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Management of Tomato Damping-off Disease in the Nursery Using of *Trichoderma asperellum*

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Abstract

The study was conducted during 2018–19 and 2019–20 to evaluate effectiveness of the *Trichoderma asperellum* liquid formulation for the management of tomato damping-off in the nursery. The experiment was conducted for two consecutive seasons in a randomized block design with seven treatments and three replications. *T. asperellum* formulation improved seed germination and controlled the damping-off diseases in tomatoes when compared with control. All the treatments performed better over control, in the first season, the antagonist's application showed 75.75% seed germination; however, it was 60.13% in control. There was 10.93 to 20.38% seedling mortality due to disease which was comparatively lower than the control (26.98%). A similar trend of seed germination and disease incidence was observed in the second season. In addition to managing the disease, the antagonist certainly promoted vegetative growth which was reflected as increased shoot and root length in comparison to control during both seasons. During the first season, shoot length ranged 10.90–12.85 cm as compared to the control (8.72 cm) and root length ranged from 3.21–3.65 cm which was greater than the control. Almost a similar trend in the vegetative growth parameters of seedlings was observed during the second season. The present investigation showed that the tested antagonist's formulation could efficiently manage the tomato damping-off as well as encouraged the vegetative growth of seedlings which ultimately ensured better and healthy seedling. This formulation can successfully be used through different methods to take care of tomato damping off.

Keywords: Damping off, growth promotion, *Trichoderma asperellum*, tomato

1. Introduction

Having a healthy and disease-free nursery is the foremost inevitability in determining profitable crop production. The damping-off disease is a global problem of seedlings in the nursery, which may cause 5-80% seedling mortality of different crops (Lamichhane et al., 2017). Usage of synthetic fungicides had been a preferred approach since long back in managing such soil-borne fungal phytopathogens. However, issues related to human and soil health, environmental hazards, and disturbed ecosystems pressurized the agriculturists to switch over towards the safer and eco-friendly plant protection approaches (Rosenzweig et al., 2001). Under such a situation, the biological control agents could be an ideal approach for disease management as they have no chance to get into the food chain and hence safe to human beings, animals, and plants (Monte and Llobell, 2003).

Genus *Trichoderma* is the most widely applied biological tool in plant protection in the agriculture sector (Sayeed Akhtar and Siddiqui, 2008). It is found in a variety of soils and with other plant materials as a major candidate (Benitez et al., 2004) and has positive effects on the plants (Hermosa et al., 2012). It is efficient in taking care of diseases of agricultural, horticultural, ornamental crops, etc caused by phytopathogenic fungi (Lewis and Lumsden, 2001), bacteria, and viruses (Hanson and Howell, 2002). It produces many enzymes and secondary metabolites (antibiotics) which are lethal to the phytopathogens. Its rhizospheric application strengthens the plants to fight against aerial fungal, bacterial, and viral diseases (Benitez et al., 2004) owing to elicitors production and resistance induction (Harman et al., 2004). This antagonist creates unfavorable conditions for the phytopathogenic fungi through acidification of the environment (Benitez et al., 2004).

It stimulates plant growth, persuades yield, vitamin production, boosts up nitrogen, and phosphorus availability as well as nutrients mobilization (Błaszczyk et al., 2014). The antagonist produces zeaxanthin and gibberellins to excel in seed germination. It also produces different acids to enhance the availability of phosphorus and other micronutrients to the plants (Harman et al., 2004). It also induces the defense system in plants (Yedidia et al., 1999). It can be used for soil application (Barari, 2016), seed treatment (Jayaraj et al.,

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2006) mixing with farmyard manure (Khan et al., 2019), and vermicompost. There are hundreds of species (Bissett et al., 2015); nevertheless, a few species have potential scope for control of phytopathogens (Hermosa et al., 2000).

Integration of *Trichoderma* sp. in plant protection strategies could manage the disease and improve plant health (Monte, 2001) which is environment friendly as well as cost-effective (Dubey et al., 2007). Keeping into consideration the disease managing potency and plant growth promotional activity of *Trichoderma* spp., the present study was planned to test its bioefficacy against the damping-off of the tomato seedlings caused by *Fusarium* sp. in the nursery.

2. Materials and Methods

The nursery trials were carried out at the research farm (29.153843 NS Latitude and 75.694118 EW Longitude), Department of Vegetable Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar for two consecutive seasons during 2018-19 and 2019-20 in the month of December to January.

The liquid formulation (10% Aqueous Suspension) of *T. asperellum* (KBN-29, accession number ITCC-7764) was prepared following a slightly modified method (Hewavitharana et al., 2018). The pure culture of the antagonist was inoculated into potato dextrose agar (Hi-Media India Pvt. Ltd.) plates, followed by proper sealing with parafilm under aseptic conditions, and incubated at 26±2°C for 5 days in a BOD incubator. Ten milliliters of double-distilled sterilized water was added to each plate and fungal biomass (conidia and mycelia) was scrapped with the help of a scrapper. The harvested biomass was further diluted with water and a conidial concentration of approximately $2x10^9$ per ml was determined through serial dilution plate inoculation technique (Figure 1). The prepared formulation was kept in the refrigerator and used within a month.

2.1. Assessment of different application methods against damping off

For bioefficacy assessment, the formulation was applied

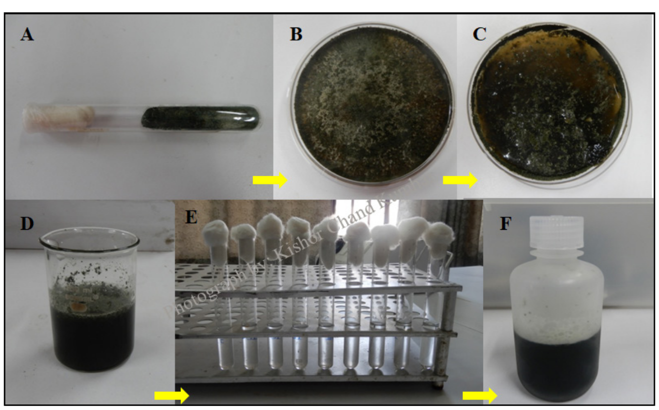

Figure 1: Steps of product formulation: A-Pure culture slant, B- Culturing on PDA plate, C- Biomass scrapping, D-Biomass collection, E-Serial dilution and F- Liquid formulation

as a seed treatment, soil application, drenching, enriched farmyard manure (FYM), and vermicompost. The treated seed was dried under shade for about two hours. In the case of soil application, the formulation was sprayed uniformly in to plot before 4-5 hr of seed sowing. For fortification of organic manures, it was mixed thoroughly with FYM and vermicompost. The fortified organic manures covered with polyethylene sheet and kept under shade for 10 days, and then applied to the beds. Carbendazim was taken as a standard check in the experiment. There were seven treatments i.e. T_1- Soil application of *T. asperellum (*1 ml l^{-1} of water), T_2- Soil drenching of *T. asperellum* (1 ml l^{-1} of water), T_3- Seed treatment *T. asperellum* (1 ml kg^{-1}), T_4- FYM enriched with *T. asperellum* (250 ml kg^{-1}), T_5- Vermicompost enriched with *T. asperellum* (250 ml kg^{-1}), T_6- Carbendazim drenching (2.5 g l^{-1} of water) and T_7- Untreated control with three replications .

The local tomato var. Selection-7 was used for experimentation. Raised seedbeds of 90 cm x 45 cm dimensions were prepared in mid-December, 2018, and 2019 (Figure 2). Two hundred tomato seeds per treatment were sown manually. After 15 days damping-off incidence was recorded. Then drenching of antagonist and carbendazim was done in respective treatments and again after two weeks, disease observation was recorded in comparison with control. After about 45 days of nursery raising, plant growth in terms of shoot and root length (cm) was measured from ten randomly sampled seedlings per treatment. The experimental data were statistically analyzed through the online package 'OPSTAT' of Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Figure 2: Performance of *T. asperellum* in tomato nursery: A- post emergence damping off, B-Vegetative growth (arrow indicating control bed)

2.2. Weather conditions during the study period

Weather data of temperature, relative humidity, wind speed, sunshine hours, and rainfall were collected from the Department of Agrometeorology, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The maximum and minimum temperatures during 2018–19 ranged from 17.1 to 21.0°C and 1.9 to 7.7°C, respectively. Morning relative humidity was 90.3 - 99.1 and during the evening it was 48.5 - 65.7 (Figure 5). During 2019 -20, the maximum and minimum temperatures ranged from 11.9 to 22.8 and 2.6 to 8.3°C. Relative humidity during the morning was recorded at 88.0 to 100 and during the evening it was 46.0 - 82.0% (Figure 6).

3. Results and Discussion

3.1. Assessment of different application methods against damping off

The investigation revealed that the liquid formulation of the *T. asperellum* could improve seed germination as well as reduce the damping-off in comparison with control in the nursery when it was applied in different ways, however, soil application, enriched vermicompost, and FYM found more promising. In the first season, the seed germinated from 63.88 to 88.75 as compared to 60.13% in control and the seedling showed 10.93 to 20.38% damping off which was less than the control (26.98%) as indicated in Table 1. Likewise, in the second season, the seed was germinated from 70.83 to 89.50% when the antagonist was applied in various ways. The seedlings suffered from damping off disease lesser (8.38-15.33%) as compared to the control bed (20.21%) as evident from Table 2 and Figure 2.

Table 1: Evaluation of *T. asperellum* for management of seedling disease of tomato under nursery (2018-19)

Treatment	Germination (%)	Damping-off incidence (%)
T_1-Soil application of *T. asperellum*	75.75 (60.51± 1.00)	11.37 (19.64 ± 0.92)
T_2-Soil drenching of *T. asperellum*	63.88 (53.10± 2.08)	20.38 (26.78 ± 1.16)
T_3-Seed treatment T. *asperellum*	65.50 (54.03± 1.23)	19.19 (25.86 ± 1.59)
T_4-FYM enriched with *T. asperellum*	72.00 (58.06± 1.03)	13.36 (21.43 ± 0.42)
T_5-Vermicompost enriched with *T. asperellum*	88.75 (70.86± 2.69)	10.93 (19.21 ± 1.14)
T_6-Carbendazim drenching	73.00 (58.79± 2.10)	12.08 (20.03 ± 2.22)
T_7-Untreated control	60.13 (50.85± 1.54)	26.98 (31.22 ± 1.49)
CD (p<0.05)	5.27	3.55

*Average of 3 replications, figures in parenthesis are angular transformed values±SE

The antagonist positively supported the vegetative growth of the seedlings in terms of higher shoot and root length in comparison with control during both seasons (Figure 2). During the first season, shoot length ranged 10.90–12.85 cm as compared to the control (8.72 cm), nevertheless, root length was observed in the range of 3.21 to 3.91 cm which was more than the control (2.70 cm). Almost a similar trend in the vegetative growth parameters of seedlings was noted during the second season (Figure 3 and 4).

Table 2: Evaluation of *T. asperellum* for management of seedling disease of tomato under nursery (2019-20)

Treatment	Germination (%)	Damping-off incidence (%)
T_1-Soil application of *T. asperellum*	83.17 (65.86 ±1.89)	8.38 (16.72±1.50)
T_2-Soil drenching of *T. asperellum*	70.83 (57.34 ±1.83)	15.33 (23.03±0.74)
T_3-Seed treatment T. *asperellum*	71.00 (57.48 ±2.34)	14.43 (22.28±1.08)
T_4-FYM enriched with *T. asperellum*	75.67 (60.45 ±1.16)	11.68 (19.95±0.53)
T_5-Vermicompost enriched with *T. asperellum*	89.50 (71.19 ±1.64)	7.82 (16.09±1.71)
T_6-Carbendazim drenching	81.00 (64.21 ±1.65)	8.54 (16.90±1.26)
T_7-Untreated control	66.83 (54.83±1.24)	20.21 (26.69±0.58)
CD (p<0.05)	3.52	3.78

*Average of 3 replications, figures in parenthesis are angular transformed values±SE

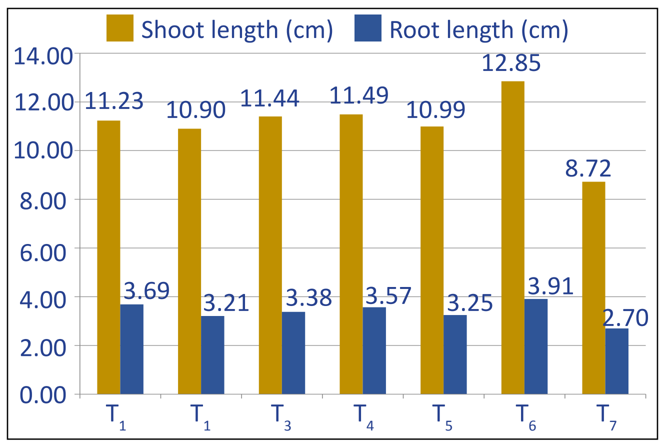

Figure 3: Effect of *Trichoderma* formulation on shoot and root growth of tomato in nursery (2018–19)

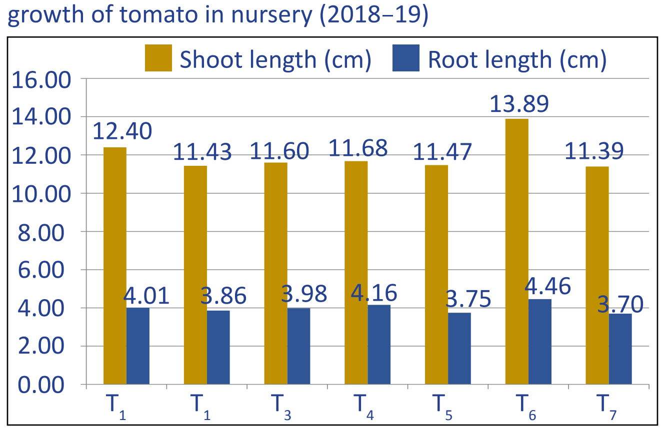

Figure 4: Effect of *Trichoderma* formulation on shoot and root growth of tomato in nursery (2019-20)

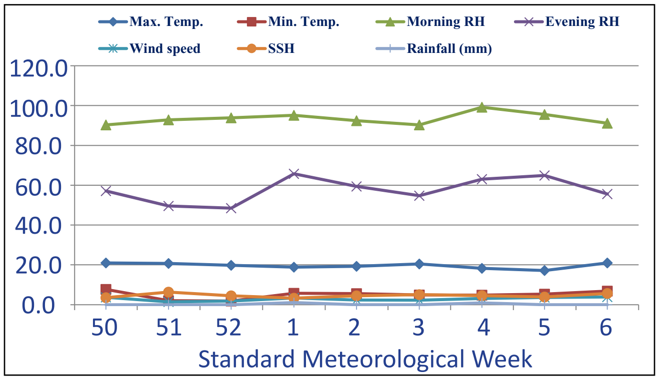

Figure 5: Weather conditions during first season (2018-19)

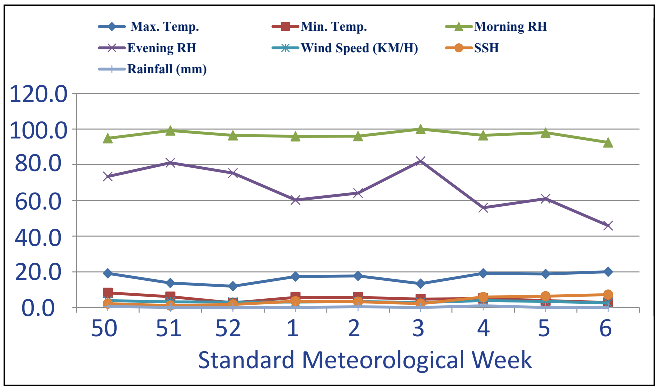

Figure 6: Weather conditions during second season (2019-20)

Trichoderma spp. was found effective in controlling the *Fusarium* wilt of tomato, watermelon, and muskmelon in the greenhouse and field (Larkin and Fravel, 1998). Sowing of tomato seeds in *T. harzianum* amended soil led to a significant decrease in the incidence of Fusarium crown and root rot disease caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Datnoff et al., 1995). Researchers also assessed this antagonist for the control of this disease of vegetable crops through various application methods and noted similar findings.

Indigenous isolates of concerned regions performed better in that particular region (Howell, 2003). Effectiveness of *T. harzianum* proven in controlling root rot (*Phytophthora capsici*) of pepper (Ezziyyani et al., 2007). *T. harzianum* and *T. virens* found potential in managing the pink rot of potato and stem rot of tomato, boosted up potato tuber yield and survival of tomato seedlings (Etebarian et al., 2000). Tomato, lettuce, pepper, bean, and tobacco showed resistance against *B. cinerea* owing to the application of *T. harzianum* (De Meyer et al., 1998).

Seed treatment, seedbed treatment, and enrichment of farmyard manure with *T. viride* and *T. harzianum* could offer a better damping-off solution of cauliflower under field conditions and improved seedling vegetative growth and health (Shabir et al., 2012).

The damping off disease of tomato could effectively manage through treatment of seed through *T. viride* and integration of other plant protection measures (Joshi et al., 2009). Farmyard manure enriched with *T. harzianum* and other species of this antagonist provided protection to the tomato seedlings against damping off (Dutta and Das, 2002).

Such treatments were found effective in terms of reduced disease and improved seedling strength and health of tomato and chilli plants (Nazir et al., 2011). Tomato seed treatment with *T. asperellum* took care of *P. aphanidermatum* (Kipngeno et al., 2015). Seed treatment with a consortium of *T. harzianum* significantly declined the damping-off incidence and enhanced the shoot length, chlorophyll content, and yield (Singh et al., 2014). Inoculation of tomato seed and nursery soil with *T. harzianum* spore suspension laid increment in the shoot as well as root length, diameter, and weight when compared to the control. There was a noticeable increase in leaf number, leaf area and chlorophyll content (Azarmi et al., 2011).

Application of *T. asperellum* in field conditions managed phytopathogenic *Fusarium* sp., and significantly increased the vegetative growth in respect of root length, shoot length, plant weight as well as chlorophyll content. It also improved total phenol, peroxidase, polyphenol oxidase, and phenylalanine ammonium lyase activity which developed plants' resistance against the pathogen (Patel and Saraf, 2017). Pre-sowing application of *T. viride* (WP) in nursery beds of tomato showed a reduction in disease incidence. Nevertheless, growth parameters showed a positive impact (Jataraf et al., 2005). Soil application of *T. viride* efficiently controlled the pre-and post-emergence damping-off of tomato (*P. aphanidermatum*) and improved vegetative growth of the plant. *T. harzianum* and *T. virens* treated tomato seed revealed up to cent percent seed germination and increased root and shoot length (Sharma et al., 2012).

Trichoderma amended compost could effectively suppress root disease causal fungal phytopathogens viz., *F. oxysporum*, *P. debaryanum*, *P. aphanidermatum*, *R. solani* in tomato and persuaded seed germination, seedling height, and overall biomass of seedling (Dukare et al., 2011). *T. harzianum* enriched organic matter could reduce the tomato damping-off intensity and increase seedling growth (Kalay et al., 2019). Concurrent application of *T. harzianum* as seed, seedbed, and soil treatment performed better than its soil application in terms of disease management and better growth parameters of eggplant and tomato. Vermicompost enriched with antagonist increased seed germination, promoted seedling vegetative growth, and minimized damping-off incidence (Uddin et al., 2009). The disease control and plant growth-promoting property of *T. asperellum* were established against *R. solani* in the cucumber plant (Ryu et al., 2006). Inoculation of *Trichoderma* in tomato nursery and field could improve plant health in terms of increased shoot weight, fruit size, yield (Nzanza et al., 2012). Furrow application of *T. harzianum* in the field conditions could reduce black scurf incidence in organically grown potatoes (Tsror et al., 2001). A few

Trichoderma strains showed good control of *P. ultimum* in pea and plant growth promotional property in terms of increased shoot weight, root weight, root length number of lateral roots (Naseby et al., 2000).

4. Conclusion

The tested fungal bioagent was effective in managing the damping off disease of tomato when it was applied through different methods. It reduced the disease incidence and enhanced the plant vegetative growth as compared to control. Hence it is concluded that for the management of tomato damping off disease in nursery it could be a promising alternative amongst the plant protection measures. This bioagent can be used as soil application, seed treatment, enrichment of organic manures, drenching etc.

5. Future Research

Being a promising biocontrol agent, its effective dose can be determined to have economic viability and wider adaptability among the growers. In addition, similar assessments can be done in other vegetable crops require nursery for further transplanting. Its survival in the soil can be studied to have an idea its existence period.

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