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Management of fungal wilt of tomato by using different formulations of bio-control agent

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A pot culture experiment was conducted at the Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar during the year 2023-2024 to manage the fungal wilt of tomato caused by *Fusarium oxysporum* by using different formulations of *Trichoderma asperellum* Kjr OUAT strain. For mass multiplication of native *Trichoderma asperellum*Kjr strain using eight solid substrates viz. rice grain, wheat grain, broken maize grain, dry cowdung powder, vermicompost, spent mushroom straw, rice, husk and wheat husk, Rice grain was the most effective substrate which recorded CFU count of 160.50×10^7/g , while broken maize grain was the least effective with CFU of 69.50×10^7/g in the first month after inoculation. In pot cultures, eight combination treatments of bioagent and fungicides were applied to tomato plants among which soil application of vermicompost-based *Trichoderma asperellum* two days after pathogen inoculation and subsequent drenching of rice grain based*Trichoderma asperellum*at ten and eighteen days after inoculation was found to be the most effective of all. It resulted in the longest shoot length (29.83 cm) and root length (7.1 cm), highest root mass (0.19 g), average leaf width (2.56 cm), increased leaf number compared to the untreated control and also highest CFU count of rhizosphere soil at the end of the experiment.

Keywords : CFU count, formulations, substrates, serial dilution, tomato, wilt

INTRODUCTION

*Solanum lycopersicum*L., or cultivated tomato, is the most consumed vegetable worldwide, essential in many dishes. Originating from wild varieties in South America, tomatoes are now grown globally to meet both local and international demands (Peralta *et al*. 2008). It is known as the “Poor Man’s Orange,” and thrives in warm climates. In 2022, global production reached approximately 186.82 million tonnes, with China and India as top producers (FAOSTAT 2022). In India, Madhya Pradesh leads production, followed by Andhra Pradesh and Karnataka (NHB 2021-22). It is rich in water, vitamins, and antioxidants, tomatoes support health and can reduce disease risks.

They are consumed fresh, cooked, or processed into products like ketchup, with waste that can be repurposed (Kiralan*et al*. 2022). Tomato crop is infected by various diseases, notably Fusarium wilt, which can cause 25-55% yield loss (Nirmaladevi *et al*. 2016). While fungicides are common, the emergence of resistance and environmental concerns have led to interest in sustainable alternatives, such as biocontrol agents like *Trichoderma* spp., which enhance plant health and growth (Naher *et al*. 2014).

The Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* is a soil-borne fungus which infects tomato crop, producing symptoms like stunted growth, leaf yellowing and wilting and brown discoloration of the xylem vessels, which may appear as lines when the stem is cut lengthwise or as dots when cut across. Other indicators are white, pink, or orange fungal growth on the surface of affected stems, particularly in damp conditions, along with root or stem decay. While the plant is alive, the vascular wilt fungus stays strictly within the xylem tissues and a few nearby cells. The fungus only spreads

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into the surrounding parenchymatous tissue and starts producing spores extensively on the plant's surface once the plant has died from the disease. As a result, *F. oxysporum* occupies a highly specific ecological niche. Fusarium wilt is a serious disease as it causes severe loss of yield. So, for the management of the disease, different formulations of biocontrol agents are suggested instead of chemical pesticides. Singh *et al.*(2017) assessed 6 fungicides (w/v) like carbendazim, captan, propiconazole, carbendazim + mancozeb, captan + hexaconazole and carboxin + thiram against *Fusarium oxysporum* which revealed that carbendazim at 50% concentration and a combination of 12% carbendazim with 63% mancozeb were particularly effective in inhibiting mycelial growth and sporulation while captan at 50% concentration demonstrated the lowest effectiveness among the fungicides evaluated. Bhujbal *et al.*(2020) reported that *Trichoderma viride* was the most effective biocontrol agent against *Fusarium oxysporum* f. sp. *lycopersici*, inhibiting growth by 84.84%. This was followed by *T. harzianum*(72.54%), *T. hamatum* (69.93%), and *T. koningii*(61.49%). Additionally, *Bacillus subtilis* and *Pseudomonas fluorescens* inhibited growth by 79.25% and 62.36%, respectively. Katyayani *et al.*(2019) found that maximum inhibition in growth (54.73%) against the tested isolate of *Fusarium oxysporum*f.sp. *lycopersici*was shown by the *Trichoderma harzianum.* A large variety of volatile secondary metabolites could be produced by *Trichoderma* spp. such as ethylene, hydrogen cyanide, aldehydes and ketones, which might play an important role in controlling various plant pathogens. Singh *et al.*(2014) recorded that at 30 days after inoculation (DAI), the highest population of *T. harzianum*T5 was in sorghum grain (64.0 x 10^7cfu/g), followed by SMC (Spent mushroom compost) (53.0 x 10^7cfu/g) and wheat grain (51.0 x 10^7cfu/g). By 180 DAI, populations in sorghum, wheat, and broken maize grains dropped to undetectable levels at a 10^{-7} dilution. In contrast, SMC maintained a significantly higher population than vermicompost and farmyard manure at both 180 and 240 DAI.

Naeimi *et al* (2020) observed that the fungus colonized nearly all substrates within a month, with the highest levels in broom sorghum grain, rice husk, rice straw, rice bran, and sugar beet pulp. Broom sorghum grain and rice straw recorded the highest populations (6.4×10^{10} and 5.3×10^{10} CFUs g^{-1}). After a year at room temperature, populations were found to be declining in all substrates. Thus, in the current study, it was attempted to analyse the effectiveness of different chemical and biological techniques to manage the fusarium wilt of tomato with the following objectives of isolation, purification and identification of fungal pathogen causing wilt in tomato, preparation of different formulations of native *Trichoderma asperellum* and field management of fungal wilt of tomato by different formulations of biocontrol agent *Trichoderma asperellum*Kjr OUAT strain.

MATERIALS AND METHODS

Collection and isolation of samples

In the present study, the infected plant samples were collected from All India Coordinated Research Project on Vegetables, OUAT Bhubaneswar (20.27°N,85.78°E). Before inoculating the diseased plant parts in petri plates, they were treated with a 0.1% sodium hypochlorite solution for 30 seconds, followed by 2-3 washes with sterile water. The infected tomato plant segments were then placed on petriplates containing Potato Dextrose Agar media The cultured plates were stored in an incubator at room temperature (around 27 ± 1°C). The cut segments were transferred aseptically to culture slants and maintained at room temperature. Throughout these procedures, strict aseptic techniques were maintained to prevent contamination and the growth of the target organism was regularly monitored.

Cultural and morphological identification of fungus

The organism of interest i.e. *Fusarium oxysporum* was isolated and identified based on its morphological characteristics. Purity was achieved through continuous sub-culturing, typically repeated 5 to 6 times, to ensure the purity and stability of the isolated organism. The pathogen's morphological identification involved examining hyphal growth and fungal structures under a microscope.

The cultured fungus was placed on a slide and observed at 40X magnification, noting characteristics like mycelia, fruiting bodies, conidiophores, and conidia. Reference cultures and monographs were used for taxonomic classification. The culture was also submitted to Indian Type Culture Collection (ITCC) ICAR, New Delhi for identification and was confirmed to be *Fusariumoxysporum*.

Pathogenicity test

Pathogenicity test for the isolated fungus from the infected tomato plant samples were conducted by inoculating in healthy tomato seedlings of variety BT-19-1-1-1 obtained from AICRP on vegetables, OUAT, Bhubaneswar. five weeks old tomato seedlings were inoculated at the basal collar region using conidial and mycelial suspension. A conidial/mycelial suspension (10^6 ml) was created by scraping and blending a seven-day-old culture grown on PDA medium, while control was inoculated with only distilled water. The inoculation was carried out using the pin prick method. The entire seedling was wrapped in polythene to maintain humidity and holes were made. All seedlings were incubated at 27±1°C for 10 days. Pots were regularly watered and checked for wilt symptoms. After the characteristic symptoms were fully expressed, the fungus was isolated from the and compared with the original which was isolated at first.

Preparation of different formulations of bio-agent

Bottles meant for spawn production (mushroom seed) were used for mass multiplication of *Trichoderma*. These were collected in bulk from local vendors and were thoroughly washed with detergent and air-dried. Three types of cereal grains were used in this experiment namely wheat (*Triticum aestivum)*, rice (*Oryza sativa*) and broken maize grain (*Zea mays*). Other solid substrates used were rice husk, wheat husk, dry cowdung powder, vermicompost and spent mushroom straw. The grains were washed and partially boiled according to their size and seed coat thickness. The wheat grains were boiled for 15 minutes, rice grains for 10 mins and broken maize grain for 30 mins. After draining, they were shade-dried to retain approximately 60% moisture. They were then mixed with chalk powder (calcium carbonate) at a ratio of 30g per kg to prevent clumping and ease the filling process. In each bottle, 200g grains were filled. The grain level in the bottles was kept below the neck for proper aeration, and the openings were sealed with non-absorbent cotton to avoid contamination. Other solid substrates like 200g each of rice husk, wheat husk, dry cowdung powder, vermicompost and 100g of spent mushroom straw substrate were added to the bottles along with sterile water to maintain about 60% moisture, which were also sealed with cotton. The bottles containing substrates were steam sterilized in an autoclave at 121.6°C (15 p.s.i) for about 20 minutes.

Isolates which were collected previously were inoculated onto PDA plates using the hyphal tip method and incubated at 26.5°C for 10 to 12 days. Afterward, 5mm sized discs of *Trichoderma asperellum*Kjr strain were cut from the plates with a cork borer, and the bottles were inoculated aseptically with one disc in each bottle. Potato Dextrose Rose Bengal Agar medium was prepared and then poured into glass petri dishes at 15 ml each and left to solidify undisturbed. One ml of substrate suspension was added to a test tube with 9 ml of sterile water for a 10^{-2} dilution and this process was repeated five times to achieve a final dilution of 10^{-7}. One milliliter from this solution was spread onto petri plates containing potato dextrose rose Bengal agar using a sterile glass spreader. This procedure was repeated monthly upto three months for taking reading of CFU count of the different formulations of *Trichoderma asperellum*Kjr strain.

Evaluation of disease management using different formulations of bioagent

Management studies were conducted in an experiment that was carried out in 5 week old tomato seedlings of BT-19-1-1-1 variety which were raised in a controlled environment in a portray and then transplanted to polybags. The test pathogen was stem inoculated at the collar region of the seedlings by pricking with a pin and applying conidial suspension of a seven day old culture of the test pathogen. The seedlings were then covered in polythene bags with holes for aeration. After two days of artificial inoculation of

pathogen in the seedlings, different treatments were applied for further studies as follows:

Treatments

Various observations like wilting percentage, height of plant, number of leaves, average width of leaf, root length and root mass were made at the end of the experiment i.e. after 30 days.

Tr no.	Treatments
T1	Soil application of dry cowdung based *Trichoderma asperellum*
T2	Drenching with rice grain based*Trichoderma asperellum*2, 10 and 20 days after inoculation
T3	Soil application of vermicompost based *Trichoderma asperellum*
T4	Soil application of wheat husk based*Trichoderma asperellum*
T5	Soil drenching with Metalaxyl+Mancozeb 0.2% @ 50ml/plant
T6	Soil drenching with Carbendazim+Mancozeb 0.2% @50ml/plant
T7	Soil application with vermicompost based *Trichoderma asperellum*2 days then subsequent soil drenching with rice grain based*Trichoderma asperellum*10 and 18 days after inoculation.
T8	Untreated control

CFU count of rhizosphere soil

One gram of rhizospheric soil sample was collected from each treatment after 30 days and added in a culturetube having 9 ml distilled sterile water. The soil sample was mixed well and diluted up to 10^{-7}dilution.. 1 ml from this soil suspension was poured on a petriplate containing potato dextrose rose bengal agar medium and spread over it by a sterile glass spreader. Petriplates were kept in an incubator at a temperature of 25 $\pm$°C for 72hrs.The petriplates were examined toobserve colonies of *T. asperellum* counted using with a colony counter. Colony forming unit was calculated using formulae as follows :

$$\text{CFU /ml} = \frac{\text{Number of colonies x Dilutionfactor}}{\text{Volume of culture plate}}$$

Statistical analysis

Data collected from various treatments were subjected to statistical analysis using established methods. When the treatment effects were determined to be statistically significant, the corresponding standard error (S.E.) and critical difference (C.D.) were calculated at a 5% significance level.

RESULTS AND DISCUSSION

Naturally infected samples of tomato were collected from AICRP on Vegetables, OUAT, Bhubaneswar which had a 14% disease incidence. The pathogen was isolated from the infected plant samples after surface disinfection and the pathogen was tentatively identified as *Fusarium oxysporum* at the Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar with the help of a compound microscope and under the guidance of the advisor, referencing relevant monographs and literature to identify the specific species of

Table 1: Mean monthly CFU in solid substrates

Tr. No.	Treatments	1st Month (cfu×10$^{7)}$/g*	2nd Month (cfu×10$^{7)}$/g*	3rd Month (cfu×10$^{7)}$/g*
T1	Rice husk	72.00	70.50	53.50
T2	Broken maize grain	69.50	48.50	47.50
T3	Vermicompost	126.00	94.00	87.50
T4	Spent paddy straw	106.00	107.00	91.50
T5	Wheat husk	128.50	114.00	77.00
T6	Rice grain	160.50	129.00	109.00
T7	Wheat grain	120.50	103.00	93.00
T8	Dry cowdung powder	135.00	125.50	100.00
	S.E.(m)±	3.46	3.09	2.47
	C.D. (5%)	11.29	10.10	8.07

*Average of three replications

the fungus. The pathogen was also identified as *Fusarium oxysporum*(ITCC I.D. No. 12077.24) at Indian Type Culture Collection (ITCC), IARI and as it caused wilt in tomato, it was tentatively identified as *Fusarium oxysporum*f.sp. *lycopersici*. The pathogen obtained from the infected tomato samples formed white tinged with light pink, fluffy and cottony, radial colonies with aerial mycelium which covered the petri plate completely. The underside of the petri plate also exhibited light pink colour which turned to dark pink or purple when the culture turned old. Under microscopic observation, it showed the presence of three types of spores: microconidia, macroconidia and chlamydospores. The hyphae of the fungus were branched, hyaline (colorless), and septate, with an average diameter of approximately 3.37ìm. Microconidia were oval to oblong, single- celled structures, measuring 4.51 x 2.18ìm, and they originated from simple phialides on the hyphae or from sparsely branched conidiophores. The macroconidia were typically straight or slightly curved, tapered towards both ends, and attached by a slender stalk, primarily consisting of three septa. On average, they measured 14.2×3.9ìm. The microconidia were abundantly present in the culture while the macroconidia were sparse in number. Chlamydospores were spherical and with average size of 6.2 ×7.6ìm. They were found at the end or within the hyphal segments (Fig. 1). The findings of the cultural and morphological characteristics of the pathogen were in close confirmity with those made by Sonkar *et al.*(2013), Isaac *et al.*(2018) and Poussio*et al* (2023)

The pathogen was inoculated into healthy BT-19-1-1-1 tomato seedlings. After covering the plants with perforated polythene for eight days, symptoms appeared: yellowing of older leaves, collar rot, and wilting, observed 10 days post-inoculation. The control seedlings remained healthy. A cross-section of the infected plants showed brown discoloration in the vascular bundles. Re-isolation and identification confirmed the pathogen's characteristics, fulfilling Koch's postulates. These observations were similar to the symptomatology observed by Isaac *et al.*(2018) and Nirmaladevi *et al.*(2016).

Eight solid substrates were evaluated for mass multiplication of native *Trichoderma asperellum*Kjr strain from OUAT, Bhubaneswar, with CFU counts taken monthly for three months as shown in 1. Rice grain recorded the highest CFU count, averaging 160.50×10w in the first month, followed by 129.00×10w and 109.00×10w in the subsequent months, making it the best substrate. Dry cow dung powder had the second highest counts: 135.00×10w , 125.50×10w , and 100.00×10w , while wheat husk followed with counts of 128.5×10w , 114.00×10w , and 77.00×10w . Vermicompost and wheat grain also performed well, while spent paddy straw and rice husk showed lower counts. Rice husk had the lowest overall counts, with broken maize grain slightly higher. Rice grain proved to be the most effective substrate, maintaining higher spore

Table 2: Evaluation of management studies using different formulations of bioagent

Tr No.	Treatment	Wilting percentage (%)*	Shoot length (cm)*	Root length (cm)*	No. of leaves*	Avg width of leaves (cm)*	Root mass (g)*
T1	Soil application of dry cowdungbased *Trichoderma asperellum*	14.76 (4.33)	17.16	5.56	29.66	1.55	0.124
T2	Drenching with rice grain based *Trichoderma asperellum* at 2, 10 and 20 days	18.50 (4.79)	6.73	3.7	6.66	1.04	0.012
T3	Soil application of vermicompost based *Trichoderma asperellum*	12.66 (4.05)	29.63	6.4	27.66	2.16	0.184
T4	Soil application of wheat husk based *Trichoderma asperellum*	20.56 (5.03)	20.73	6.2	26.66	2.26	0.251
T5	Soil drenching with Metalaxyl+Mancozeb 0.2% @ 50ml/plant	14.66 (4.32)	20.76	5.13	27.33	1.76	0.129
T6	Soil drenching with Carbendazim+Mancozeb 0.2% @50ml/plant	15.86 (4.47)	26.83	4.43	20.66	2.03	0.189
T7	Soil application with vermicompost based *Trichoderma asperellum* 2 days then subsequent drenching with rice grain based *Trichoderma asperellum* after 10 and 18 days	10.83 (3.78)	29.86	7.1	29.66	2.56	0.193
T8	Untreated control	52.16	5.33	1.8	5.33	1.06	0.009
	SE (m) +	0.27	0.54	0.12	0.56	0.03	0.002
	CD (5%)	0.82	1.62	0.36	1.69	010	0.006
	CV	4.59%	4.78%	4.20%	4.50%	3.37%	2.66%

*Average of three replications

Table 3 : CFU count of rhizosphere soil applied with different formulations of bioagent

Tr no.	Treatments	CFU count (cfu×10^7)/g*
T1	Soil application of dry cowdung based *Trichoderma asperellum*	10.66
T2	Drenching with rice grain based*Trichoderma asperellum*2, 10 and 20 days after inoculation	7.66
T3	Soil application of vermicompost based *Trichoderma asperellum*	11
T4	Soil application of wheat husk based*Trichoderma asperellum*	9.66
T5	Soil drenching with Metalaxyl+Mancozeb 0.2% @ 50ml/plant	0
T6	Soil drenching with Carbendazim+Mancozeb 0.2% @50ml/plant	1.66
T7	Soil application with vermicompost based *Trichoderma asperellum*2 days then subsequent soil drenching with rice grain based*Trichoderma asperellum*10 and 18 days after inoculation.	14.33
T8	Untreated control	2
	SE(m)	0.03
	CD (5%)	0.11
	CV	1.01%

*Average of three replications

viability through the third month (Table 1 &Fig.2). Mustafa *et al* (2009) also evaluated wheat bran and rice husk which were deemed effective as substrates. Patel and Singh (2021) reported that rice grain gave appreciable growth of *Trichoderma* which coincides with the result obtained in the present study. The result in present study is consistent with Pandya *et al* (2018) who reported wheat grain alongwith dry cowdung, vermicompost and paddy straw proved better sources for mass multiplication of *Trichoderma.* The population and shelf life of *Trichoderma* was excellent in vermicompost which is in close confirmity with that reported by Bora *et al* (2010). The lowest cfu/g was recorded in broken maize grain in the present study which aligns with that of Dhruw*et al* (2023) who reported minimal growth of *Trichoderma* in crushed maize seed+straw substrate.

The effectiveness of different formulations of *Trichoderma asperellum*Kjr strain was compared with chemical fungicides (w/v) for the management of fungal wilt of tomato in pot culture experiment. Various parameters were observed viz. wilting percentage, shoot length, root length, number of leaves, average width of leaves, root mass and CFU count of rhizosphere soil of each treatment. The fungal pathogen was inoculated at the stem of 5 week old tomato seedlings of BT-19-1-1-1 variety by pin-prick method and all the treatments were applied two days after

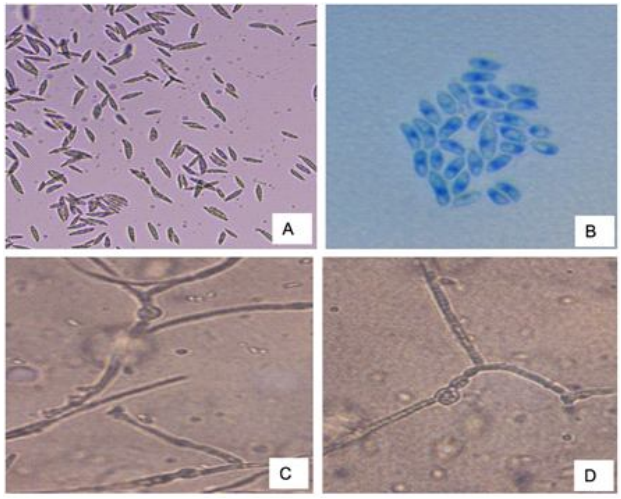

Fig 1: Microphotographs of *Fusarium oxysporum*fsp. *Lycopersici*. Macroconidia (A), Microconidia (B) and Chlamydospores (C& D)

dry cow dung-based *Trichoderma* (14.76%), rice grain-based *Trichoderma* drenching (18.5%), and wheat husk-based *Trichoderma* (20.56%). The untreated control had a wilt incidence of 52.16%(Table 2 & Fig.3). The inhibitory effect of the fungal biocontrol agent might be due to antibiosis and/ or competition. Out of eight treatments, maximum shoot length was recorded in soil application with vermicompost based *Trichoderma asperellum* and subsequent drenching with rice grain based *Trichoderma asperellum* with average of 29.86cm, followed by soil application of vermicompost based *Trichoderma asperellum* with 29.63cm, drenching with carbendazim +mancozeb with 26.83cm, soil drenching with metalaxyl +mancozeb with 20.76cm, soil

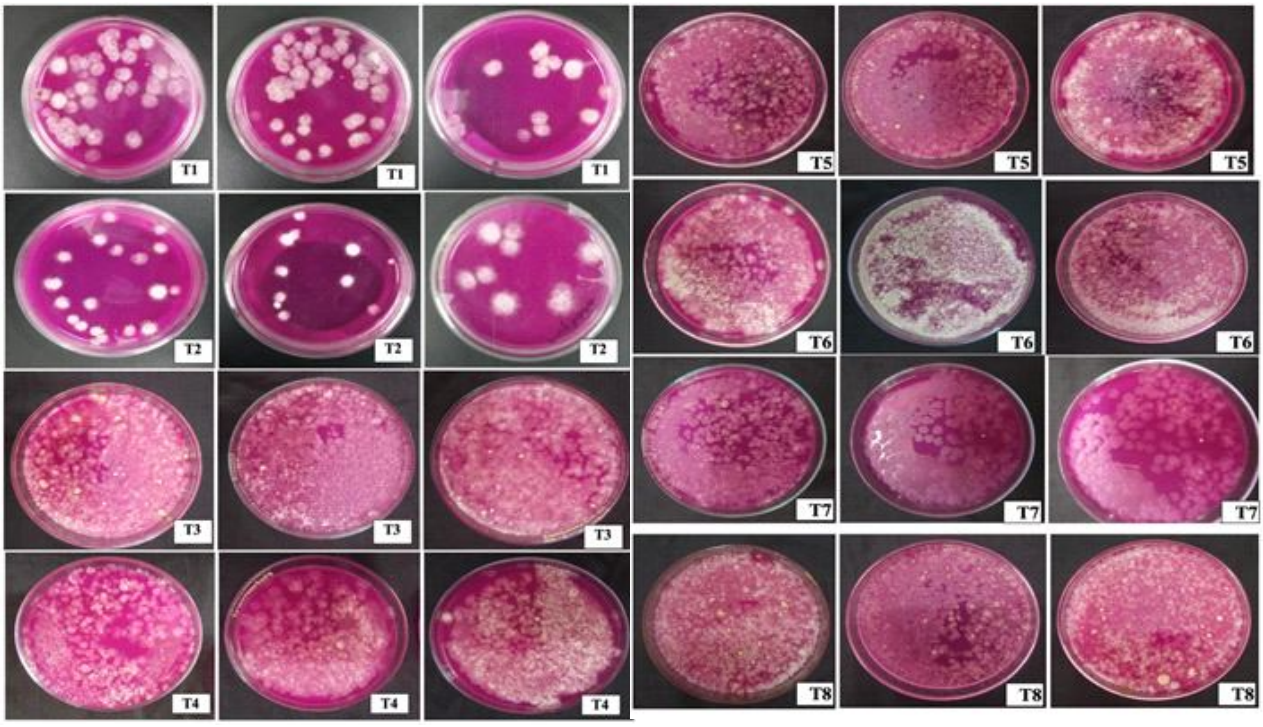

Fig. 2 : CFUcountofdifferentsubstrates(T1- Rice husk; T2- Broken maize grains; T3-Vermicompost:T4- Spent paddy straw: T5- Wheat husk; T6- Rice grain; T7- Wheat grain & T8- Dry cow dung powder) inoculatedwith *Trichodermaasperellum*in 1st(1st and 4th column), 2nd(2ndand 5th column) and 3rd(3rd and 6th column) month of inoculation

inoculation. The observations were recorded when the plants were two months old.

The lowest wilt incidence among the eight treatments was 10.83% with soil application of vermi compost-based *Trichoderma asperellum* followed by drenching with rice grain-based *Trichoderma* (12.66%). Other treatments included drenching with metalaxyl+mancozeb (14.66%), carbendazim+mancozeb (15.86%), application of wheat husk based *Trichoderma asperellum* with 20.73cm, soil application of dry cowdung based *Trichoderma asperellum* with 17.16cm. The least shoot length was recorded in soil drenching with rice grain based *Trichoderma asperellum* with an average of 6.73cm as compared to untreated control with 5.33cm.

Out of eight treatments, maximum root length

Fig.3 : Evaluation of management studies using different formulations of bioagent

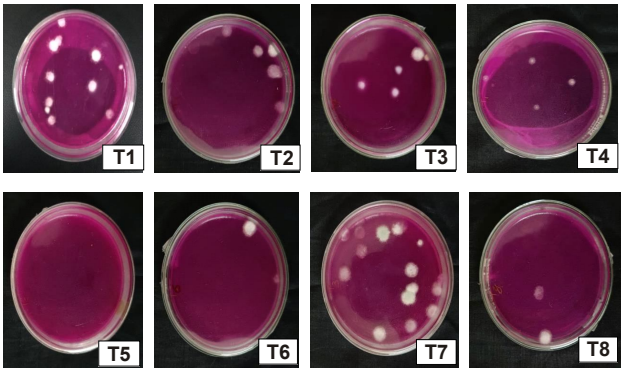

Fig. 4 : CFU count of rhizospheres oil applied with different formulations of bioagent

was recorded in soil application with vermicompost based *T. asperellum* and subsequent soil drenching with rice grain based *T. asperellum* with 7.1cm followed by soil application of vermicompost based *T. asperellum* with 6.4cm, soil application of wheat husk based *T. asperellum* with 6.2cm, soil application of dry cowdung based *T. asperellum* with 5.56cm, soil drenching with metalaxyl+mancozeb with 5.13cm, soil drenching with carbendazim +mancozeb with 4.43cm. The least root length was recorded in soil drenching with rice grain based*T. asperellum* with 3.7cm as compared to control with 1.8cm.

Out of eight treatments, highest average number of leaves were recorded in soil application with vermicompost based *T. asperellum* and subsequent soil drenching with rice grain based *T. asperellum* and soil application of dry cowdung based *T. asperellum* with 29.66, followed by soil application of vermicompost based *T. asperellum* with 27.66, soil drenching with metalaxyl + mancozeb with 27.33, application of wheat husk based *T. asperellum* with 26.66, soil drenching with carbendazim+mancozeb with 20.66. The least number of leaves was recorded in soil drenching with rice grain based *T. asperellum* with 6.66 as compared to control with 5.33.Out of eight treatments, maximum average width of leaves was recorded in soil application with vermicompost based *T. asperellum* and subsequent soil drenching with rice grain based *T. asperellum* with 2.56cm, followed by application of wheat husk based *T. asperellum* with 2.26cm, soil application of vermicompost based *T. asperellum* with 2.16cm, soil drenching with carbendazim+mancozeb with 2.03cm, drenching with metalaxyl+mancozeb with 1.75cm, soil application of dry cowdung based *T. asperellum* with 1.55cm. The minimum average width of leaves was recorded in soil drenching with rice grain based *T. asperellum* with 1.04cm as compared to control T8 with 1.06cm.

Out of eight treatments, maximum root mass was recorded in by soil application of wheat husk based *T. asperellum* with 0.251g followed soil application with vermicompost based *T. asperellum*and subsequent drenching with rice grain based *T. asperellum* with 0.193g, drenching with carbendazim+mancozeb with 2.03cm with 0.189g, application of vermicompost based *T. asperellum* with 0.184g, drenching with metalaxyl+mancozeb with 0.129, soil application of dry cowdung based *T. asperellum*with 0.124g. The minimum root mass was recorded in drenching with rice grain based*T. asperellum*with 0.012g as compared to untreated control T8 with 0.009g.

Comparison of the different treatments with respect to all parameters are given in Table 2 and Fig.3.

Li et. al (2018) reported that the *Trichoderma asperellum* strain CHF 78 significantly reduced the severity of fusarium wilt in the inoculated tomato seedlings. They also reported that the biocontrol agent significantly increased plant height and growth due to presence of various plant-growth promoting factors and increased nutrient uptake. In this study, the increase in shoot and root growth, luxuriant growth of leaves maybe due to production of growth promoting factors like

auxins, gibberlins, cytokinins, etc. or indirect enhancement of nutrient uptake or production of siderophores. Similar results on increased plant growth on application of *Trichoderma gamsii* on cereals and legumes were obtained by Rinu*et al*. (2014). The different substrates for the formulations of *Trichoderma* are for increasing the efficacy and shelf-life of the biocontrol agent. The vermicompost fortified *Trichoderma asperellum* showed better results as vermicompost is rich in organic matter which in turn helps in the growth of the plant by providing the required nutrients. The possible reason for the efficiency of rice gran fortified *Trichoderma asperellum* may be because of the rich carbohydrates provided by the rice grain and better water retaining capacity.

The CFU count of *Trichoderma* sp. from rhizosphere soil of each treatment of the pot culture was recorded at 10^{-7} dilution at the end of the experiment and results are presented in the table. Out of eight treatments, maximum CFU count was recorded in soil application with vermicompost based *T. asperellum* and subsequent drenching with rice grain based *T. asperellum* with 14.33×10^7 followed by drenching with vermicompost based *T. asperellum* with 11.00×10^7, then followed by soil application of dry cowdung based *T. asperellum* with 10.66×10^7, soil application of wheat husk based *T. asperellum* with 9.66×10^7, in drenching with rice grain based *T. asperellum* with 7.66×10^7, untreated control with 2.00×10^7, drenching with carbendazim+mancozeb with 1.66×10^7 while 0 in drenching with rice grain based *T. asperellum* (Table 3 and Fig.4). The findings in the present study aligns with the data of Bisen *et al* (2023) which indicated that tomato plants treated with vermicompost enhanced with *Trichoderma* exhibited the longest root length at 14.95 cm after 15 days of sowing, followed by vermicompost biofortied *Bacillus subtilis* at 11.25 cm and vermicompost with *Pseudomonas flourescens* at 9.85 cm. The highest dry weight was also recorded in the plants treated with *Trichoderma*-fortified vermicompost. These findings suggest that tomato plants treated with biofortified vermicompost experienced significant reductions in disease incidence and enhanced growth.

CONCLUSION

In the present study conducted, it was revealed that *Fusarium oxysporum*f.sp. *lycopersici* is the causal organism of fungal wilt of tomato. It was also revealed that rice grain followed by dry cowdung powder had great potential as substrates for mass multiplication of *Trichoderma asperellum* Kjr strain and also provided a better shelf-life. Soil application with vermicompost based *Trichoderma asperellum* at two days after inoculation and subsequent soil drenching with rice grain based *Trichoderma asperellum* at ten and eighteen days after inoculation in polybags proved to be the best treatment for the management of fungal wilt of tomato and can be recommended for further studies in field condition to determine it's efficacy. Further research can also be conducted using various supplements to enhance solid substrates for better multiplication and increased shelf-life of the biocontrol agent.

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