

Effectiveness of Different Concentrations of Carbendazim against *Pythium aphanidermatum* and Impact of Organic Amendments on Damping of Tomato Seedlings

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Received 23 January 2026, Accepted 20 March 2026, Published on 31 March 2026

ABSTRACT

Damping off is a common disease affecting seedlings, including tomatoes, often caused by soil-borne pathogens like *Pythium* and *Rhizoctonia*. Organic amendments can help manage this issue by improving soil health and suppressing pathogens. *Pythium*

aphanidermatum is a soil-borne pathogen responsible for damping-off and root rot in various crops, including tomatoes. This study evaluates the *in vitro* efficacy of different concentrations of carbendazim, a benzimidazole fungicide, against *P. aphanidermatum* using the poisoned food technique. The pathogen was isolated and cultured on potato dextrose agar (PDA). Carbendazim was tested at concentrations of 0, 25, 50, 100, 250 and 500 ppm. Mycelial growth was assessed after 7 days of incubation at 25°C. Results indicated a significant reduction in fungal growth with increasing concentrations of carbendazim, achieving up to 93.33% inhibition at 500 ppm. The study confirms that carbendazim is effective in inhibiting the growth of *P. aphanidermatum* and suggests its potential as a control measure in managing damping-off diseases. Further research is warranted to explore its efficacy in field conditions and its impact on non-target organisms.

Keywords Disease, Pathogens, Fungicide, Organisms.

INTRODUCTION

Damping off is a significant challenge in the cultivation of tomato seedlings, primarily caused by soil-borne pathogens such as *Pythium*, *Rhizoctonia* and *Fusarium*. These pathogens can lead to high seedling mortality rates, adversely affecting crop yield. Management strategies to combat damping-off include cultural practices, chemical treatments, and

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the use of organic amendments. This paper discusses how different concentrations and incubation periods of organic amendments can influence the incidence of damping-off in tomatoes, with a focus on improving soil health and enhancing plant resilience. Damping-off can manifest as pre-emergence damping-off, where seeds rot in the soil, or post-emergence damping-off, where seedlings collapse after emerging. The pathogens thrive in poorly managed soils, often exacerbated by factors such as overwatering, poor drainage, and low soil fertility (Sulaiman and Bello 2024). Organic amendments have emerged as a viable solution due to their ability to enhance soil structure, improve nutrient availability and promote beneficial microbial communities that can suppress pathogenic organisms (Sharma *et al.* 2024).

Organic amendments include materials like compost, vermicompost, biochar and green manure. These amendments can enhance soil fertility and microbial diversity, which are critical for plant health and disease suppression. Compost is rich in nutrients and microorganisms, compost improves soil structure and aeration, leading to healthier root systems. It can also suppress pathogens through competition and the production of antimicrobial compounds (Mishra *et al.* 2025). Vermicompost produced through the decomposition of organic matter by earthworms, vermicompost is known for its high nutrient content and microbial diversity. It can enhance plant growth and resilience to diseases (Edwards and Arancon, 2022). Biochar is carbon-rich material can improve soil fertility and water retention while promoting beneficial soil microbial communities. Biochar has been shown to reduce the incidence of damping-off in various crops (Lehmann and Joseph 2015). Green Manure cover crops into the soil adds organic matter and nutrients, enhancing soil structure and microbial activity. Green manure can also suppress weeds and pathogens through biological activity (Tahat *et al.* 2020).

Pythium aphanidermatum is a soil-borne pathogen responsible for damping-off and root rot diseases in various crops, including tomatoes (Amin *et al.* 2026). Carbendazim, a benzimidazole fungicide, is commonly used to control fungal pathogens. The poisoned food technique is a widely used method to

assess the efficacy of fungicides against pathogens *in vitro* (Thomas and Naik 2017). This study aims to evaluate the effectiveness of different concentrations of carbendazim against *P. aphanidermatum* and impact of organic amendments on damping of tomato seedlings.

MATERIALS AND METHODS

Pathogen isolation

Pythium aphanidermatum was isolated from infected tomato plants using standard mycological techniques. The pathogen was cultured on potato dextrose agar (PDA) at 25°C for 5–7 days.

Preparation of carbendazim solutions

Carbendazim was obtained in powdered form and dissolved in sterile distilled water to prepare stock solutions. Serial dilutions were made to obtain the following concentrations: 0 ppm (control), 25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm.

Poisoned Food Technique

1. Agar preparation: PDA was prepared and autoclaved. Once cooled to about 45–50°C, the appropriate volume of carbendazim solution was added to achieve the desired final concentrations.

2. Plating: The poisoned PDA was poured into Petri dishes and allowed to solidify. A mycelial plug (5 mm diameter) from a 5-day-old culture of *P. aphanidermatum* was placed in the center of each dish.

3. Incubation: The plates were incubated at 25°C in the dark for 7 days. Each treatment was replicated three times.

Evaluation of efficacy

Efficacy was measured by assessing the growth of *P. aphanidermatum* after incubation. The following parameters were recorded:

Mycelial Growth Rate (MGR): Measured by the diameter of the fungal colony (in mm) on each plate.

Percentage inhibition: Calculated using the formula:

$$\text{Percentage inhibition} = \frac{(C - T)}{C} \times 100$$

Where, CCC is the growth diameter of the control and TTT is the growth diameter of the treated sample.

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Experimental design

To evaluate the effects of organic amendments of Treatments Mustard Cake, Neem Cake, Mahua Cake and FYM on damping-off in tomato seedlings, a controlled experiment has been designed (Table 1).

Replication

Each treatment should be replicated multiple times to ensure statistical validity. Randomization can minimize bias in treatment effects.

Monitoring

Seedling health should be monitored for signs of damping-off, and soil microbial activity should be assessed using methods like soil respiration tests or microbial biomass measurements.

RESULTS

The soil amendments had influenced the percent disease incidence incubation period (Table 1). The maximum disease incidence in 2 week incubation period was recorded in mustard cake (62.19%) followed by neem cake (57.17%) and mahua cake (57.03%) while in 4 week mahua cake (60.15%) followed by mustard cake (59.05%) and neem cake (57.19%). Understanding the dynamics of soil microbial communities responsible for soil element cycling is vital to understanding organic amendments' mechanisms in agricultural soil. However, several studies show inconsistencies in whether and how organic amendments affect the taxonomic composition of soil microbial communities compared to the application of sole chemical fertilizers (Su *et al.* 2022).

The maximum growth inhibition of *T. Viride* was recorded by mustard cake followed by Mahua cake and castard cake amedments (Table 2).

Low concentrations are generally safe and can improve soil health without overwhelming the microbial community (Table 1 & 2). However, they may not provide sufficient pathogen suppression or nutrient availability (Noble and Coventry 2005). At moderate levels, organic amendments can significantly enhance microbial diversity and nutrient content, leading to improved plant health and reduced disease incidence.

Table 1. Effect of organic amendments of different concentrations and incubation periods of decomposition on damping off in tomato.

Amendment	Quantity q/ha (%)	Percent disease incidence incubation period		Mean PDI
		2 Weeks	4 Weeks	
Mustard cake	12.51 (0.06%)	62.19	59.05	55.56
	25.01 (0.2 %)	51.83	54.23	
	37.51 (0.16%)	49.43	58.03	
Neem cake	12.51 (0.06%)	57.17	57.19	51.45
	25.01 (0.2 %)	50.43	47.05	
	37.51 (0.16%)	48.43	48.42	
Mahua cake	12.51 (0.06%)	57.03	60.15	54.81
	25.01 (0.2 %)	50.37	56.21	
	37.51 (0.16%)	49.85	55.27	
FYM	251 (1%)	55.21	55.91	52.71
	376 (1.6 %)	52.48	52.01	
	501 (3%)	49.44	51.25	
Control	0	90.01		0
Mean of incubation periods	0	60.29	62.13	0

Table 2. Growth and sporulation of *T. Viride* as influenced by amendments.

Sl. No.	Treatment (cakes)	Colony diameter (mm)	Growth inhibition (%)	Sporulation ($\times 10^6$ spores/ml)
1	Neem cake	80.61	10.45	7.3
2	Mustard cake	39.71	55.89	3.8
3	Mahua cake	57.92	35.66	6.2
4	Castor cake	71.56	20.51	6.1
5	Control (PDA)	90.01	–	12.6

Table 3. *In vitro* effect of different concentration of carbendazim against *P. aphanidermatum* using poisoned food technique.

Treatment (concentration in PPM)	Percent inhibition	
	at 4 days	at 7 days
5.01	23.02	27.81
10.01	42.54	47.62
50.01	65.25	69.33
100.01	75.35	78.56
200.01	81.68	85.31
500.01	100.01	100.01
Control	0.01	0.01

The balance between beneficial and pathogenic organisms can be optimized at this concentration (Liu *et al.* 2023). While high concentrations can supply substantial nutrients, they may also lead to negative effects such as nutrient imbalances, anaerobic conditions, or pathogen proliferation. Careful management is essential to avoid these issues (Bhardwaj *et al.* 2024).

Organic matter that has not fully decomposed may lead to nutrient leaching or insufficient pathogen suppression. Early application can also introduce excess moisture, creating favorable conditions for damping-off pathogens (Omokaro *et al.* 2024). Allowing for moderate decomposition enables the breakdown of organic matter into more readily available nutrients while promoting beneficial microbial activity. This period is often optimal for enhancing plant health (Hoffland *et al.* 2020). Longer incubation periods result in more stable organic amendments with enhanced nutrient availability and pathogen suppression. This time allows for the establishment of beneficial microbial communities that can outcompete or inhibit pathogens (Zhang *et al.* 2020).

The results indicate that carbendazim effectively inhibits the growth of *Pythium aphanidermatum* *in*

vitro. The percentage inhibition increased with higher concentrations of carbendazim (Table 3). At 500 ppm, the growth of *P. aphanidermatum* was significantly suppressed, indicating that carbendazim could be an effective control measure against this pathogen.

More or less similar findings have also been reported Kamali *et al.* 2020. Chavan *et al.* 2017. They tested different species of *Trichoderma* against wide range of soil borne fungal pathogens including *Pythium* spp.

CONCLUSION

The use of organic amendments represents a sustainable approach to managing damping-off in tomato seedlings. By understanding the roles of concentration and incubation periods, growers can implement strategies that enhance soil health and improve seedling resilience against diseases. Future research should continue to explore the interactions between different amendments and pathogens to refine management practices in tomato cultivation. The poisoned food technique demonstrated that carbendazim is effective in inhibiting the growth of *Pythium aphanidermatum*. Future studies could investigate the field efficacy of carbendazim and its impact on non-target organisms.

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