The Pharma Innovation Journal 2023; 12(4): 1966-1969

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1966-1969

www.thepharmajournal.com
Received: 07-01-2023
Accepted: 11-02-2023

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Studies on efficacy of bioagents and fungicide against damping-off of tomato caused by *Pythium aphanidermatum*

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Abstract
The present study was aimed to evaluate the efficacy of biological agents (*Trichoderma asperellum* and *Pseudomonas fluorescens*) and a fungicide, Carbendazim (12%) + Mancozeb (63%) application in resisting damping-off disease. The experiment was conducted under pot culture conditions to observe the effects of bioagents and fungicide against *P. aphanidermatum.* 10 treatments were taken up with 3 replication and data collected were analysed using CRD. The treated seeds were sown in pathogen inoculated soil @10 seeds per pot and irrigated daily. Pots with no pathogen inoculation served as control. The results revealed that seed treatment with fungicide and bioagents against damping-off (*Pythium aphanidermatum*) as compared to control were significant. Maximum germination was observed in Carbendazim (12%) + Mancozeb (63%) @ 3 g/kg (91%) and among the bioagents *T. asperellum* @ 4 g/kg (88%) followed by *P. fluorescens* @ 4 g/kg (86%) as compared to control (56%).

Keywords: *Trichoderma asperellum*, *Pseudomonas fluorescens*, bioagents, fungicide, *P. aphanidermatum*

Introduction
Tomato (*Solanum esculentum* Miller) is a solanaceous fruit vegetable and the second most significant crop after potatoes. It is commonly called as "Poor man's orange" and is extensively cultivated and traded worldwide. Tomatoes are consumed in various forms, including raw, cooked, and processed products such as paste, powder, ketchup, sauce, soup, and canned whole fruits. Tomatoes are highly nutritious and contain a variety of essential elements, including vitamins A, B, C, and iron. Damping-off disease is one of the most severe and widespread diseases that affects seedlings of various vegetable crops raised in nurseries. This disease causes significant yield losses, especially under favourable conditions.

Damping-off disease in tomato occurs in two phases: pre-emergence and post-emergence. Jukte *et al*. (2016) [10] proved that in the pre-emergence phase, the seedlings are killed just before reaching the soil surface. In contrast, the post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level. The infected tissues become soft, water-soaked, and the seedlings topple over the ground. *Pythium* species, which are essentially soil-borne, pose a significant problem in disease management due to their wide host range, soil-borne nature, prolonged survival in soil saprophyte growth, and resting structure. As a result, controlling this pathogen is difficult.

The use of bio-agents for plant protection has assumed greater importance in recent years all over the world due to environmental pollution and health hazards associated with the indiscriminate use of synthetic fungicides, use of fungicides can be minimized by the integrated approach towards the management of plant diseases. This study aimed to assess the effectiveness of various bioagents and a fungicide at different doses against damping-off disease in tomato by treating the seeds and to find the safe and cheap way to control damping-off of tomato.

Material and Methods
Survey
In order to investigate the variation in disease incidence, tomato plants exhibiting symptoms of damping-off disease were gathered from tomato fields in Parbhani district *viz*. Khanapur, Pimpari Deshmukh, Selu, and Pedgaon. The diseased plants were carefully placed in plastic bags and transported to the laboratory to isolate the fungi responsible for causing the disease.

Data on disease incidence were collected and recorded during the survey.

In each field randomly 10 m^2 area was selected and number of infected plants in selected area were counted. Disease incidence of each field was calculated by using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants showing disease symptoms}}{\text{Total number of plants observed}} \times 100$$

Isolation

The tissue segment method was used to isolate the fungus responsible for tomato damping-off from the collar region of diseased plants (Vaartaja and Bumbieris, 1964). The single hyphal tip method was used to purify it. Small portions of sick and healthy tissue were removed from the collar region (3 mm) with a sterile scalpel. The pieces were treated with 0.1 percent sodium hypochlorite solution for 30 seconds to surface sterilize them. The tissue fragments were then washed three times in sterile distilled water. The tissue bits were surface sterilized, placed on Petri dishes with PDA medium, and incubated in BOD at 28±2 °C with growth being checked often. Purification of *P. aphanidermatum* isolates were done on PDA and maintained in slants for further use.

Mass Multiplication

Sand: Maize medium (3 part partially broken maize grains + 1 part sand + distilled water to moisten the medium) was prepared, filled into polypropylene bags (9 x 12 cm) and autoclaved at 20 lbs pressure for 30 minutes, for two consecutive days (Muthuswamy, 1972) [14]. After cooling at room temperature, the sterilized sand: maize medium in bags was inoculated with 8-10 mycelial discs (5 mm dia.) of the test pathogen obtained from a week-old culture and incubated at room temperature for two weeks and used for making the soil sick with the test pathogen.

Pathogenicity Test

Pathogenicity of the test fungus was confirmed by sick soil method in pots under screen house conditions. Pure culture of test pathogen multiplied on sand: maize medium was uniformly mixed @ 100 gm/kg soil with sterilized potting mixture of soil: sand: FYM (2:1:1). For this purpose, two pots were cleaned with a 5 percent formaldehyde solution, then they were filled with potting mixture. Pots without the culture of *P. aphanidermatum* was kept as the uninoculated control. Surface sterilized (0.1% $HgCl_2$) healthy seeds of tomato cv. PKM- 1 were sown (10 seeds/pot). After 10-15 days of sowing, observations on pre-emergence seed rot and post-emergence seedling mortality were recorded. The test pathogen isolate was re- isolated aseptically on PDA plates to fulfil Koch's postulates.

Effect of biocontrol agents and fungicide on plant growth promotion of tomato against *P. aphanidermatum*

To evaluate the efficacy of biocontrol agents and fungicide for germination percent, root length, shoot length, vigour index and disease incidence, pot culture studies were conducted under screen house conditions in Department of Plant Pathology, VNMKV, Parbhani. Sand: maize medium multiplied with *P. aphanidermatum* were mixed thoroughly in top 5-6 cm layer of the potting mixture. Surface sterilized healthy seeds of tomato treated with bioagents and fungicide were sown (10 seeds/pot) in these pots, watered lightly and maintained in the screen house. Untreated seeds sown in pots without pathogen inoculum served as control. The data was recorded on percent germination, disease incidence, root length, shoot length and vigour index were recorded after 30 days of sowing.

Pot Experiment details

Design	:	CRD
Replication	:	3
Treatments	:	10
Variety	:	PKM-1
Seed rate	:	10 seeds / pots

Result and Discussion survey

A survey was conducted in various locations within Parbhani district to estimate the prevalence of damping-off disease, caused by *P. aphanidermatum*, in tomato fields. The village of Khanapara had the highest disease incidence at 24.25 percent, followed by Pimpari Deshmukh at 23.00 percent and Selu at 21.25 percent, compared to other localities (Fig 1). This may have been caused by the planting of susceptible tomato cultivars in heavy black soil. Additionally, it was observed that the frequent watering of seedlings or their exposure to waterlogged conditions for extended periods increased the likelihood of damping-off disease. Hafiz (1986) reported that soil-borne diseases resulted in significant yield loss in tomato crops each year.

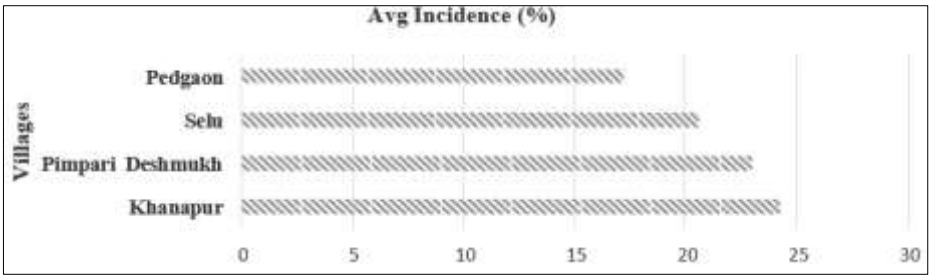

Fig 1: Incidence of damping-off in Parbhani district

Isolation and Purification

During the collection of disease samples, naturally infected tomato seedlings that displayed the typical symptoms of damping-off were brought to the laboratory. The pathogen was isolated from diseased seedlings using tissue-segment method on PDA and later purified using hyphal tip technique under aseptic conditions and isolates were maintained in PDA slants. Similar fungi have been isolated from infected tomato seedlings showing characteristics and symptoms of damping-off from farmer's fields (Arya, 2004) [1]. Karmel and

Muthukumar (2019) [13] studied 20 isolates causing *P. aphanidermatum* from tomato growing region of Tamil Nadu.

Pathogenicity Test

Pathogenicity test of *P. aphanidermatum* by sick soil method in pots recorded 30 percent pre-emergence mortality and 60 percent post-emergence mortality (Table 1). No mortality recorded in uninoculated pots. Germination of the seeds was started after the 11th day of sowing and ungerminated seeds were considered as pre-emergence mortality. Ungerminated seeds were found to be soft and rotten. After 19th day, germinated seedlings initially produced small water-soaked lesions on collar regions near the soil line and lesions enlarged after three days, which covered the newly emerged leaves resulting in girdling and rotting of leaves and stem. Damped off seedlings were considered as post-emergence mortality. Isolated culture was similar with the test pathogen culture isolated from naturally diseased tomato seedlings. Hence, pathogenicity of *P. aphanidermatum* was proved. Similarly, Ramamoorthy *et al.* (2002) [15] proved the pathogenicity of *P. aphanidermatum* on tomato by soil inoculation method. Karmel and Muthukumar (2019) [13] studied 20 isolates causing *P. aphanidermatum* from major growing region of Tamil Nadu and confirmed the pathogenicity in pots under polyhouse conditions.

Table 1: Pathogenicity test of *P. aphanidermatum* on tomato seedlings by sick soil method

Inoculation method	No. of seeds used	Pre-emergence damping off (%)	Post-emergence damping off (%)
Soil inoculation method	10	30.00	60.00
Control	10	0.00	0.00

Table 2: Effect of different bioagents and a fungicide on damping-off disease and plant growth of tomato

Tr. No.	Treatments	Dose (g/kg)	Germination (%)	Disease incidence (%)	Mean root length (cm)	Mean shoot length (cm)	Vigour index
T1	*T. asperellum*	3	79	21	4.85	6.02	858.33
T2	*T. asperellum*	4	88	12	7.65	8.01	1378.08
T3	*T. asperellum*	5	82	18	5.50	5.94	938.08
T4	*P. fluorescens*	3	76	24	5.65	5.70	862.60
T5	*P. fluorescens*	4	86	14	7.20	7.65	1277.10
T6	*P. fluorescens*	5	85	15	6.05	6.20	1041.25
T7	Carbendazim (12%) + Mancozeb (63%)	2.5	91	9	8.07	8.05	1466.90
T8	Carbendazim (12%) + Mancozeb (63%)	3	93	7	7.20	8.10	1422.90
T9	Carbendazim (12%) + Mancozeb (63%)	3.5	91	9	7.40	8.19	1418.69
T10	Control	--	56	44	4.15	4.55	487.20
	S.Em ±		1.74	1.13	1.04	1.02	1.03
	C.D. @ 0.01		3.04	1.29	1.08	1.04	1.05

*Values presented are means of 3 replications

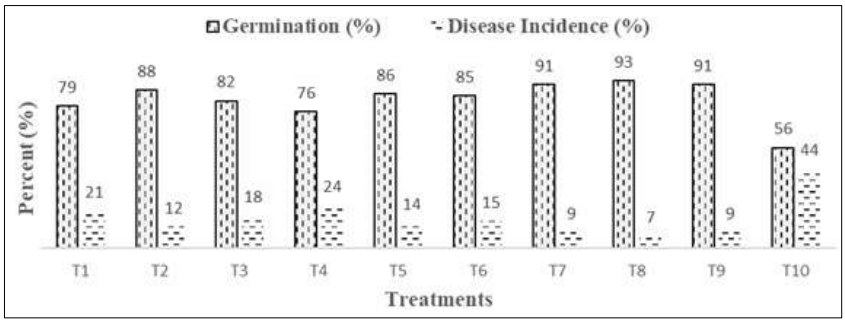

Fig 2: Effect of bioagents and a fungicide on germination and damping-off disease incidence on tomato

Effect of bioagents and a fungicide on damping-off disease tomato plant growth

Bioagents and fungicide tested for their efficacy in the management of damping-off and plant growth promotion of tomato under screen house conditions. Results (Table 2) indicated that all fungicides and biocontrol agents tested as seed treatment were able to control damping off caused by *P. aphanidermatum* and showed a significant effect on germination (Fig 2) and vigour index of tomato plants. Fungicide tested could control damping off more than bioagents. Carbendazim (12%) + Mancozeb (63%) @ 2.5 g/kg was most effective in all the aspects among all other treatments which is line with observations of Dar *et al.* (2015) [3]. Whereas, Hanif *et al.* (2015) [8] reported that amongst, the bioagents tested the application of *T. asperellum* @ 4g/kg followed by *P. fluorescens* @ 4g/kg proved superior over other bioagent treatments.

Carbendazim (12%) + Mancozeb (63%) treatment proved its efficacy as fungistatic under pot culture conditions. Chemical fungicides were found to be most effective than bioagents but it has an adverse effect on environment. While biocontrol agents exhibit antagonistic as well as plant growth promoting activity, therefore it could be more effective in combating plant diseases. The antagonistic fungi can also induce systemic resistance in plants, making resistant to a variety of disease attacks and thus boosting plant defense response. (Harman *et al.* 2004) [9]. Similarly, Zagade *et al.* (2012) [17] found Carbendazim more effective in inhibiting the growth of *P. aphanidermatum* than *Trichoderma* spp. Likewise, El-Kholy *et al.* (2021) [4] reported that chemical fungicides were

significantly more effective than the biocontrol agents. Muthukumar *et al.* (2011) [13] reported the maximum growth inhibition of *P. aphanidermatum* in treatment with *Trichoderma* spp. While, Ramamoorthy *et al.* (2002) [15] reported that *Pseudomonas fluorescens* suppressed damping off in tomato through antagonism against *Pythium* invasion. The results were in harmony with earlier workers *viz.*, El-Samasidy *et al.* (2008) [5], Kamala and Indira (2011) [11], Gholve *et al.* (2014) [6] and Bora and Deka (2020) [2].

The overall results indicated that the seed treatment with Carbendazim (12%) + Mancozeb (63%) was found effective than the bioagents, but in the long run the continuous use of chemicals is hazardous to soil health as well as pollutes the environment and leads to develop resistant strain of pathogenic organisms. There is a growing need to find a sustainable and cost-effective approach for managing diseases and reducing reliance on synthetic agrochemicals. Our findings suggest that *Trichoderma asperellum* and *Pseudomonas fluorescens* are effective antagonists against *P. aphanidermatum*, the pathogen responsible for tomato damping-off, in pot culture conditions. While fungicides are more effective than bioagents, their use can have detrimental effects on both the environment and human health. Therefore, it is important to explore environmentally safe alternatives for disease control.

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