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PLANT PROTECTION

Performance of Native *Trichoderma* spp. and Copper Enriched Fermented Whey Preparation for Management of Tomato Damping-off Disease

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ABSTRACT

Tomato is an economically crucial nutritious vegetable crop grown in various states of our country, including Haryana. Its nursery is raised to get seedlings for transplanting in the main field. The nursery seedlings are adversely affected by damping off disease which is caused by several fungal species. In the present investigation, the evaluation of native *Trichoderma* spp. and copper-enriched fermented whey preparation was studied in the laboratory, as well as nursery conditions for the management of fungal pathogen responsible for damping off disease. The associated pathogen was isolated from diseased tomato seedlings and identified as *F. oxysporum* f. sp. *lycopersici* on the basis of cultural and morphological characters as well as through pathogenicity. Out of forty-three soil samples, five samples showed the presence of *Trichoderma* sp. These isolates were identified as *T. harzianum*, *T. viride* and *Trichoderma* sp. based on cultural and morphological characteristics. The antagonists could inhibit the mycelia growth of the pathogen in the range of 52.89 to 61.17%, and copper-enriched fermented whey preparation showed 42.28% inhibition. Among *Trichoderma* isolates, *T. harzianum* exhibited higher inhibition as compared to *T. viride*. Native *T. harzianum* was assessed through soil application, seed treatment, drenching, enrichment of farmyard manure as well as vermicompost. Copper-enriched fermented whey preparation was also taken as one of the treatments. The results showed that the antagonist was efficient in managing the disease in terms of lower disease incidence than the control. Damping-off incidence ranged from 4.35 to 9.76 percent in antagonist applied treatments, which was lower than in control (20.48%). The second preparation was also better than control, where the incidence of disease was 10.47%.

HIGHLIGHTS

- Native *T. harzianum* and copper enriched fermented whey preparation efficiently managed tomato damping-off disease under different conditions.

Keywords: Tomato, nursery, *Fusarium oxysporum* f. sp. *lycopercici*, damping off, *Trichoderma harzianum*, *T. viride*, copper enriched fermented whey preparation, mycelia growth inhibition, disease management

Tomato (*Lycopersicon esculentum*) is an important vegetable crop that contributes a lot to the human diet. It is a very good source of lycopene, ascorbic acid, flavonoids, a-tocopherol, and potassium in the human diet (Bramley 2000; Willcox *et al.* 2003). It belongs to the family Solanaceae and native of South America. It helps to lower the chances of cancer and cardiovascular disease (Klipstein-Grobusch *et al.* 2000; Giovannucci *et al.* 2002).

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It is cultivated in several states of our country in approximately 789.15 hectares with production of 19759.32 MT. Odisha is the top state followed by Madhya Pradesh, Karnataka, Chhattisgarh, Andhra Pradesh, West Bengal, Maharashtra, and Telangana. Haryana is at 9th position with approximately 34.99 hectares and 753.72 MT production (Anonymous, 2018).

Nursery raising is the foremost step to getting the desired planting material in terms of both its health and quantity. Seed and newly emerged seedlings face the challenge of soil-borne fungal phytopathogens like *Fusarium*, *Pythium*, *Rhizoctonia, Phytophthora* (Chaudhary *et al.* 2012; Karima and Nadia 2012), etc. which are responsible for seedling diseases such as seed rot, damping-off, root rot etc. These pathogens can effectively be managed through the application of azoxystrobin, metalaxyl-M, and pyraclostrobin fungicides (Salman and Abuamsha 2012). Their over-usage has increased human health risks many-fold and imbalanced the overall environment to a significant extent (Nicolopoulou-Stamati *et al.* 2016; Tripathi *et al.* 2019). And hence, such synthetic fungicides are not permitted for organic crop production. Under such a situation, therefore, biological control of such diseases is an ideal approach. Among biocontrol agents, species of *Trichoderma* have been efficient in controlling numerous phytopathogens (Domingues *et al.* 2000; Kumar *et al.* 2017) of different crops through different modes of action (Elad *et al.* 1982; Ridout *et al.* 1988) in an eco-friendly manner. *Trichoderma harzianum*, *T. viride,* and *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* are capable to manage *Alternaria solani*, *Fusarium solani*, *F. oxysporum*, *M. phaseolina* and other fungal sp. of tomato and other plants (S. El-Mougy *et al.* 2012). Application of *Trichoderma* formulations can be applied as a seed treatment, soil treatment, enrichment of farmyard manure (FYM), and vermicompost for the control of diseases in vegetables in a nursery which are either seed or soil-borne in nature (Nirmalkar *et al.* 2018). *T. viride, T. harzianum,* along with FYM, *Azospirillum*, and phosphate solubilizing bacteria (PSB) could significantly improve tomato seed's germination and seedling vigor (Thakur and Tripathi 2015).

Looking into the usefulness of *Trichoderma* spp. as a compelling candidate in controlling vegetable crop soil and seed borne diseases, the present investigation was undertaken to evaluate the bio effectiveness of it and copper-enriched fermented whey preparation under laboratory and nursery conditions against *Fusarium* sp. causing damping off.

MATERIALS AND METHODS

Isolation and identification of *Fusarium* sp. and *Trichoderma* sp.

Isolation of *Fusarium* spp.

Fusarium species was isolated from diseased tomato seedlings (Hammami *et al.* 2013). The seedlings that showed clear-cut damping symptoms were collected in sterilized polyethylene bags, brought to the biocontrol laboratory, Department of Plant Pathology, and kept in the refrigerator. For isolation of the pathogen, the collected seedlings were first washed with the running tap water, then cut into small pieces and surface sterilized with 1.5% sodium hypochlorite for 20 minutes (Jan *et al.* 2013); followed by washing with distilled sterilized water and then water was soaked by placing them between two sterilized blotting papers. The surface-sterilized pieces were transferred individually into solidified potato dextrose agar plates. The inoculated plates were incubated at 28±2 °C for 5-7 days. The developed colonies were identified and subcultured into PDA slants and identified microscopically based on the shape of macroconidia.

Pathogenicity of *Fusarium* sp.

The study was conducted using autoclaved field soil. The sterilized soil was applied into the seed beds. Seed (var. Selection 7) sowing was done and watered as and when required. After ten days of sowing, 10 ml potato dextrose broth culture ($1x10^7$cfu/ml) per liter of water was drenched (Isaac *et al.*, 2018). Disease observation was recorded after 25 days of sowing.

Isolation of *Trichoderma* spp.

Forty-three soil samples from the research farm, Deendayal Upadhyay Centre of Excellence for Organic Farming, Chaudhary Charan Singh Haryana Agricultural University, Hisar were processed to isolate the species of *Trichoderma* by applying the standard technique (Askew and

Laing, 1993; Cherkupally *et al.* 2017; Awad 2018). The soil samples were serially diluted up to 5th dilution and 500 microlitres were inoculated into Petriplates containing solidified potato dextrose agar medium followed by incubation at 28±2 ^{0}C up to one week (Cherkupally *et al*. 2017). The appeared fungal colonies were visually observed and tentatively identified based on growth rate and cultural characteristics, then subcultured on PDA plates. Further based on cultural characteristics and microscopic visualization, the antagonist was identified (Rifai 1969; Bissett 1991; Shah *et al*. 2012). The hyphal tip culture method was followed for the purification of cultures of both microorganisms, and their pure cultures were maintained on PDA slant at 5±1 ˚C in the refrigerator (Cherkupally *et al.* 2017).

In vitro bioefficacy evaluation of *Trichoderma* sp. against *Fusarium* sp.

The dual culture technique (Kunova *et al.* 2016) was employed to assess the bio-efficacy of *Trichoderma* species. The experiment was conducted in Biocontrol Lab, Department of Plant Pathology, in a complete randomized block design (CRD). There were seven treatments i.e., T_1-*T. harzianum*-1, T_2-*T. harzianum*-2, T_3-*T. viride*-1, T_4-*T. viride*-2, T_5-*Trichoderma* sp., T_6-copper enriched fermented whey preparation and T_7- untreated control, each treatment was replicated thrice.

Five-millimeter discs of both fungi (*Trichoderma* sp. and *Fusarium* sp.) were inoculated at equidistance in the center of the plates; in control, the only bit of pathogen was inoculated, followed by incubation at 28 ± 2 °C for one week. Observations on the pathogen's mycelial growth were recorded after one week and growth inhibition was worked out using the following formula:

Mycelial growth inhibition =

$$\frac{\text{Colony dia. in control} - \text{Colony dia. in treatment}}{\text{Colony dia. in control}} \times 100$$

Development of *Trichoderma* preparation

The liquid preparation of promising *Trichoderma* isolate was developed (Fig. 1A) by adopting the slightly modified method of earlier researchers (Niranjana *et al.* 2009; Khan *et al.* 2011; Kumhar *et al.* 2014). The concentration (active ingredient) of the liquid formulation was determined as 2×10^9 cfu/ml through serial dilution plate inoculation technique using routine medium (Fig. 1A).

Copper enriched fermented whey preparation was prepared using fermented whey and copper wire. The copper wire was added to the whey container and autoclaved; then, it was kept for one week to react (Fig. 1B). The developed preparation was evaluated for controlling damping-off pathogen in the laboratory using 5 and 10 ml per liter of water. Its effective dose was drenched into tomato nursery. Each treatment was replicated thrice, and a randomized block design (RBD) was adopted for the field study. Observations on seed germination and disease incidence (damping off) were recorded after ten and twenty-five days of sowing.

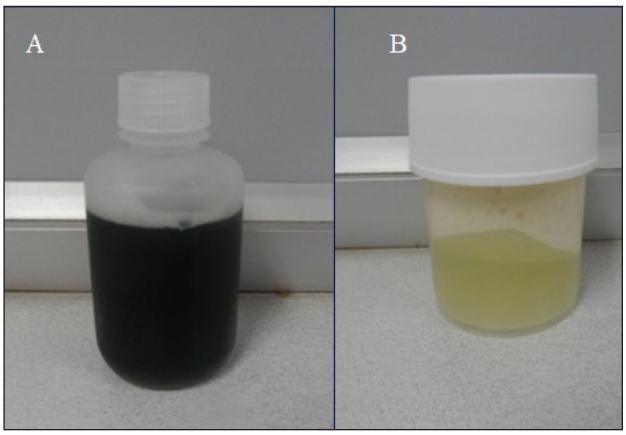

Fig. 1: Preparation developed for evaluation against damping-off pathogen (**A:** *T. harzianum,* **B:** copper enriched fermented whey formulation

Bio-efficacy of *Trichoderma* formulation against the damping-off disease of tomato under nursery conditions

The bioefficacy of the liquid formulation was carried out at the research farm of Deendayal Upadhyay Centre of Excellence for Organic Farming in a tomato nursery to assess its effect on the control of the damping-off disease. It was comprised of seven treatments i.e. T_1-*T. harzianum* soil application, T_2-*T. harzianum* drenching, T_3-seed treatment with *T. harzianum,* T_4-*T. harzianum* enriched FYM, T_5-copper enriched fermented whey preparation, T_6-*T. harzianum* enriched vermicompost and T_7- untreated control, each treatment was replicated thrice.

The soil application of *T. harzianum* formulation was made one day before seed sowing in the beds. Seed

treatment was done at the rate of 50 µl *T. harzianum* formulation per 10 g seed. The treated seed was air-dried in the shade and sown. Its drenching was done after one week. For enrichment of organic manures (farmyard manure and vermicompost), the bioagent's preparation was added to it (2% v/w) and was kept for 10 days under shade, ensuring proper moisture. Each treatment was replicated thrice in a randomized block design (RBD). Observations on seed germination and disease incidence (damping off) were recorded after 10 and 25 days of sowing, respectively.

RESULTS AND DISCUSSION

Isolation and identification of *Fusarium* spp. and *Trichoderma* spp.

Isolation of *Fusarium* spp.

The damping-off disease causing fungus *F. oxysporum* f. sp. *lycopersici* was isolated from diseased tomato seedlings (Fig. 2A). It was identified based on its cultural, morphological, and pathogenic characteristics. The native isolate produced slight fluffy and submerged mycelial growth. The color of mycelia was initially white and turned light pink in a later stage (Fig. 2B). It produced conidia ranging from 5.33×10^6 to 11.3×10^6 spores/ml on potato dextrose agar medium. It produced micro and microconidia; the macroconidia were 4-6 septate and sickle-shaped, whereas the microconidia were 0–1 septate (Fig. 2C).

The present study proved that *F. oxysporum f.* sp. *lycopersici* played the key role in the causation of tomato damping in the nursery. Similarly, it has already been established that *F. solani* was responsible for damping off as well as root rot, and it was isolated from tomato using potato dextrose agar and were preliminarily identified morphologically with the help of a microscope (Thilagam *et al.* 2018). Several isolates of *Fusarium* species were isolated from infected tomato roots in Iran and were identified as *F. oxysporum*, *F. redolens*, *F. proliferatum* and *F. verticillioides* morphologically and proven pathogenic against host crop under polyhouse conditions (Chehri 2016). In Mexico, tomato wilt causing phytopathogenic fungi were isolated from diseased seedlings and identified as *F. oxysporum* and *Fusarium* sp., morphologically as well as molecularly (Isaac *et al.* 2018). Numerous isolates of *F. oxysporum* were obtained from the infected plant and characterized molecularly in Uttar Pradesh, India, which were found pathogenic to its tomato plant (Joshi *et al*. 2013).

Fusarium species associated with rooibos damping-off were recovered from 12 nurseries in the Western Cape Province of South Africa. All 121 isolates resembled *F. oxysporum* morphologically. 25 *F. foetens* and 33 *F. oxysporum* were among the 58 representative isolates identified through sequence analyses of the EF-1 gene area, and these two species were obtained from 11 and 12 nurseries, respectively. Under glasshouse conditions, twenty isolates of each *Fusarium* sp. were tested for their ability to cause damping-off of rooibos and the rotation crops lupin and oat. Both *Fusarium* spp. caused damping off in rooibos seedlings but not lupin or oat seedlings. Both species could be isolated from lupin but not from oat. This is the first time

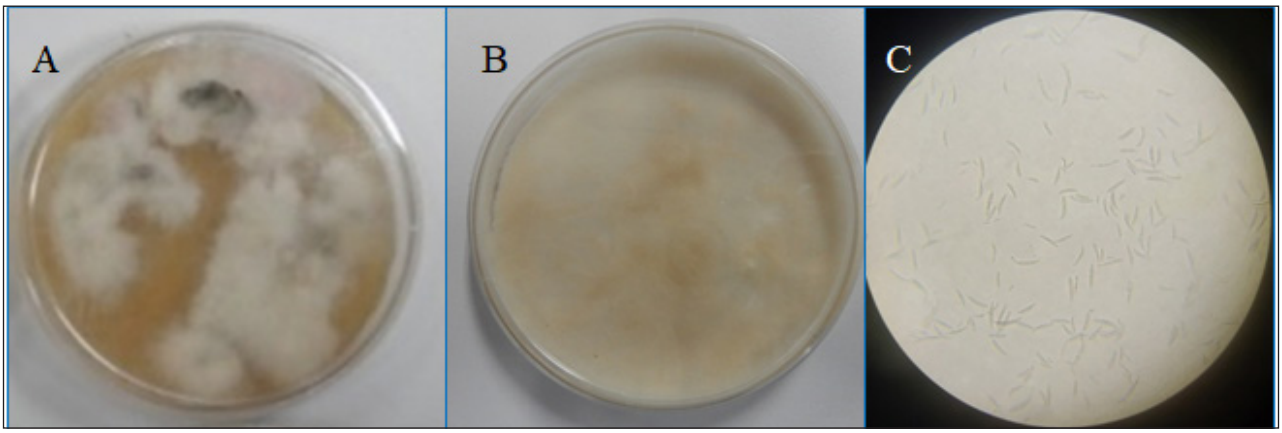

Fig. 2: Isolation and identification damping off pathogen (**A:** Mycelia of pathogenic fungus growing out from diseased tomato seedling, **B:** Pure culture of fungal pathogen, **C:** Micro and macroconidia)

these two *Fusarium* spp. have been reported as pathogens of rooibos seedlings, as well as the first time *F. foetens* has been reported in South Africa (Lamprecht and Tewoldemedhin, 2017).

Infected squash and tomato plants garnered *R. solani* and *F. solani* (Mart.) Sacc, respectively. Initially, *R. solani* isolates predominated and caused the most severe damping-off in squash plants. In addition, the isolate of *F. solani* (Mart.) Sacc. The most common one found in the rotten root of tomatoes had the highest pathogenic severity (Helal 2017). In and around the Udaipur district of Rajasthan, an estimated 40 to 50 damping-off affected diseased onion seedlings were collected from the onion crop nursery and adjacent main fields. These samples were put through a fungal isolation procedure on potato dextrose agar (PDA) medium in an *in vitro* environment. Fungi with a distinct colony were selected, and culturing was performed on them. *F. solani* and *S. rolfsii* were identified as the fungi isolated (Rathore and Patil, 2019).

The fungal phytopathogenic isolate could cause damping off disease of tomatoes under nursery conditions when potato dextrose broth culture (10 ml per liter of water) (1×10^7cfu/ml) was drenched after 10 days of seed sowing. After 15 days of its application, it could show 53.50 percent disease (Table 1).

Table 1: Pathogenicity of *Fusarium* sp. for tomato damping off disease

Treatment	Per cent damping off incidence*
F. oxysporum f. sp. *lycopersici*	53.50
Control	0.0

**Average of three replications.*

Isolation of *Trichoderma* spp.

Out of 43 soil samples, only five showed the presence of *Trichoderma* spp. In addition, species of *Fusarium*, *Alternaria,* and *Curvularia* were also present in soil samples (Table 2).

The recovered *Trichoderma* isolates were identified based on their cultural and morphological characters. *T. harzianum* formed 1-2 concentric rings with green-colored conidial production. Conidial production was denser in the centre than margins. In case of *T. viride* there was no formation of a concentric ring. Green-colored conidia distributed throughout the Petri plate. Irregular yellow zone with conidia formed in Petriplate. In case of *T. harzianum* the conidia were globose to sub-globose, light green colored (Fig. 3A). Its phialides were flask-shaped, arranged in divergent groups of 2-4. The conidia

Table 2: Status of *Trichoderma* spp. in collected soil samples

Soil sample No.	*Trichoderma* sp.	Soil sample No.	*Trichoderma* sp.	Soil sample No.	*Trichoderma* sp.
1	*T. harzianum*-1	16	–	31	–
2	–	17	–	32	–
3	–	18	–	33	–
4	–	19	–	34	–
5	–	20	–	35	–
6	–	21	–	36	–
7	–	22	–	37	–
8	–	23	–	38	–
9	–	24	–	39	–
10	–	25	*T. viride*-1	40	–
11	*T. harzianum*-2	26	–	41	*T. viride*-2
12	–	27	–	42	–
13	–	28	–	43	–
14	–	29	*Trichoderma* sp.		
15	–	30	–		

- indicates absence of Trichoderma sp. in soil sample

of *T. viride* were globose, light green colored (Fig. 3B). Its phialides were slender, comparatively shorter, and arranged in a divergent group of 2-4 (Fig. 3C-D).

Five local isolates of *Trichoderma* spp. were successfully obtained from collected soil in this study and were identified based on their specific characteristics as *T. harzianum*, *T. viride* and *Trichoderma* sp. Several isolates of *T. viride* and *T. harzianum* were recovered from soil followed by their characterization as per required parameters for identification and antagonistic potency against soil airborne fungal phytopathogens (Kumar *et al.* 2012). A few *Trichoderma* isolates were isolated from the agricultural soils in Egypt. Their identity was established morphologically and molecularly (Hassan and El-Awady 2011). In China, some of the antagonistic fungal strains were isolated from the rhizospheric soil of some vegetable crops through the serial dilution plate technique (Pradhan and Sukla 2006; Fan *et al.* 2020).

Numerous *Trichoderma* spp. were recovered from different samples, including soil in the North Western Province of Sri Lanka. These were characterized and evaluated against the phytopathogens under lab conditions (Kannangara *et al.* 2017). It has been isolated and evaluated by some other workers as well in various parts of the world (Ibarra-Medina *et al.* 2010; Liu *et al.* 2020; Mukherjee *et al.* 2014; Khang *et al.* 2013; Hamed *et al.* 2015; Nadarajah *et al.* 2014; Mendoza-Mendoza *et al.* 2015). The success of *Trichoderma* depends on its virulence, type of formulation, storage conditions, handling, cost-effectiveness, shelf life, etc. A virulent isolate of *Trichoderma* needs a suitable formulation with the desired quality for its application, to get desirable results in managing plant pathogens.

In vitro bioefficacy evaluation of *Trichoderma* sp. against *Fusarium* sp.

In dual culture, *Trichoderma* spp. could efficiently inhibit the growth of tomatoes damping-off pathogens in the laboratory. The growth inhibition ranged from 52.89 to 61.17%; however, the maximum inhibition (61.17%) was observed in the case of *T. harzianum*-1 followed by *T. harzianum*-2 (59.67%) as indicated in table 3. The preparation made from whey plus copper wire showed 3.31 to 42.28% inhibition when used at 5 and 10% v/v concentrations, respectively (Table 3). It was an interesting observation that its 10% concentration demonstrated good control of the pathogen (Fig. 4).

The indigenous *T. harzianum* showed comparatively better control of the pathogen when compared with *T. viride* in the present investigation. The *T. harzianum* found as a successful antagonist against the Fol in the laboratory, and its application along with vermicompost could lower the disease incidence as well as boost the vegetative growth of the plant and its production potential (Basco *et al.* 2017). In Iran, the local isolates of *T. harzianum* controlled the mycelia growth of Fol in the laboratory. The antagonist, when applied in the greenhouse on tomato crops, resulted in less disease incidence and promoted plant growth (Barari 2016).

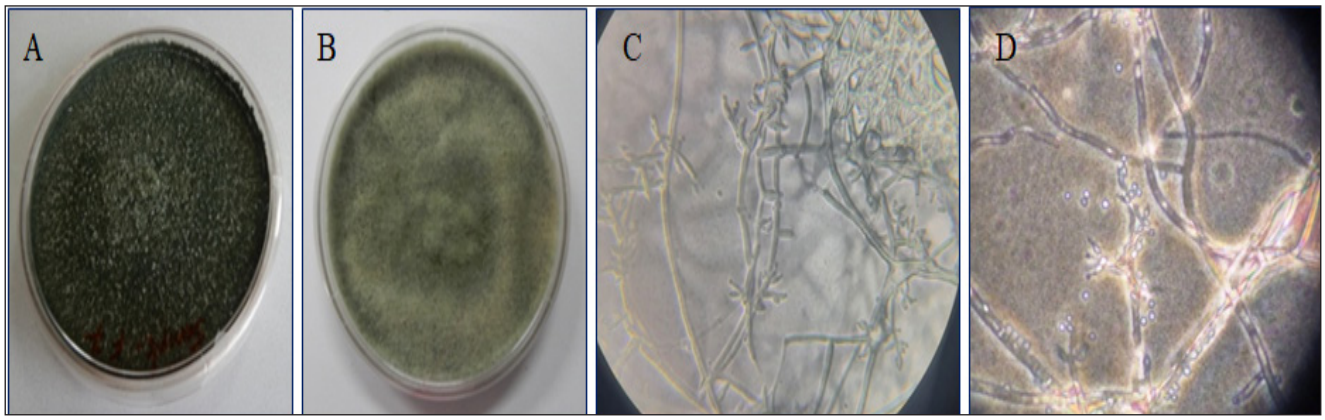

Fig. 3: Cultural and morphological characters of local *Trichoderma* isolates (**A:** *T. harzianum,* **B:** *T. viride,* **C-D:** Phialides and conidia)

The bio-efficacy of *T. harzianum* was also proven successfully against wilt disease-causing fungal phytopathogen of tomatoes under lab as well as greenhouse conditions (Mwangi *et al.* 2019). Treatment of tomato seedlings with *T. asperellum* minimized the incidence of *F. oxysporum* f. sp. *lycopersici* in the greenhouse. Its application improved the crop yield potency and enhanced the level of the genes responsible for disease resistance reactions in the plants (El Komy *et al.* 2016). Soil amendment with *T. harzianum* enriched farmyard manure was found effective in managing the tomato wilt incited by *F. oxysporum* f. sp. *lycopersici* (Ali Salim *et al.* 2017). *T. harzianum, T. asperellum* and *T. virens* found as potent biocontrol agents against the wilt of *Lycopersicon esculentum* Mill. caused by *F. oxysporum* f. sp. *lycopersici* under lab conditions (Prasad *et al.* 2016).

Usage of both *T. harzianum* and *T. viride* for root dipping and soil application resulted in to better disease control. Its application resulted in to improved vegetative growth in terms of shoot as well as root length and fruit yield (Dubey *et al.* 2020). In Iran, *T. harzianum* was found effective in managing the wilt pathogen of tomatoes in the lab and greenhouse (Ismael and Mahmood 2016). *T. harzianum* and *T. asperellum* are reasonable biological control agents of soil-borne fungal phytopathogenic genera viz., *F. solani*, *R. solani* and *S. sclerotiorum* (Qualhato *et al.* 2013).

Table 3: *In vitro* bioeffectiveness of *T. harzianum* and copper-enriched fermented whey preparation on the growth of *F. oxysporum* f. sp. *lycopersici*

Treatment	Mycelial growth inhibition (%)*
T_1 - *T. harzianum*-1	61.17 (51.43)
T_2 - *T. harzianum*-2	59.67 (50.63)
T_3 - *T. viride*-1	55.63 (48.21)
T_4 - *T. viride*-2	56.03 (48.58)
T_5 – *Trichoderma* sp.	52.89 (46.65)
T_{6A} - Copper enriched fermented whey preparation (0.5% v/v)	3.31 (10.02)
T_{6B} - Copper enriched fermented whey preparation (1.0% v/v)	42.28 (40.39)
T_7 - Untreated control	0.0
C.D. > 5%	3.27

Average of 3 replications, figures in parenthesis are angular transformed values.

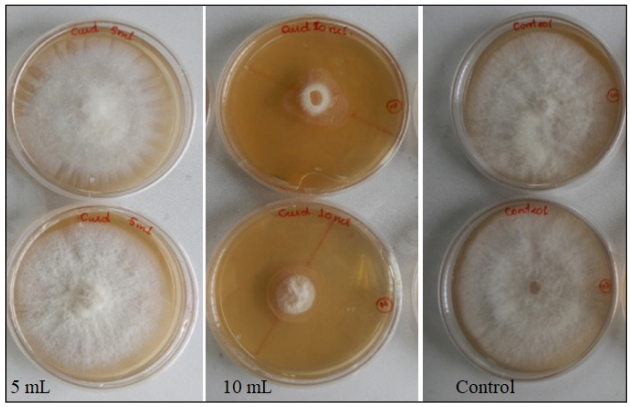

Fig. 4: *In vitro* bio-efficacy of copper enriched fermented whey preparation against damping off pathogen

Bio-efficacy of *Trichoderma* formulation against the damping-off disease of tomato under nursery conditions

Being the most effective antagonist, *T. harzianum*-1 was chosen for preparation of liquid formulation which contained 2 × 10^9cfu per ml. When applied in different ways, the *Trichoderma* formulation was efficient in enhancing the seed germination and reducing the damping off incidence under field conditions as compared to control. The seed germination due to application of *Trichoderma* as soil application, drenching, seed treatment, enrichment of farmyard manure (FYM) as well as vermicompost showed 75.78 to 86.67 germination against 67.56 percent in control. Drenching with copper-enriched fermented whey preparation was found inferior to fungal biocontrol agent but superior to control.

The damping-off incidence ranged from 4.35 to 9.76 percent in various treatments of *Trichoderma*, which was lesser than the control (20.48%), proving the potency of this antagonist in managing the disease. The drenching with indigenous fungicide formulation was found lesser effective as compared to the antagonist; however, it was better than the control (Table 4).

Trichoderma-based liquid and another formulation composed of fermented whey and copper wire was developed for lab and field assessment against damping off the pathogen. The coconut and rice washed water was used as the liquid carrier. However, coir dust, sawdust and parboiled rice were used as the solid carrier for its mass production. The solid carriers were found to be better than liquid in terms of stability (Hewavitharana *et al.*

Table 4: Bio-efficacy of *T. harzianum*-1 against damping-off of tomato in nursery

Treatment	Seed germination (%)	Disease incidence (%)
T_1 - *T. harzianum* soil application (0.5%)	86.67 (68.87)	5.15 (13.08)
T_2 - *Trichoderma* drenching (0.5%)	79.11 (62.89)	9.76 (18.08)
T_3 - Seed treatment with *T. harzianum* (50µl/10g seed)	84.89 (67.34)	7.13 (15.31)
T_4 - *Trichoderma* enriched FYM (2%)	75.78 (60.53)	6.12 (14.29)
T_5 - Copper enriched fermented whey preparation (1.0% v/v)	71.22 (57.66)	10.47 (18.81)
T_6 - *Trichoderma* enriched vermicompost (2%)	81.22 (64.38)	4.35 (11.98)
T_7 - Control	67.56 (55.30)	20.48 (26.81)
C.D. >0.5	3.49	3.93

**Average of 3 replications, figures in brackets are angular transformed values.*

2018). A combi-formulation using *T. harzianum* and *Streptomyces rochei* was developed with 3.5 × 10^8 spores/ml of the first antagonist. It contained a mixture of plantation soil and vermiculite and is efficient up to 30 °C. The shelf life of it was reported up to two years at room temperature (Ezziyyani *et al.* 2007).

Microencapsulation of *Trichoderma* was formulated to improve its shelf life and efficiency for seed treatment purpose (Cumagun 2014). It can be mass multiplied using various substrates like wheat, rice bran, rice chaffy grain, farmyard manure, banana pseudostem, and dried banana leaf as basic carrier material to support *T. harzianum* growth. The addition of 10% w/v jaggery to substrate fasten the multiplication rate of the antagonist, which survived for more than half a month on the stored substrate (Thangavelu *et al.* 2004; Niranjana *et al.* 2009). It can also be mass multiplied on various other substrates such as molasses (Khan *et al.* 2011) and its formulation can be developed using chalk powder, organic oils, agricultural wastes etc. (Khan *et al.* 2011; Subash *et al.* 2014).

Our investigation indicated that the formulation of antagonists in various application methods was capable of controlling tomato disease. Another formulation formulated by fermented whey and copper wire also showed better disease-controlling trends than control.

In Cameroon, common bean seeds treatment with *T. gamsii* demonstrated an advantageous effect on the plant emergence by reducing the damping-off disease fungal phytopathogens including *F. oxysporum*, *F. solani*, *M. phaseolina* and *Pythium ultimum* (Bedine Boat *et al.* 2020). *T. harzianum* isolates of Rajasthan, Kerala, and Andhra Pradesh were effective against damping off and wilt-causing fungal pathogens of tomatoes under lab conditions. Under greenhouse and field conditions, seed treatment with antagonists resulted in growth promotion and crop production (Sain and Pandey 2016). The use of *T. harzianum* (BHU-51), *T. harzianum* (BHU-105), and a consortium of both as seed treatment of tomato could efficiently reduce damping off (*R. solani*) and support its vegetative growth and crop production (Singh *et al.* 2014).

Tomato seed treatment with *B. subtilis* and *T. asperellum* was established as an efficient practice to manage seedling damping-off disease caused by *P. aphanidermatum* is the most important in Kenya (Kipngeno *et al.* 2015). Tomato seed coating with *T. asperellum* and *B. subtilis* successfully managed seedling disease incited by *P. aphanidermatum* in the greenhouse (Kipngeno *et al.* 2015). *T. harzianum* could control damping-off, *Fusarium* wilt, *Rhizoctonia* wilt, leaf spot, late blight, and *Septoria* leaf spot of tomatoes under lab, greenhouse, and field conditions (Sain and Pandey 2016). Fennel, peppermint, oregano, and ginger oils are reported as a natural biocide for the control of *R. solani* and *F.solani* inciting damping-off disease of tomato. Biocides formulations were prepared from the essential oils of fennel, peppermint, oregano, and ginger. These formulations were effective under lab and field conditions (Helal 2017).

CONCLUSION

The fungal phytopathogen, *F. oxysporum* f. sp. *lycopersici* found to be associated with damping-off seedlings of tomato, and its pathogenicity was proven successfully. Antagonist *Trichoderma* sp., *T. harzianum*, and *T. viride* were found in five local

soil samples. These isolates were found efficacious against the damping off pathogens under laboratory and nursery conditions. Copper-enriched fermented whey preparation also demonstrated a reasonable control of the pathogen at its higher concentration under tested conditions. This investigation concludes that species of *Trichoderma* and second preparation could be options of synthetic fungicides and applied where chemical fungicides are restricted. In addition, these are eco-friendly, pollution free and natural in occurrence.

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