



Role of *Trichoderma asperellum* enriched organic manures in controlling tomato damping off disease and enhancing plant growth

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ABSTRACT

Damping off disease of tomato is one of major constraints for getting desired healthy seedlings in the nursery. This disease is primarily caused by certain soil borne fungal species which can be controlled through application of certain chemical fungicides. Since recent past, organic vegetable production getting attention of researchers as well as consumers. It is so because of a number of well-known side effects on overall environment and human health. For organic vegetable production, genus *Trichoderma* is one of the promising microbial agents that can manage this disease effectively. An indigenous *T. asperellum* in combination with farmyard manure and vermicompost was tested against tomato damping off disease in pots. Findings showed that *Trichoderma* enriched organic manures reduced disease and their efficacy increased with incubation time. Fifteen days incubation of antagonist along with vermicompost resulted in to 88.3%, 1968.4, 7.6%, 22.4 cm, 4.5 cm, 3.01 g and 0.231 g seed germination, vigour index, disease incidence, seedling height, root length, fresh and dry weight, respectively followed by farmyard manure treated with antagonist and incubated for same period. In control these parameters were as 58.3%, 790.8, 25.8%, 13.6 cm, 2.9 cm, 1.44 g and 0.109 g, respectively. It is concluded from this study that *T. asperellum* can be an option for the management of damping off disease if it is mixed



with organic manures preferably vermicompost followed by fortnight incubation. In case vermicompost is not available it can also be mixed with farmyard manure for same purpose.

Introduction

Tomato (*Solanum lycopersicon* L.) is an important vegetable crop nationally as well as globally (Pritesh and Subramanian, 2011; Bawa, 2016). It possess numerous vitamins and minerals in abundant quantity (Pramanik and Mohapatra, 2017; Boccia et al., 2019) which are necessary for healthier life of human being. Its fruits are consumed in various ways. For getting optimum crop production its healthy seedlings are transplanted in to main field. Healthy seedlings are possible only through disease free nursery. While raising nursery, the seedlings are subjected to infection of soil resident pathogens especially species of *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium* etc. (Hassanisaadi et al., 2021; Shalaby et al., 2022; Ma et al., 2023; Srinivas et al., 2019; Kumhar et al., 2022; Soliman et al., 2023). Such pathogens infect the young seedlings in early stage and cause damping off disease. Damping off disease is one of the major concerns limiting production of tomato under field conditions ultimately.

This disease appears as pre- and post- emergence. In pre-emergence phase, the seeds get infected with pathogens fail to emerge out while in post emergence phase, the pathogen cause infection at collar region of young seedling and infected seedlings toppled down at soil surface which remained unfit for transplanting.

Fungicides namely difenoconazole, tetraconazole, pyraclostrobin + metiram, trifloxystrobin, metiram, mancozeb, flutolanil etc are effective against *F. oxysporum* f. sp. *lycopersici* (El-Samadisy et al., 2008). This disease can be managed through seed treatment with chemical fungicides such as emisan, captan, thiram (Anonymous, 2017). However these synthetic fungicides are not permitted when crop is cultivated organically.

Under organic crop production system biological control is one of applicable strategies for managing damping off. Species of *Trichoderma* are of great significance as far as biological control of phytopathogens is concerned (Pal and Gardener, 2006; Contreras-Cornejo et al., 2016; Zhang et al., 2022). Genus *Trichoderma* is found in natural on various sources (Druzhinina et al. 2011; Kubicek et al. 2008) and efficient in controlling number of soil inhabitant fungal pathogens (Olowe et al., 2022; Sharma et al., 2022). Soil application of this biocontrol agent along with farmyard manure and vermicompost efficiently protects the seedlings from soil borne fungal pathogens (Kumhar et al., 2022). Such organic



manures support its growth and development through ensuring nourishment. Hence, in this study, *T. asperellum* enriched farmyard manure and vermicompost incubated for different time was evaluated for the control of tomato damping off in the nursery and its effect on growth parameters.

Material and methods

Efficacy of *T. asperellum* enriched FYM, vermicompost and neem

Pot experiments were carried out under screen-house conditions at Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during year 2022–23 using *T. asperellum* (KKHT-3) and neem. There were a total of twelve treatments i.e. FYM enriched with *T. asperellum* (0 day incubation), FYM enriched with *T. asperellum* (5 days incubation), FYM enriched with *T. asperellum* (10 days incubation), FYM enriched with *T. asperellum* (15 days incubation), Vermicompost enriched with *T. asperellum* (0 day incubation), Vermicompost enriched with *T. asperellum* (5 days incubation), Vermicompost enriched with *T. asperellum* (10 days incubation), Vermicompost enriched with *T. asperellum* (15 days incubation), Neem leaves extract, Control (FYM), Control (vermicompost) and Absolute control.

For experiment, autoclaved field soil was filled in the sterilized pots. Pathogens's inocula was prepared first by growing the phytopathogenic fungus (*Fusarium oxysporum*) in potato dextrose broth in 250 ml capacity conical flask. The fungal biomass (mycelia and conidia) was harvested after 15 days of incubation. It was homogenized in a blender for about 10 -15 seconds and suspension was prepared by filtering through double layer of muslin cloth. The suspension was quantified with a haemocytometer and spore load was adjusted to 1×10^6 cfu/ml by adding sterile distilled water.

Well decomposed farmyard manure and vermicompost were enriched with *T. asperellum* liquid preparation at a concentration of 20 ml/kg substrate and incubated for 0, 5, 10 and 15 days.

To initiate disease, two hundred millilitre inocula solution of *F. oxysporum* (1×10^6 cfu/ml) was added to each pot.

On the second-day biocontrol agent enriched FYM as well as vermicompost (500 grams / pot) and freshly prepared neem preparation was added to in to the pots according to decided treatments. Tomato seeds were sown and all agronomic practices were followed. Observations on seed germination (after one week), vigour index, disease incidence (after two weeks), root and shoot length, fresh and dry weight (after one month) were recorded and calculated using different formula.



$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Vigour index} = \text{Germination (\%)} \times \text{seedling length (cm)}$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tomato seedlings}}{\text{Total number of tomato seedlings}} \times 100$$

The seedlings were carefully uprooted after a month and shoot and root lengths were measured.

Immediately after seedling uprooting, the fresh shoot weight was recorded. Seedlings were dried in the oven at 65–70°C for 16 hours to record dry weight of the seedlings.

Statistical analysis

An online statistical software “OPSTAT” of CCSHAU, Hisar was applied to work out analysis of variance (ANOVA) of organized data.

Results

Effect of *T. asperellum* enriched FYM, vermicompost and neem on tomato seed germination, vigour index, damping off disease and seedling weight

Tomato seed germination, vigour index

Extended incubation time improved the performance of *T. asperellum* in combination with farmyard manure and vermicompost. Its pot application resulted in higher seed germination and vigour index. Fifteen days incubated *Trichoderma* treated vermicompost exhibited the highest seed germination (88.3%) and vigour index (1968.4) which were higher than FYM with same antagonist and incubation period. Neem application also enhanced seed germination and vigour index as compared to absolute control where it was 58.3% and 790.8, respectively (Table 1).

Damping off incidence

Increasing the incubation time of the biocontrol agent applied organic manures better protected the tomato seedlings from damping off disease in comparison to absolute control. Vermicompost enriched with biocontrol agent with incubation time of 15 days showed the lowest disease incidence (7.6%)



followed by FYM enriched with antagonist (10.1%). There was 15.5% disease incidence in neem applied pots as compared to 25.8% in control (Table 2, Photo 1).

Plant height and root length

Among all the experimental treatments, *T. asperellum* enriched vermicompost which was incubated for one fortnight showed the maximum highest plant height (22.4 cm) and root length (4.5 cm). FYM with this antagonist and same incubation period ranked the 2nd in performance where plant height and root length was 20.3 cm and 4.1 cm, respectively. Neem application also had positive effect on enhancement of plant height as well as root where both were 17.9 cm and 3.3 cm, respectively. In absolute control the plant height and root length were minimum (Table 3).

Seedling fresh and dry weight

Fifteen days incubation of *Trichoderma* treated vermicompost found the best in supporting increased seedling weight. The fresh and dry weight of seedlings was 3.01 g and 0.231 g, respectively. With same incubation period of FYM enriched with antagonist showed 2.94 g fresh weight and 0.209 g dry weight. In case of neem application fresh and dry weight was 2.13 g and 0.195 g, respectively as compared to absolute control where it was 1.44 g and 0.109 g, respectively (Table 4)

Discussion

In the present experiment, effectiveness of *T. asperellum* (KKHT-3) in suppressing damping off was assessed by enriching the FYM and vermicompost with the *T. asperellum* (KKHT-3) at a concentration of 2% for different incubation time of 0, 5, 10 and 15 days. Neem leaf extract was also included as one of treatments for this purpose. Study revealed that vermicompost enriched with biocontrol agent with incubation time of 15 days showed lowest disease incidence and significantly higher seed germination, disease reduction, vigour index, seedling length, shoot length, root length, fresh weight and dry weight as compared to control. Neem also performed better than the control. The findings of our study are similar to the findings of Kumhar *et al.* (2022) who tested indigenous *T. asperellum* in nursery and found that there was higher seed germination and lesser as compared to control. Kareem and Matloob (2019) tested *T. harzianum* under field conditions and observed that seed germination was increased and disease incidence was reduced in comparison to control. Yücel *et al.* (2016) evaluated *T. harzianum* isolates under field conditions and observed that damping-off incidence was reduced to 15-23% as compared to control.



Singh and Yaduman, (2016) evaluated the efficacy of plant leaf extracts against the damping-off at 40 and 60% concentrations in field conditions and found that among all the extracts tested, neem leaf extract significantly reduced the damping-off incidence as compared to the control. Islam and Faruq (2012) assessed neem, bel, allamonda, katamehedi, ginger, kalijira, turmeric and onion bulb extract for their effects on damping-off, seed germination, and the growth characteristics of tomato, eggplant, and chilli seedlings and observed that neem leaf extract as the most effective one in reducing damping-off disease incidence along with improvement plant growth parameters. Lalremruatpuii and Banik (2022) evaluated the effect of *Trichoderma* on tomato plant growth in combination with vermicompost, NPK, and farmyard manure (FYM). They found that 50% NPK + 50% vermicompost had highest *Trichoderma* population, mean plant height, number of leaves and other plant growth metrics.

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Table 1: Performance of *T. asperellum* incubated organic manures and neem on tomato seed germination and vigour index

Treatment	Germination (%)	Vigour index
FYM enriched with <i>T. asperellum</i> (0 day incubation)	73.3	1237.4
FYM enriched with <i>T. asperellum</i> (5 days incubation)	78.3	1433.8
FYM enriched with <i>T. asperellum</i> (10 days incubation)	81.7	1537.5
FYM enriched with <i>T. asperellum</i> (15 days incubation)	85.0	1715.9
Vermicompost enriched with <i>T. asperellum</i> (0 day incubation)	78.3	1356.2
Vermicompost enriched with <i>T. asperellum</i> (5 days incubation)	81.7	1527.2
Vermicompost enriched with <i>T. asperellum</i> (10 days incubation)	83.3	1675.8
Vermicompost enriched with <i>T. asperellum</i> (15 days incubation)	88.3	1968.4
Neem leaves extract	75.0	1340.0
Control (FYM)	61.7	898.3
Control (vermicompost)	63.3	907.4
Absolute control	58.3	790.8
C.D (p = 0.05)	4.1	-

Mean of three replications

**Table 2: Effect of *T. asperellum* incubated organic manures on tomato damping off**

Treatment	Disease incidence (%)
FYM enriched with <i>T. asperellum</i> (0 day incubation)	15.9 (23.7±1.6)
FYM enriched with <i>T. asperellum</i> (5 days incubation)	14.9 (22.6 ±1.5)
FYM enriched with <i>T. asperellum</i> (10 days incubation)	12.2 (20.5± 0.2)
FYM enriched with <i>T. asperellum</i> (15 days incubation)	10.1 (18.4 ±1.7)
Vermicompost enriched with <i>T. asperellum</i> (0 day incubation)	15.0 (22.6 ±1.9)
Vermicompost enriched with <i>T. asperellum</i> (5 days incubation)	12.2 (20.5 ±0.2)
Vermicompost enriched with <i>T. asperellum</i> (10 days incubation)	11.5 (19.8 ±0.2)
Vermicompost enriched with <i>T. asperellum</i> (15 days incubation)	7.6 (15.1 ±2.1)
Neem leaves extract	15.5 (23.1 ±1.310)
Control (FYM)	24.4 (29.6± 0.4)
Control (vermicompost)	23.7 (29.1 ±0.4)
Absolute control	25.8 (30.5 ±0.5)
C.D (p = 0.05)	3.7
C.V	9.4

Mean of 3 replications ± SE

**Table 3: Effect of *T. asperellum* incubated organic manures on tomato plant growth**

Treatment	Plant height (cm)	Root length (cm)
FYM enriched with <i>T. asperellum</i> (0 day incubation)	16.9±1.2	3.3±0.3
FYM enriched with <i>T. asperellum</i> (5 days incubation)	18.390±0.1	3.4±0.2
FYM enriched with <i>T. asperellum</i> (10 days incubation)	18.893±0.6	3.7±0.3
FYM enriched with <i>T. asperellum</i> (15 days incubation)	20.3±0.2	4.1±0.4
Vermicompost enriched with <i>T. asperellum</i> (0 day incubation)	17.3±0.4	3.5±0.1
Vermicompost enriched with <i>T. asperellum</i> (5 days incubation)	18.7±0.1	3.5±0.1
Vermicompost enriched with <i>T. asperellum</i> (10 days incubation)	19.4±0.3	3.9±0.1
Vermicompost enriched with <i>T. asperellum</i> (15 days incubation)	22.4±0.8	4.5±0.1
Neem leaves extract	17.9±0.1	3.3±0.0
Control (FYM)	14.6±0.4	3.0±0.1
Control (vermicompost)	14.3±0.540	2.9±0.1
Absolute control	13.6±0.0	2.9±0.0
C.D (p = 0.05)	1.5	0.4

Mean of 3 replications ± SE

Table 4: Effect of *T. asperellum* enriched FYM, vermicompost and neem on fresh and dry weight of seedling

Treatment	Fresh weight (g)	Dry weight (g)
FYM enriched with <i>T. asperellum</i> (0 day incubation)	2.06±0.09	0.140±0.03
FYM enriched with <i>T. asperellum</i> (5 days incubation)	2.15±0.19	0.156±0.01
FYM enriched with <i>T. asperellum</i> (10 days incubation)	2.40±0.44	0.197±0.03
FYM enriched with <i>T. asperellum</i> (15 days incubation)	2.94±0.30	0.209±0.02
Vermicompost enriched with <i>T. asperellum</i> (0 day incubation)	2.11±0.08	0.129±0.01
Vermicompost enriched with <i>T. asperellum</i> (5 days incubation)	2.44±0.31	0.171±0.02
Vermicompost enriched with <i>T. asperellum</i> (10 days incubation)	2.52±0.25	0.189±0.01
Vermicompost enriched with <i>T. asperellum</i> (15 days incubation)	3.01±0.17	0.231±0.01
Neem leaves extract	2.13±0.04	0.195±0.01
Control (FYM)	1.86±0.16	0.125±0.01
Control (vermicompost)	1.71±0.21	0.119±0.03
Absolute control	1.44±0.20	0.109±0.01
C.D. (p = 0.05)	0.7	0.04

Mean of 3 replications ± SE

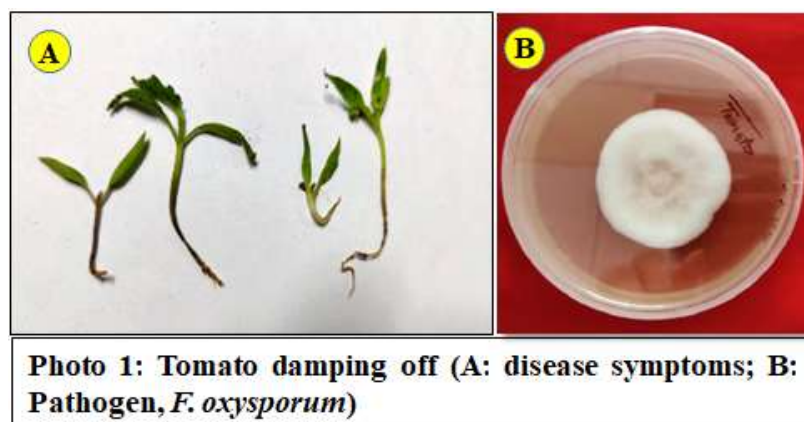


Photo 1: Tomato damping off (A: disease symptoms; B: Pathogen, *F. oxysporum*)