, the objectives of this study were: to isolate and identify disease causing *Fusarium* spp. from infected muskmelon plants and *in vitro* evaluation of fungicide against the *Fusarium solani.*

Materials and Methods

Sample collection

The infected plants showing typical symptoms of the disease were collected from the Hosallii village, Srinivas Pura taluk, Kolar district of Karnataka during the year 2020. The infected samples were brought immediately to the laboratory and isolated the pathogen on potato dextrose agar media.

Isolation of the pathogen

For isolation, the infected tissue showing typical symptoms of the disease was used for the isolation of the pathogen. The plant parts showing brown discoloration of vascular tissues were cut into small pieces and washed thoroughly in running tap water. The infected pieces were surface sterilized with 1% Sodium hypochlorite solution for 60 seconds and such pieces were transferred to Petri dishes containing sterile water successively for three times to remove traces of 1% Sodium hypochlorite. Such few bits were aseptically transferred into Petri dishes containing potato dextrose agar medium and the plates were incubated at 26 ±1 ºC for 7 days. Pure culture of the fungus was obtained by single spore isolation.

Pathogenicity test

Fungal spore suspension was prepared in the laboratory by inoculating 5 mm disc to the potato dextrose broth which was cut at periphery of the actively growing culture and incubated at 26±1 ºC for 21 days. The conidial suspension was prepared by adjusting conidial concentration to 4 x 10^6 cfu/ml by adding sterile distilled water to the inoculum. After preparing, inoculum was brought to the poly house. The seven days old healthy susceptible seedlings were used in this study. Apparently healthy seeds of muskmelon were sown in portrays consisting cocopeat media and allow it to grow for 7 days. After that, seedlings were uprooted and the roots were washed thoroughly in running tap water to remove the adhering particles to the root surface. The roots were injured by using sterile blade for penetration of the pathogen. The injured roots of seedlings were dipped in the conidial suspension of 4x10^6 cfu/ml for 60 minutes. Control plants were dipped in sterile distilled water and planted separately in the grow pot containing sterilized soil. Symptoms produced on artificially inoculated plants were recorded and compared with those observed on naturally infected plants. The fungus was re-isolated from the inoculated plants of sweet pepper on PDA medium to fulfill Koch's postulate.

individuals, which is one of the components of population

DNA extraction

The genomic DNA was isolated from mycelium of 8 days old culture using Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method described by Descenzo and Harrington, 1994 [5] with slight modifications as follows: Approximately 100 mg of fungal mycelia was added into mortar. The mycelia were ground using pestle by adding 1ml of CTAB extraction buffer (2%w/v CTAB, 1.4 M Nacl 0.2% v/v 2-Mercapitoethanol, 20 mM EDTA and 100 mM Tris HCL, Ph 8.0) which was pre-heated to 60 ºC for 10 min. About 750 µl of the sample was poured into a 1.5 ml Eppendorf tube and the samples were kept in hot water bath at 60 ºC for 30 min with occasional stirring. The sample were then mixed with an equal volume (750 µl) of chloroform: isoamyl alcohol (24:1) and centrifuged at 13000 rpm for 10 min. Top aqueous phase was transferred into new Eppendorf tube. The step can be repeated if required. DNA was precipitated by adding 300 µl (0.6 volume) of cold isopropanol and incubated at -20 ºC for at least one hour. Samples were centrifuged at 13000 rpm at 4 ºC for 10 min and the supernatant was poured off by taking care that not to lose the pellet formed at the bottom of the tube. The pellet was washed with 500 µl of 70 percent ethanol by vertexing and then centrifugation at 13000 rpm for 5 min. The ethanol was removed and pellet was air dried for 15 min at room temperature and the dried pellet was suspended in100 µl of 1X TE buffer. DNA can be treated with ribonuclease (*RNase* 10 mg/ml) or proteinase (10 mg/ml) if the contamination occurred by RNA or protein respectively and stored at -20 ºC until use. All the DNA extracts were further diluted using sterilized double distilled water before used for PCR amplifications. The DNA was quantified either by gel electrophoresis or Nano drop. The qualitative check for the presence of DNA in samples was confirmed through agarose gel electrophoresis using 0.8% agarose. Purity of DNA was analysed by $A_{260}/A_{280\ ratio}$ using Nano drop (Thermo Scientific).

PCR condition

The ITS region of *Fusarium solani* was amplified with primersITS1 (50-TCCGTTGGTGAACCAGCG G-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) (White *et al*., 1990) [22]. The PCR was performed in a 25 µl reaction mixture, consisting of 12.5 µl GoTaq G2 Hot Start Green Master Mix (dNTPs, Buffer, MgCl2, Taq Polymerase), 2.0 µl of each primer (10 µM), 2 µl DNA template and 6.5 µl water. The PCR conditions included an initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 60s, followed by primer extension for 30s at 72°C and final extension at 72°C for 10 min. later PCR products are purified and it yielded approximately 582bp was sequenced in laboratory .Sequencing data was blast searched and compared for percent homology of rDNA sequence with similar DNA sequences retrieved from NCBI Genebank database. The phylogenetic analysis was conducted using the multiple sequence alignment tools and a maximum likelihood tree was generated using MEGA 6 software (Tamura *et al*., 2013; Sikder *et al.*, 2020) [20, 18].

Efficacy of bio-control agents and fungicides against *F. solani*

The bio-control agents- *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*, *Aspergillus niger* were (Nene and Thaplyal, 1982) [12]. A requisite quantity of fungicides was added to the medium with a concentration of 500 ppm, 1000 ppm, 1500 ppm, 2000ppm and 2500 ppm. The percent inhibition of growth of test fungus over control will be determined using the formula given by Vincent (1947) [21].

PI = 100 (C-T) / C

Where,
PI= percent inhibition,
C= Growth of the fungus in control,
T= Growth of the fungus in treatment.

Statistical analysis

Data obtained in the investigation for various parameters was subjected to ANOVA for *in vitro* following completely randomized block design. The data generated from various experiments were statistically analysed using WASP and OPISTAT software.

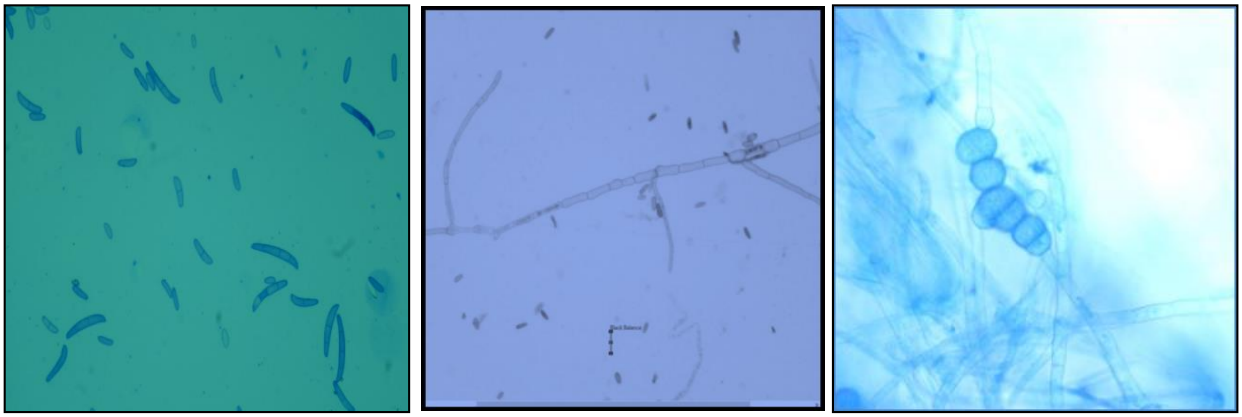
evaluated against mycelial growth of the fungus using dual culture technique (Raza *et al.*, 1976) [16]. The effect of fifteen (Table 1) fungicides against growth of the *F. solani* was determined on PDA medium using the food poison technique

Results and Discussion

Identification and pathogenicity of the fungal pathogen

Symptoms of the disease on muskmelon caused by the fungus have shown in plate 2. The main disease symptoms are wilting, followed by chlorosis and subsequently necrosis of the leaf interveinal regions. Plants that were infected early did not set fruit, while those that were infected later produced little, deformed fruits. On sick vines, cracks are common. The colony was more or less circular or slightly irregular depending on culture media, with abundant milky white-colored conidial masses on upper surface, and under surface of plate slightly oranges colour. (Plate 1A and B). The hyphae of the fungus were hyaline and septate (Plate 1E). The conidia were born on distinct, hyaline conidiophores. Microconidia were oval ellipsoidal shaped with no septation while macroconidia were sickle shaped, hyaline, with 2- 3 septation (Plate 1C and D). Based on morphological features, it was identified as *Fusarium* spp. It was quite difficult to identify the fungus at species rank without molecular characterization**.**

Topside view of plate(A) **Backside view of plate(B)** **Micro conidia (40 X)(C)**



Macro conidia (40X) (D) **Septate mycelia (40X) (E)** **Chlamydospore (40X)**

Plate 1: Morphological and cultural characterization of *Fusarium solani* (Mar.) Sacc.

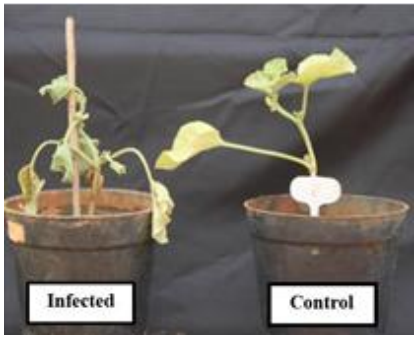


Plate 2: Proving pathogenicity of muskmelon plants

Table 1: List of fungicides used for *in vitro* evaluation against *F. solani* (Mart.) Sacc. by poisoned food method

Fungicides	Sl. No.	Technical name	Trade name	Concentration (ppm)
Systemic fungicides	1	Hexaconazole 5%SC	Contaf plus	500, 1000, 1500, 2000, 2500, 3000
	2	Tebuconazole 25.9% EC	Folicure	500, 1000, 1500, 2000, 2500, 3000
	3	Propiconazole 25% EC	Tilt	500, 1000, 1500, 2000, 2500, 3000
	4	Thiophanate methyl 70% WP	Roko	500, 1000, 1500, 2000, 2500, 3000
	5	Azoxystrobin 23% SC	Amistar	500, 1000, 1500, 2000, 2500, 3000
Contact fungicides	6	Captan 50% WP	Captaf	500, 1000, 1500, 2000, 2500, 3000
	7	Chlorothalonil 78% W/W	Kavach	500, 1000, 1500, 2000, 2500, 3000
	8	Copper hydroxide 53.8% DF	Kocide	500, 1000, 1500, 2000, 2500, 3000
	9	Mancozeb 75% WP	Indofil M-45	500, 1000, 1500, 2000, 2500, 3000
	10	Zineb 75% WP	Indofil Z-78	500, 1000, 1500, 2000, 2500, 3000
Combination fungicides	11	Hexaconazole 4%+Zineb 68%	Avatar	500, 1000, 1500, 2000, 2500, 3000
	12	Metalaxyl 4% + Mancozeb 64% (68%WP)	Redomil gold	500, 1000, 1500, 2000, 2500, 3000
	13	Carbendazim 25% +Mancozeb 50%	Sprint	500, 1000, 1500, 2000, 2500, 3000
	14	Captan 70% + Hexaconazole 5% WP	Toqat	500, 1000, 1500, 2000, 2500, 3000
	15	Tricyclazole 18% + Mancozeb 62% WP	Merger	500, 1000, 1500, 2000, 2500, 3000

a) Drooping of leaves | b) Chlorosis and necrosis of leaves

Plate 2: Symptoms of *Fusarium* wilt of muskmelon under poly house condition

In the present study, the results of the pathogenicity test revealed that *Fusarium solani* was found to be pathogenic. The symptoms induced by pathogenic *Fusarium solani* isolate developed within 20 days after inoculation. Necrosis and brown discoloration were observed on roots, stem as well as a browning of the vascular tissues (Plate 3). The re-isolation of the fungal pathogen proved the pathogenic nature of a particular fungus *F. solani.* After submission of nucleotide sequence of the studied fungus, accession number OP492078, OP492079, OP492080, OP492081was received and identified as *Fusarium solani.* After the amplification, the PCR products were sequenced and dendrogram was constructed (Sriram *et al.*, 2015) [19]. In the BLAST analysis, DNA sample isolated from Kolar (Hosahalli) location of Karnataka showed nucleotide sequence identity or sequence homology of 91.49 percent with the *Fusarium solani* (Mart.) Sacc. HG938368.

The evolutionary tree, commonly known as phylogenetic tree showed that *Fusarium solani* (Mart.) Sacc. (Kolar isolate) was closely related to isolates HG938368 (*Fusarium solani*) and MW045615.1 (*Fusarium solani*), with a similarity of 91.49 percent and 89 percent, respectively (Plate 3b). This relationship confirmed the pathogen's identity. Also, it shows similarity with MZ298791 isolate (*Fusarium udum*) of about 75.8 percent, with *Fusarium oxysporum* f. sp. *vasinfectum* 66.8% percent and MW074253.1 *Fusarium oxysporum* f. sp. *ricini* 68.2% similarity respectively. The evolutionary

ancestry of the pathogen was concluded using the Neighbor-Joining method. Percentage of trees which are replicated where in there is a group of taxa which are related in the bootstrap test (1000 replicates) is shown. Analysis of evolutionary relations was conducted in MEGA - X. The fungal pathogen was documented on a gel using the ITS1 primer and a 100bp ladder (Gene direx). M-Molecular Weight Marker (100bp) as depicted on Plate 3.

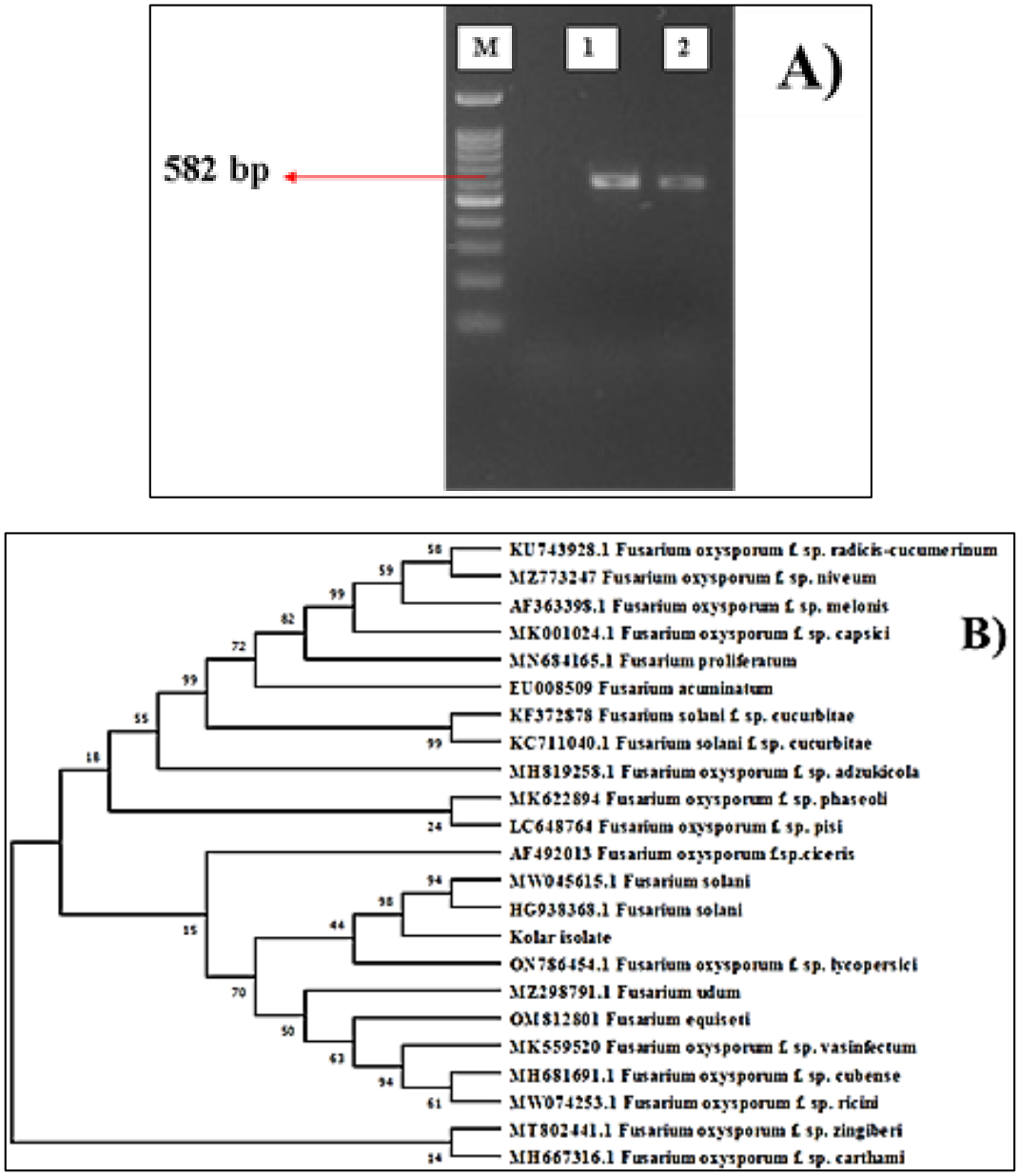


Plate 3: a) Polymerase chain reaction products of fungi samples with ITS 1 and ITS4, M: 100 bp Ladder, 1 and 2: DNA isolated from *F. solani* (Mart.) Sacc. b) Dendrogram showing the phylogenetic relationship *F. solani* (Mart.) Sacc. Kolar isolate with the selected GenBank accession

Efficacy of fungicides against the fungal pathogen

In vitro fungicide screening is a useful tool for evaluating a large number of fungicides at various concentrations. The laboratory evaluation of fungicides using the poison food technique showed significant results for several fungicides evaluated at different concentrations in the present study. Fifteen fungicides were tested at six different concentrations: 500, 1000, 1500, 2000, 2500 and 3000 ppm. On the basis of fungal growth in test plates, the percent inhibition over control was calculated. The resulting data is provided in the table below (Table 2). Efficacy of fungicides is increased with the increased concentration of fungicide (3000 ppm).

Five systemic fungicides were tested against *F. solani* (Mart.) Sacc., among which tebuconazole 25.9% W/W gave the best result by maximum inhibition of 98.88 percent at 3000 ppm (Table 2, Plate 4a), which was significantly superior to all other fungicides and the least inhibition of the mycelial growth was in azoxystrobin (42.22%). Among the contact fungicides tested, captan recorded considerable highest mycelial inhibition of 64.07, 67.40, 77.77, 82.96, 84.07 and 86.29 percent inhibition at 500, 1000, 1500, 2000, 2500 and 3000 ppm respectively, followed by zineb of about 55.55, 67.40, 69.63, 71.48, 75.55 and 75.18 percent inhibition at 500, 1000, 1500, 2000, 2500 and 3000 ppm respectively while least percent inhibition was observed in case of chlorothalonil 57.40 percent inhibition at 3000 ppm (Table 2, plate 4b). Among the combination products, carbendazim 25% + mancozeb 50% showed 86.29, 90.00, 92.22, 93.70, 95.18 and 96.29 percent inhibition at 500, 1000, 1500, 2000, 2500 and 3000 ppm respectively, followed by captan 70% +

hexaconazole 5% WP of about mycelial growth inhibition of 94.44 percent inhibition at 3000 ppm. The least percent inhibition of 75.92 percent was showed by hexaconozole 4% + zineb 68% recorded of at 3000 ppm (Table 2 and Plate 5).

Among all three groups of fungicides, systemic fungicides were found to be more effective than contact and combination fungicides. Among all fungicide tested tebuconazole 25.9% WW maximum mean inhibition of 97.74 percent followed by propiconazole 25% EC 97.40 percent mean inhibition, while least percent mean inhibition (28.70%) was observed in case of copper hydroxide 53.8% DF as compared to control. There was positive correlation between concentration and inhibition of growth of pathogen. It is inferred from the data presented in Table 2 and Fig. 1 that all the fungicides are significantly effective in inhibition of test pathogen. The results of present investigation have similar findings with the earlier records of Ravichandran and Hegde (2015) [15] tested for four systemic fungicides against Fusarium wilt in chickpea and among all fungicides carbendazim and tebuconazole inhibited complete mycelial growth of pathogen followed by hexaconazole (88.61%). Golkiya *et al.* (2018) [6] tested six systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri.* Tebuconazole inhibited highest mycelial growth (89.19%) followed by carbendazim (67.30%). Tebuconazole (97.74% inhibition) belongs to the triazole fungicide. The other scientists like group and produced the best results among all fungicides tested, making it the best fungicide for Fusarium wilt.

In vitro efficacy of biological organisms against the fungal pathogen

In Table 3 an interactions of *F. solani* (Mart.) Sacc. with three fungal antagonists and two bacterial antagonists were studied under laboratory conditions by inoculating the culture on the opposite ends of Petri plates containing PDA medium as described under material and methods. The results of the study indicated that all the antagonists significantly inhibited the growth of *Fusarium solani*. The percent inhibition *Fusarium solani* ranged from 64.07 to 86.29 percent. The maximum inhibition was observed in *T. viride* (86.29%) followed by *Psedomonas fluorescens* (72.22%) as shown Table 3 and plate 6.

Table 3: *In vitro* evaluation of bio control agents against *F. solani* (Mart.) Sacc.

Sl. No.	Bioagents	Growth of the pathogen (mm)*	Growth of the antagonists (mm)**	% Inhibition of colony growth over control
1	*Trichoderma viride*	12.33	77.66	86.29(68.31) ***
2	*Pseudomonas fluorescens*	25.00	65.00	72.22 (58.24)
3	*Aspergillus niger*	26.66	63.33	70.37(57.01)
4	*Bacillus subtilis*	30.33	59.66	66.29(54.49)
5	*Paecilomyces lilacinus*	57.66	32.33	64.07(53.16)
	Control	90		
	S.Em ±			1.995
	CD at 1%			8.939

***** Average diameter of pathogen ** Average diameter of antagonists.
***Figures in parenthesis are arc sine transformed values.

Table 2: *In vitro* evaluation of fungicides against *F. solani* (Mart.) Sacc. causing *Fusarium* wilt.

	Sl. No.	Fungicide	Percent inhibition of mycelial growth Concentration						
			500ppm	1000ppm	1500ppm	2000ppm	2500ppm	3000ppm	Mean
I	1	Metalaxyl 4%+Mancozeb 64% WP	60.00 (50.77)	64.07 (53.16)	68.51 (55.87)	74.07 (59.39)	75.18 (60.13)	80.37 (63.71)	70.37
	2	Hexaconazole 4%+Zineb 68%	51.85 (46.04)	53.33 (46.89)	55.55 (48.17)	59.25 (50.35)	62.96 (52.53)	75.92 (60.62)	59.81
	3	Captan 70% + Hexaconazole 5% WP	80.00 (63.74)	85.18 (67.61)	88.88 (70.50)	91.11 (72.71)	92.22 (74.33)	94.44 (76.38)	88.64
	4	Tricyclazole 18% + Mancozeb 62% WP	05.55 (13.17)	16.66 (23.88)	25.92 (30.49)	51.85 (46.04)	54.07 (47.32)	88.88 (70.50)	40.49
	5	Carbendazim 25% + Mancozeb 50%	86.29 (68.31)	90.00 (71.55)	92.22 (73.77)	93.70 (75.45)	95.18 (77.31)	96.29 (78.89)	92.28
II	6	Captan 50% WP	64.07 (53.16)	67.40 (55.16)	77.77 (61.86)	82.96 (65.59)	84.07 (66.45)	86.29(68.31)	77.09
	7	Copper hydroxide 53.8% DF	07.40 (15.57)	14.07 (21.98)	19.63 (26.25)	28.51 (32.22)	31.48 (34.09)	71.11(57.47)	28.70
	8	Chlorothalonil 78% W/W	12.59 (20.65)	19.25 (25.96)	23.70 (28.86)	50.74 (45.40)	52.59 (46.47)	57.40(49.02)	43.33
	9	Mancozeb 75% WP	10.37 (18.75)	16.66 (24.01)	37.77 (37.87)	51.11 (45.61)	64.44 (53.41)	79.63(61.85)	72.46
	10	Zineb 75% WP	55.55 (48.23)	67.40 (55.16)	69.63 (56.54)	71.40 (57.73)	75.55 (60.37)	75.18(60.11)	29.38
III	11	Azoxystrobin 23% SC	21.11 (27.27)	23.70 (29.07)	24.07 (29.37)	29.25 (32.72)	35.92 (36.80)	42.22(40.50)	97.74
	12	Tebuconazole 25.9% WW	96.66 (79.90)	97.03 (80.19)	97.40 (80.93)	97.77 (81.87)	98.70 (83.46)	98.88 (83.91)	74.75
	13	Hexaconozole 5% SC	64.07 (53.16)	74.07 (59.47)	74.07 (59.39)	77.77 (61.96)	78.88 (62.69)	79.63(63.19)	84.44
	14	Thiophanate methyl 70% WP	74.07 (57.18)	80.00 (60.25)	82.96 (65.66)	84.07 (66.46)	92.96 (74.65)	98.51(83.07)	97.40
	15	Propiconazole 25% EC	93.70 (75.45)	97.40 (78.07)	97.40 (80.74)	98.51 (83.07)	98.51 (83.07)	98.86 (83.91)	43.33
		Mean	54.22	57.35	62.37	69.48	72.85	81.58	
			S. Em±		CD @ 1%				
		Fungicide (F)	0.950		3.461				
		Concentration (C)	0.601		2.189				
		F X C	2.327		8.477				

Values in parenthesis are arc sine transferred values. Whereas **i:** Combination fungicide **ii:** Contact fungicide, **iii:** Systemic fungicide

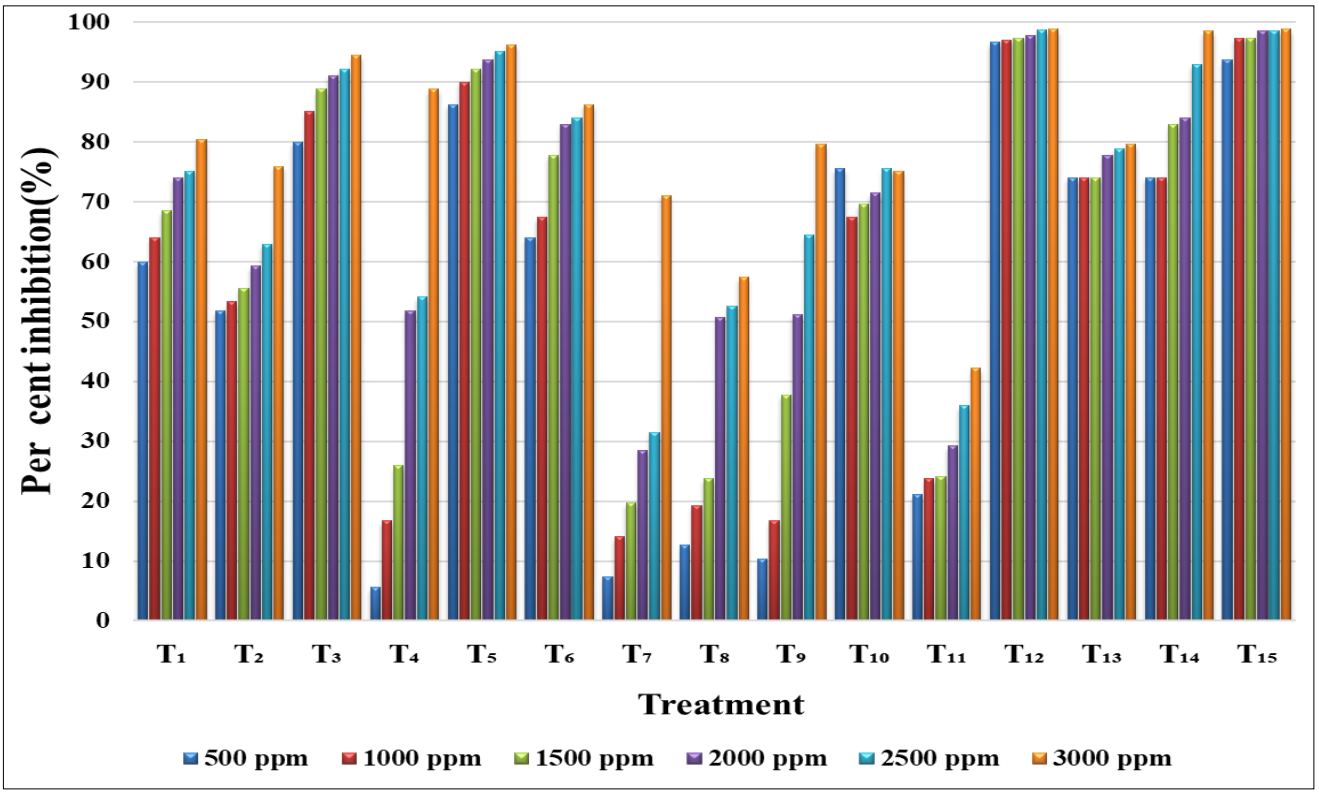


Fig 1: *In vitro* evaluation of bio control agents against *F. solani* (Mart.) Sacc.

Treatments

T_1 -Metalaxyl 8% +Mancozeb 64% WP , T_2- Hexaconozole 4%+Zineb 68% T_3 - Captan 70% + Hexaconazole 5% WP, T_4 -Tricyclazole 18% + Mancozeb 62% WP, T_5- Carbendazim 25% +Mancozeb 50%, T_6- Captan 50% WP, T_7 - Copper hydroxide 53.8% DF, T_8- Chlorothalonil 78% W/W, T_9- Mancozeb 75% WP, T_{12} - Tebuconozole 25.9% EC, T_{13}- Hexaconazole 5% SC, T_{14} – Thiophenate methyl 70% WP, T_{15}- Propinconozole 25% EC.

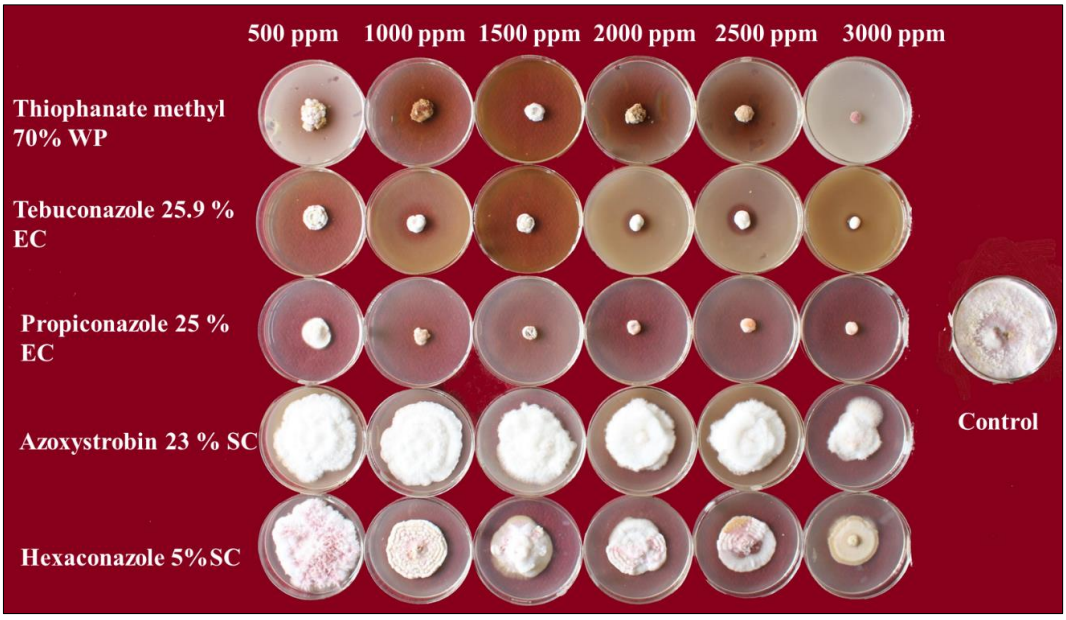


Plate 4a: *In vitro* evaluations of systemic fungicides against *F. solani* (Mart.) Sacc.

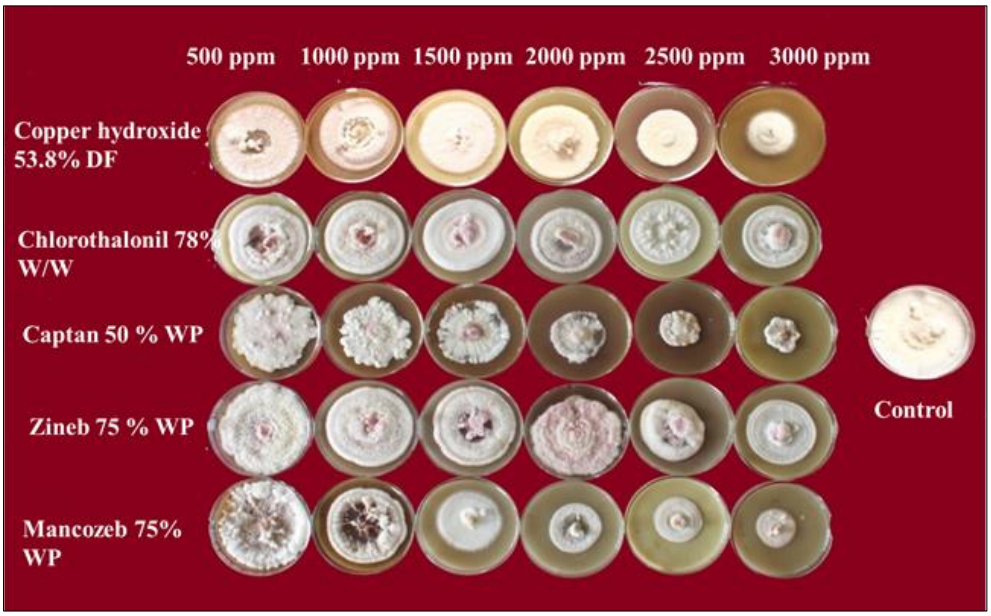


Plate 4b: *In vitro* evaluations of contact fungicides against *F. solani* (Mart.) Sacc

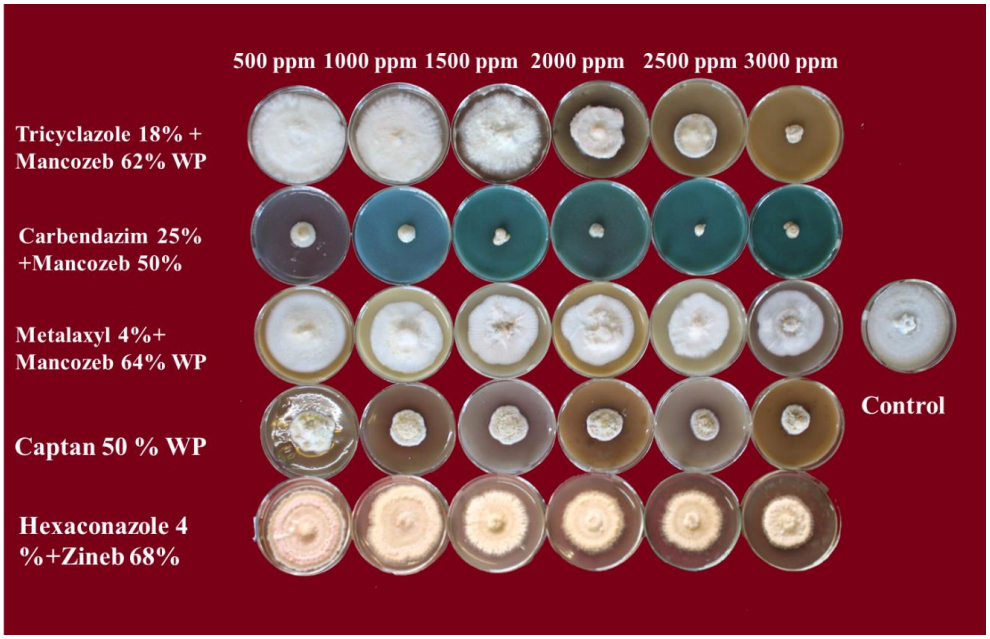


Plate 5: *In vitro* evaluations of combination fungicides against *F. solani* (Mart.) Sacc.

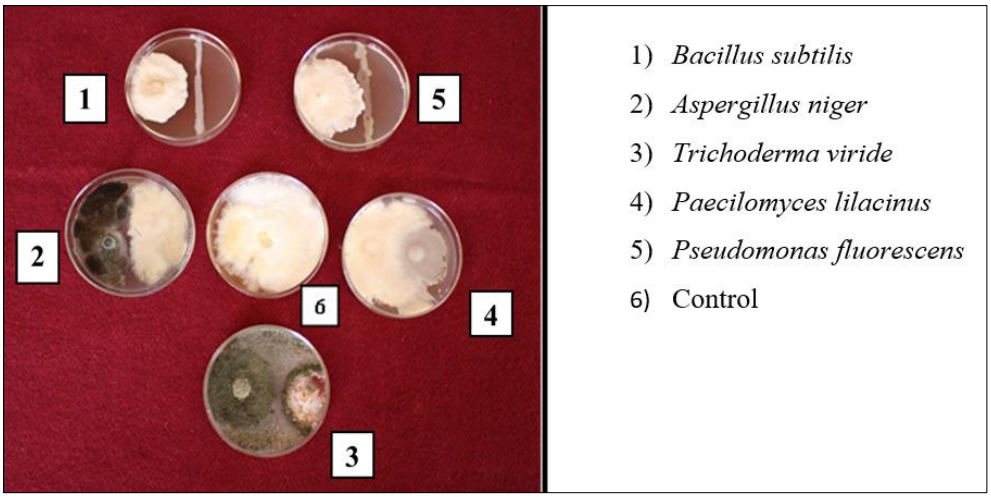


Plate 6: *In vitro* evaluation of bioagents against the *F. solani* (Mart.) Sacc.

In the present investigation, *Trichoderma viride* showed the highest reduction in colony growth of *Fusarium solani* (Mart.) Sacc. when compared to all other bio-agents, followed by *Pseudomonas fluorescens*. *Paecilomyces lilacinus* inhibited least against *Fusarium solani* (Mart.) Sacc. and *Trichoderma* species inhibited the organism more when compared to bacterial antagonists. The current findings were consistent with those of Shrivastava and Agrawal (2010) [17], Kala *et al*. (2016) [7] and Pushpavathi *et al*. (2016) [14] reported that *Trichoderma* spp. was the most effective against *Fusarium* wilt disease in crop plants. The current study's findings on the antagonistic effects of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium oxysporum* are consistent with previous findings Amara *et al*. (1996) [3].

Conclusion

Fusarium sp. was isolated from root and stem of naturally diseased muskmelon plants grown in Kolar region of Karnataka. Based on the morphological characteristics, the isolate was identified as *F. solani* (Mart.) Sacc. From the pathogenicity test *F. solani* (Mart.) Sacc. was identified as the major causal agent of wilt in muskmelon. PCR-ITS primers analysis used in this study a convenient tool for characterization and analyzing variations of *Fusarium* spp. This study also explored *in vitro* efficacy of several bio-control agents and fifteen commercial fungicides against *F. solani*. This study could be the basis for field trials to find out the efficacy of these tested bio-control agents and fungicides to manage Fusarium wilt disease in muskmelon.

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