



Research Article

Comparative Efficacy of Biological, Botanical and Chemical Treatments Against Damping Off Disease of Tomato in Chitwan

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Keywords: *Pythium* spp.; damping off; *Trichoderma*; disease incidence

Abstract

A field experiment on “Comparative Efficacy of Biological, Botanical and Chemical Seed Treatments against Damping Off Disease of Tomato in Bhojad, Chitwan” was conducted from 1st October to 1st November 2020. The test was placed on Randomized Complete Block Design (RCBD) with five treatments and four responses. The treatments were included *Trichoderma harzianum* (10^7 cfu ml⁻¹) as a bio control agent, Bavistin (2g/litre of water) as chemical treatments and Neem extract as botanical treatments. The germination percentage, pre, and post-emergence damping-off, plant height, root weight and shoot weight, dry root and dry shoot weight of tomato seedlings were recorded. There was a significant increment in germination percentage (86.25%), reduction in pre and post disease incidence (23% and 5.16%), highest plant height (41.20 cm), highest fresh root weight, and fresh shoot weight (0.9725g and 12.300g), highest dry root and dry shoot weight (0.1975g and 1.393g) respectively were found when the Bavistin treatment was given however *Trichoderma harzianum* (10^7 cfu ml⁻¹) treatment was also found significantly effective for controlling damping-off of tomato as compared to control which is non-hazardous to environment and the human being so, is suggested to use for organic agriculture.

Introduction

Nepal is a country having a wide range of agro-climatic and topographical conditions from subtropical, temperate to cold climate, it has a high potentiality of growing different vegetable crops. Nepal produces vegetables worth NRs 55 billion annually and around 70 % of total households are being involved in vegetable farming with about NRs 12

billion invested in farming every year in the nation. Terai is considered to be the largest growing vegetable area with an annual production of 1,437,921 mt followed by the hilly region of Nepal with 1,261,041 mt. Of the total production, 39% (1.10 million mt) is used for domestic food, and 61% (1.71 million mt) are sold. (Chalise & Bhandari, 2014).

Among the various vegetable crops, tomato is the most important vegetable crop having high market potentialities. The tomato (*Lycopersicon esculentum*), one of the members of the nightshade family (Solanaceae), which includes potato, pepper, and eggplant, was first considered to be poisonous to humans (André Lévesque & De Cock, 2004). The average national consumption of tomatoes in Nepal was 11.97Kg/person/year. It is an important vegetable crop with an estimated global production of 120 million metric tons and Iraq produces only 913493 tons (FAOSTAT, n.d.). The National Agriculture Statistics Service reported that the value of the new tomato market in 2005 was about \$1.6 billion (Anonymous, 2006). Tomato is a warm-season crop; it requires a warm and cool climate. The plant cannot withstand frost and high humidity. The average temperature for Tomato cultivation is 21-24°C and the average rainfall is all 194mm. Loam and Sandy Loam soils are best for Tomato cultivation, but these types of plants grow in all types of soil except heavy clay. Around 7,500 tomato varieties are grown for numerous functions (Garibaldi, 2012).

Even though the tomato is commercially grown throughout the globe, there is no region where the plant is disease-free. A number of the primary constraints of tomato, production is the damage due to pathogens, inclusive of viruses, bacteria, nematodes, and fungi, which result in severe losses in production (Moretti et al., 2002). Damping-off is the most common disease of the seedling's vegetables. (Buchenaer, 1998) reported that Damping-off is an important soil-borne disease attacking plants. It is caused by several important vegetable pathogens and is very common during the spring. The two fungi which might be most usually related to damping-off are *Rhizoctonia solani* and *Pythium* species, other fungi that may be related to seedling or transplanting damping-off are *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *S.minor*, *Alternaria sp*, *Phytophthora sp*, *Fusarium sp*, *Thielaviopsis basicola*. Three species of *Pythium* causing damping-off disease are *P. aphanidermatum*, *P. debaryanum*, *P. ultimum*. Amongst them, the damping-off because of *Pythium aphanidermatum* in nurseries is a first-rate constraint inside the production of tomatoes. *Pythium* spp. is essential soil-borne (Report, 2010) and pose a greater problem in disease management. Damping-off disease caused by *Pythium* species usually begins as root rot. *Rhizoctonia solani* is positioned in most agricultural soils and survives among plants on plant residues and as micro *sclerotia*. This pathogen assaults seedling at or near the soil surface. Damping-off can kill seedlings before they ruin the soil line (pre-emergent damping-off) or kill seedlings soon after they emerge (post-emergent damping off). In general, *Phytophthora* spp. and *Pythium* spp. are more likely to cause damping off in cool wet over watered soil. Pre- and post-emergence damping-off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide. One of the major causes of seedling

loss is damping-off, a disease that is caused by a variety of fungi, including the fungal-like organism, *Pythium myriotylum* Drechsler. Because of the ability of *Pythium* spp. to travel through water and to survive long, harsh periods as resting spores has threaten the production of tomato. Rapid germination of sporangia of *Pythium* after exposure to exudates or volatiles from seeds or roots followed by immediate infection makes management of *Pythium* very difficult (Fontem, 1993). surveyed 67 tomato fields and found that damping-off fungi *Rhizoctonia solania* and *Verticillium albo-atrum* attacked all tomato nurseries.

The most aggressive species of *Pythium* that causes important plant diseases is *P. aphanidermatum*. It is a soil as well as seed born pathogen. They are the most important and responsible for seedling before and after germination. Diseases caused by *P. aphanidermatum* vary depending on the host plant, causing damping-off before and after the emergence of various plants. It also causes seed rot, root rot, cottony leak, cottony blight, stem rot, etc. Moreover, new hosts and diseases caused by this destructive pathogen are continuously reported. (André Lévesque & De Cock, 2004) reported that a significant number of studies on *P. aphanidermatum* have been carried out in different parts of the world. It affects nearly every crop grown in every part of the world (Ben-Yephet & Nelson, 1999).

In writings of early civilizations, as far as 4000 B.C., there have been documentations on the preparation and use of plants for medicinal uses, and the 3 antimicrobial properties of some of these plants have been documented for efficacy in controlling disease (Bishop & MacDonald, 1951). Different organisms can be successfully used as biocontrol agents, such as *Trichoderma* spp. (Abdel-Monaim et al., 2011), *Bacillus* spp. (Cuevas & Banaay, 2022) and *Pseudomonas* spp. (Manorantitham et al., 2001). Seed treatment is defined as the process in which you take untreated seeds, which have low germination capacity and treat them to make them capable to germinate in any condition. When the seed is treated, the essential oils of *Chenopodium ambrosioides* (Chenopodiaceae) can reduce the damping-off of tomato in soil infested with *Pythium aphanidermatum* or *P. debaryanum* by 67 and 100%, respectively (Bardin et al., 2004). Damping-off caused germination and emergence problems (Whipps & Lumsden, 1991) suggested Topsin-M and Benlate as the most effective fungicides in laboratory tests against *Rhizoctonia* on tomatoes. (Forcelini et al., 2001) and (Deising et al., 2008) focused on the increased use of chemicals which has become an alarming issue in an increase in fungicide resistance of plant pathogens in several crops in modern agricultural practices in several countries. Biological control methods along with the use of natural antagonists of pathogens and predators, parasitoids and parasites have been suggested as a safe and suitable alternative method of chemical method of disease and insect control. Different

management practices such as cultural practices, biological control, chemicals and integrated disease management practices can reduce the incidence of different kinds of diseases in crops but it should be considered that the impact of the use of chemicals on animals and human health, effect the environment, cost-effectiveness as well as sustainability in production (Magar et al., 2020). Due to adverse effects on human health and the environment, the use of pesticides, including fungicides, has come under increasing public demand in many lands, especially in the European Union (Bourguet & Guillemaud, 2016). Biocontrol can also be used as an alternative to common fungicides, especially if this is not effective or can cause any fungal problems such as seed phytotoxicity from fungicides (Burns & Benson, 2000). Many species within the genus *Trichoderma* are well known for their biological control capabilities against a

wide range of commercially important plant pathogens (Whipps & Lumsden, 1991)

Materials and Methods

The experiment was conducted in the sick plot of Nepal Polytechnic Institute (NPI) Bhojad-11, Chitwan (Fig. 1). To include biological, botanical and chemical for seed treatment of tomato. Five treatments included were *Trichoderma*, Neem extract, Cow urine, Bavistin and control. These materials were used as a seed treatment. Treatments effects would be evaluated by different parameters. Treatments effects would submit to variance analysis and when significant will be adjusted by regression equations. The details of the treatments that were used in the experiment are given in Table 1.

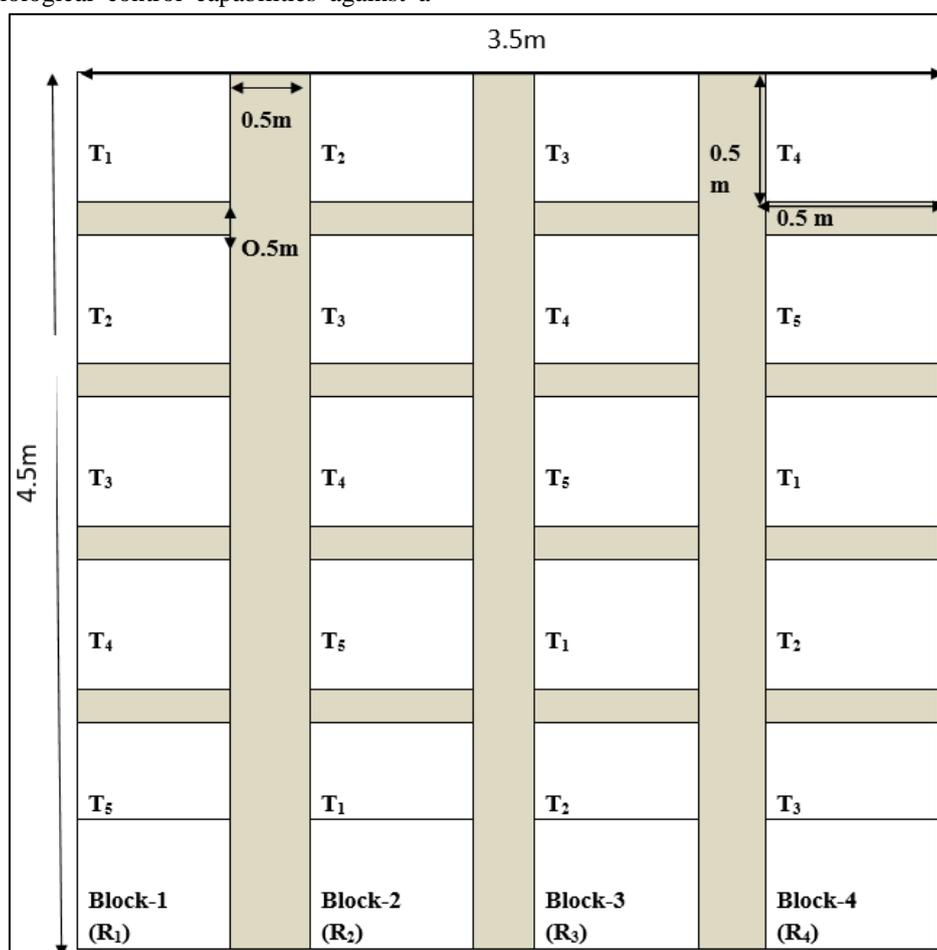


Fig. 1: Layout of the experimental plot

Table 1 : Detail of the treatments used in the experiment

SN	Seed Treatments	Symbol
1	<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	T1
2	Neem extract @100g plant parts +200ml distilled water	T2
3	Cow urine @1lit + 5lit water	T3
4	Bavistin 0.1% AI	T4
5	Control	T5

The commonly grown variety “Srijana hybrid” was brought from the local market for the experiment. Biocontrol agent *Trichoderma harzianum* was collected in liquid form from the local market. The liquid form *Trichoderma harzianum* was diluted for seed treatment by adding 2ml per liter water and seeds were treated for 2 hrs. Neem leaves were collected fresh and grinded in a blender and juice was extracted from the leaves. Extract 100 gm of leaves mixed with 200 ml of distilled water and tomato seeds soaked for 2 hours. Fresh cow urine was collected from the cow shade of NPI. It was fermented for 4 weeks and was mixed with water at the ratio of 1:5 for seed treatment. Seeds were dipped in solution for 12 hrs. Systemic fungicides Bavistin (Carbendazim 50%) were collected from the local market of Narayanghat. Chemical fungicide Bavistin at the rate of 2g/lit water was taken for the seed treatment and seeds were dipped in the suspension for 10 minutes. The experiment was conducted in RCBD with 4 replications and five different treatments. The total area for the field experiment was 5.5 m*6.5 m. There were 20 plots with an individual plot size of 0.5 m². Interblock and inter plot spacing were 50cm*50cm respectively. The outer border was left 1m from each side. Treatments were randomly allocated in experimental units.

The observation was done on regular basis to record the data of germinated seedlings and to record the data for other parameters (seedling height, root weight, shoot weight, dry root weight, and dry shoot weight). Germination and non-germination % were also observed at the same time. Ten sample plants were randomly selected and tagged for further observation.

$$\text{Germination \%} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Disease incidence \%} = \frac{\text{No. of infected seedlings}}{\text{Total no. of seedling in a plot}} \times 100$$

The data were recorded based on different parameters like germination percentage, the incidence of damping off, plant height, shoot weight, root weight and dry root and scientifically shoot weight. The recorded data were tabulated in an Excel datasheet and were analyzed by using the Gen stat software program. The data entry was done to develop an ANOVA table and different treatments were compared through Duncan’s multiple range test. All the figures and graphs were prepared by using Microsoft excel.

Results and Discussion

Effect of Different Treatments on Germination Percentage of Tomato at Nursery

Germination percentage was recorded on 5, 10, 15, 20, 25 and 30 days after sowing respectively. Results showed a significant difference in mean germination % concerning different treatments (Table 2). The highest germination % was recorded in seed treatment with Bavistin followed by

Trichoderma harzianum, cow urine and neem extract. However, there was no significant difference in germination percentage of tomato seed treated with *T. harzianum* and cow urine. Similarly, there was no significant difference between germination percentage when seeds were treated with Neem extract and that of Control. Bavistin resulted in highest germination percentage of seed (61.75%) and (15.50%) was recorded lowest in control on the 5th day after sowing. The germination percentage of seed reached up to 86.25% with Bavistin whereas seed treatment with *Trichoderma harzianum* could reach only 66.50% germination and Control resulted significantly lowest germination percentage 40.75%.

The chemical seed treatment with Bavistin helped the higher germination percentage (86.25%). Chemical fungicides have been used for a long time as it gives quick response and effective control for disease management. Carbendazim @ 0.25% was found to achieve 20 and 25% inhibition (Zalte et al., 2013). The *Trichoderma harzianum* was also found effective in reducing disease incidence and increasing crop germination (Ismael & Mahmood, 2016). The germination percentage of seed treated with *Trichoderma harzianum* was (66.50%). Possible mechanisms of antagonisms employed by *Trichoderma* spp. include, genetic and niche competitions, antibiosis for the production of flexible components and consistent antibiotics that block the broad range of soil-borne fungi, as well as parasitism (Abdel-Monaim et al., 2011; Sy et al., 2008). The present research indicated that the use of seed treatment with Bavistin and *Trichoderma harzianum* was the best option for the management of damping-off disease of tomato seedlings in the nursery among other treatments. It also helped to increase germination for the production of healthy seedlings in the nursery.

Effect of Seed Treatments on Pre-Emergence and Post Emergence Damping-Off

In the context of eliminating the emergence of diseases caused by *Pythium* spp., Analysis of Variance (ANOVA) revealed significant differences between treatments. The lowest pre-emergence damping-off disease percentage was recorded in seed treatment with Bavistin (23%) which is followed by seed treatment with *Trichoderma* (32%), seed treatment with neem extract (44.50%) respectively (Table 3) Also, in case of post-emergence damping-off disease incidence caused by soil pathogens, Analysis of Variance (ANOVA) revealed a significant difference between the treatments. The lowest post incidence damping-off disease percentage was recorded in seed treatment with Bavistin (5.16%). Seed treatment with *Trichoderma* (6.50%), cow urine (9.14%), and control had the highest (24.65%) post-emergence disease incidence.

The effect of chemical fungicide Bavistin and biological control agent *Trichoderma* resulted in significantly better

options for the management of pre-emergence damping-off and post-emergence damping-off of tomato seedlings in the nursery as compared to other treatments. The *Trichoderma harzianum* was found effective in reducing disease incidence and increasing crop germination (Manorantitham et al., 2001). The effect of Bavistin was found to be best in controlling pre-emergence damping-off (23%) and post-

emergence damping-off (5.16%) caused by *Pythium* spp. Successful management of damping-off of seedlings caused by *Pythium* spp. in various crops by application of *Trichoderma harzianum* has been previously supported by (Jayaraj et al., 2006) which is in agreement with our present findings.

Table 2: Effect of different treatments on germination percentage of tomato at nursery

Seed Treatments	Germination %					
	5 DAS	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS
Bavistin 0.1% AI	61.75 ^a	66.75 ^a	76.75 ^a	80.00 ^a	83.25 ^a	86.25 ^a
<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	41.00 ^b	46.00 ^b	56.00 ^b	60.00 ^b	64.50 ^b	66.50 ^b
Cow urine @1:5 ratio	37.00 ^{bc}	42.25 ^{bc}	52.25 ^{bc}	56.25 ^{bc}	64.00 ^b	66.00 ^b
Neem extract @1:2 ratio	24.00 ^{cd}	29.00 ^{cd}	39.00 ^{cd}	43.00 ^{cd}	47.00 ^c	49.00 ^c
Control (plain water)	15.50 ^d	20.50 ^d	30.50 ^d	34.50 ^d	38.50 ^c	40.75 ^c
SEM (±)	4.75	4.78	4.78	4.63	4.92	5.15
P-Value	<.001	<.001	<.001	<.001	<.001	<.001
LSD (≥ 0.05)	14.32	14.42	14.42	13.96	14.83	15.51
CV (%)	26.5	23.4	18.8	16.9	16.6	16.7

CV=coefficient of variation, LSD=Least significant difference, SEM=Standard Error of mean, Letters denoted by the same letter do not differ significantly.

Table 3: Effect of treatments on the percentage of pre-emergence and postemergence damping-off disease of tomato in the seedling stage

Seed Treatments	Pre-emergence Damping-off (%)	Post-emergence Damping-off (%)
Bavistin 0.1% AI	23.00 ^a	5.16 ^a
<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	32.00 ^a	6.50 ^b
Cow urine @1:5 ratio	45.00 ^b	9.14 ^{ab}
Neem extract @1:2 ratio	44.50 ^b	11.72 ^{ab}
Control (plain water)	48.00 ^{bc}	24.65 ^c
SEM (±)	3.63	2.590
P-Value	<.002	<.001
LSD (≥ 0.05)	11.17	5.644
CV (%)	18.8	32.0

CV=coefficient of variation, LSD=Least significant difference, SEM=Standard Error of mean, Letters denoted by the same letter do not differ significantly

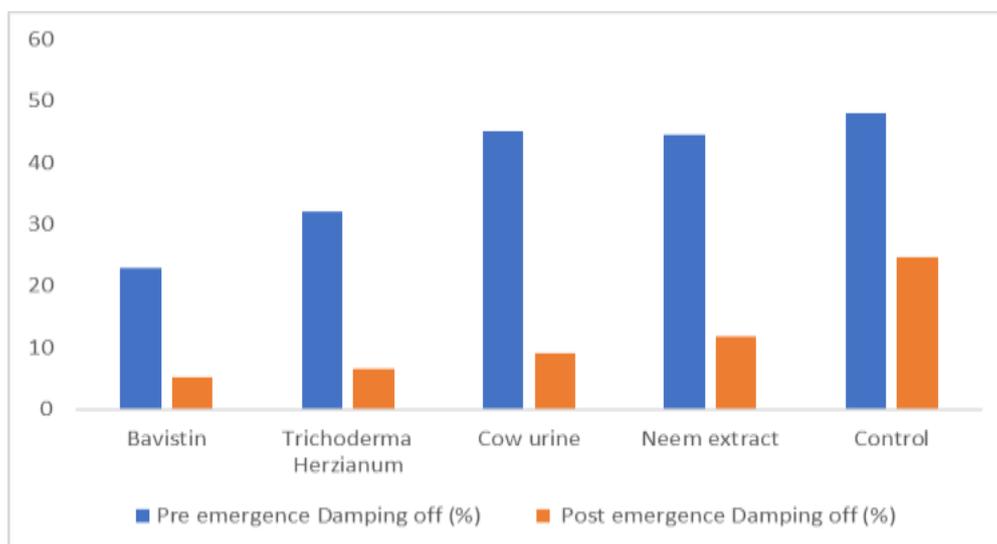


Fig. 2: Effect of treatments on Pre-emergence and Post-emergence Damping-off disease on tomato seedlings

Effect of Seed Treatments on Plant Height of Tomato Seedlings

Plant height was recorded on 10, 15, 20, 25, and 30 days after sowing respectively. Results showed a significant difference in plant height for different treatments. The highest plant height was recorded in seed treatment with Bavistin followed by *Trichoderma harzianum*, cow urine and neem extract (Table 4). However, there was no significant difference in plant height of tomato seed treated with *T. harzianum* and cow urine. There was a significant difference between plant height when seeds were treated with Neem extract and that of Control. Bavistin resulted in the highest plant height of seedlings (8.300 cm) and (4.050 cm) was recorded lowest in control on 10 days after sowing. The plant height of seed treated reached up to 41.20 cm with Bavistin whereas seed treatment with *Trichoderma harzianum* could reach only 38.35cm plant height and Control resulted significantly lowest plant height 37.77cm. This result was supported by (ISMAEL & MAHMOOD, 2016) Bal and ; (Hanson, 2000) as they reported the same positive effects of *Trichoderma* spp. on the growth and development of seedlings of vegetable and non-vegetable crops, namely cabbage, cucumber, lettuce and cotton and antagonistic behavior to pathogen *Pythium* spp.

Colonization of the root system by the rhizosphere capable of *Trichoderma* leads to increased development of roots and/or air systems and crop yields. (Harman, 2006).

Effect of Treatment on Root Weight and Shoot Weight of Tomato Seedlings

In 30 DAS, the tomato seedlings were uprooted, fresh root weight and shoot weight of sampled seedlings were recorded simultaneously. Results showed a significant difference in root weight and shoot weight to different treatments (Table 5). The highest root weight and shoot weight were recorded in seed treatment with Bavistin followed by *Trichoderma harzianum*, cow urine and neem extract. Bavistin resulted in the highest root and shoot weight of seed (0.9725g) and (12.300g) respectively and (0.4250g) and (5.000g) was recorded lowest in control on 30 days after sowing. All *Trichoderma* had significant positive effects on root weight and significantly increased root weight of seedlings, positively affecting the shoot weight. (Manorantitham *et al.*, 2001) . Successful management of damping-off of seedlings caused by *Pythium* spp in various crops by application of *Trichoderma harzianum* has been previously supported by(Lamont, 2005) which is in agreement with our present findings.

Table 4: Effect of seed treatments on plant height of tomato seedlings

Seed Treatments	Plant height				
	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS
Bavistin (WP) 0.1% a.i	8.300 ^a	13.30 ^a	20.20 ^a	30.80 ^a	41.20 ^a
<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	6.250 ^b	11.25 ^b	17.35 ^b	27.45 ^b	38.35 ^b
Cow urine @1:5 ratio	5.150 ^{bc}	10.12 ^{bc}	17.05 ^b	26.35 ^{bc}	38.05 ^b
Neem extract @1:2 ratio	5.125 ^{bc}	10.10 ^{bc}	16.15 ^{bc}	26.32 ^{bc}	37.15 ^{bc}
Control (plain water)	4.050 ^c	9.05 ^c	14.75 ^c	25.25 ^c	35.77 ^c
SEM (±)	0.547	0.565	0.543	0.547	0.540
P-Value	<.001	.001	<.001	<.001	<.001
LSD (≥ 0.05)	1.648	1.715	1.635	1.648	1.629
CV (%)	18.9	10.5	6.3	4.0	2.8

CV=coefficient of variation, LSD=Least significant difference, SEM=Standard Error of mean, Letters denoted by the same letter do not differ significantly

Table 5: Effect of treatment and on root weight and shoot weight of tomato at seedlings stage

Seed Treatments	Root weight (g)	Shoot weight (g)
Bavistin 0.1% AI	0.9725 ^a	12.300 ^a
<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	0.8425 ^{ab}	10.125 ^{ab}
Cow urine @1:5 ratio	0.6800 ^{bc}	8.200 ^{bc}
Neem extract @1:2 ratio	0.6100 ^{cd}	6.975 ^{cd}
Control (plain water)	0.4250 ^d	5.000 ^d
SEM (±)	0.0673	0.983
P-Value	<0.001	0.001
LSD (≥ 0.05)	0.2028	2.964
CV (%)	19.1	23.1

CV=coefficient of variation, LSD=Least significant difference, SEM=Standard Error of mean, Letters denoted by the same letter do not differ significantly

Table 6: Effect of treatment and on dry root weight and dry shoot weight of tomato at seedlings stage

Seed Treatments	Dry Root weight (g)	Dry Shoot weight (g)
Bavistin 0.1% AI	0.1975 ^a	1.393 ^a
<i>Trichoderma harzianum</i> @10 ⁷ cfu ml ⁻¹	0.1725 ^{ab}	1.125 ^{ab}
Cow urine @1:5 ratio	0.1650 ^{ab}	1.090 ^{ab}
Neem extract @1:2 ratio	0.1525 ^{ab}	0.878 ^{bc}
Control (plain water)	0.1050 ^b	0.655 ^c
SEM (±)	0.0265	0.1359
P-Value	0.213	0.018
LSD (≥ 0.05)	0.0800	0.4096
CV (%)	33.5	26.4

CV=coefficient of variation, LSD=Least significant difference, SEM=Standard Error of mean, Letters denoted by the same letter do not differ significantly

Effect of Treatment on Dry Root Weight and Dry Shoot Weight of Tomato Seedlings

In 30 DAS, roots and shoots of tomato seedlings were separated and fresh roots and shoots were covered with newspaper and kept in a hot air oven to make them dry. After 24 hrs dry root and dry shoot of sampled seedlings were recorded simultaneously. Results showed a significant difference in dry root weight and dry shoot weight concerning different treatments. The highest dry root weight and dry shoot weight were recorded in seed treatment with Bavistin followed by *Trichoderma harzianum*, cow urine, and neem extract Table 6). Bavistin resulted in the highest dry root and dry shoot weight of seed (0.1975g) and (1.393g) respectively and (0.1050g) and (0.655g) was recorded lowest in control when measured on weighing balance. Root enhancement caused by *T. harzianum* treatment has also been reported for sweet peppers, cucumber plants with 24% dry roots and 45% root growth. (Idowu et al., 2016)

Conclusion

Damping-off is the most common disease he vegetable seedlings. It is one of the major diseases causing a huge loss in tomato production. Damping-off caused by *Pythium* spp. infects the seedlings and destroy the whole nursery. The present study was conducted to find out better management options other than chemicals for the management of damping-off disease and to produce healthy seedlings of tomato in Bharatpur, Chitwan. Among the different treatments, although the chemical Bavistin showed the best result, however, biological control was also found to significantly better control measure as compared to other treatments. Moreover, the use of the biological agent *Trichoderma* also showed a significant reduction in pre and post-emergence disease incidence and resulted in a higher germination percentage, with higher dry root weight and dry shoot weight. The chemical although being effective is hazardous to the environment and soil. Therefore, the bio-control method could be suggested to be used by the farmer

for the management of damping-off of tomato seedlings in the nursery which is effective and eco-friendly.

Conflict of Interest

The authors declare no conflict of interest

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