Studies on the compatibility of *Trichoderma asperellum* (Tr-9) with nematicide Carbofuran and Cassava based biopesticide, Nanma under *in-vitro* conditions

Abstract

The compatibility of *Trichoderma asperellum* isolate Tr-9 with Carbofuran 3G and cassava-based biopesticide, Nanma was investigated to evaluate their potential for integrated nematode management. Carbofuran at 100 ppm showed no inhibition of *Trichoderma* growth, indicating full compatibility, while minimal inhibition (6.98%) was observed at 200 ppm. Inhibition increased slightly (15.25%-19.75%) at 400–800 ppm, but *Trichoderma* remained moderately compatible at 1000 ppm (36.2%). Conversely, Nanma demonstrated high incompatibility with *Trichoderma*, with complete inhibition of mycelial growth at concentrations up to 400 ppm, attributed to the antifungal properties of neem oil. At lower concentrations, growth inhibition ranged from 70.32% - 78.55%. These findings highlight that Carbofuran 3G is suitable for integrating *Trichoderma* in nematode management. It offers a sustainable approach for managing root-knot nematode in tuber crops.

Keywords: *Meloidogyne incognita*, *Trichoderma asperellum* Tr-9, Carbofuran 3G, Cassava based biopesticide-Nanma, compatibility

1. Introduction:

Root-knot nematodes (*Meloidogyne* spp.) are one of the most destructive plant-parasitic nematodes, significantly affecting tuber crops (Sathyarajan et al., 1966; Mohandas, 1994; Mohandas & Siji, 2012 and Kolombia et al., 2017). These nematodes invade the roots and tubers, induces the formation of characteristic galls that impair the plant's ability to absorb water and nutrients. This results in stunted growth, reduced yield, and poor tuber quality. Yield losses due to root-knot nematodes in tuber crops can range from 20% to 50%, depending on the severity of infestation. In yam, yield reduction was around 24%–80% due to *M. arenaria* (Gao, 1992). In case of cassava, yield losses ranged from 42% to 98% due to root-not nematode infestation (Akinsanya et al., 2020). Nematode-infested tubers are smaller in size, malformed due to deformities and blemishes and significantly reducing their market value (Nwauzor & Fawole, 1981; Kolombia et al., 2020). In addition to lowering tuber yield and quality, the damage caused by these nematodes predisposes plants to secondary

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Comment [DC3]: Use recent references in the introduction part.

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Comment [DC5]: Add recent references

infections by fungal and bacterial pathogens, increasing yields losses (Akinlesi, 2014). Furthermore, the nematodes continue to multiply after harvest and during storage, leading to severe infestations that can cause planting material to rot or dry up by the next planting season (Sasser & Carter, 1985).

Only few nematicides are available in the market and carbofuran is one among them for the management of root knot nematode, limited use is necessary since it is hazardous to the environment and human health. Hence, there is a need to adopt an alternate economical and eco-friendly methods *viz.*, biopesticides to combat these problems. Biopesticides based on living microorganisms, plant extracts and other natural compounds represent non chemical alternatives and eco-friendly in nature (Kumar et al., 2018). Among biopesticides, soil dwelling microorganisms like bacteria and fungi have been successfully used as bioagents against plant parasitic nematodes appears to be a promising alternative strategy in the management of root- knot nematode (Hussain et al., 2017). A wide range of bacterial and fungal agents have been used to reduce a wide range of plant-parasitic nematodes (Hallmann et al., 2001; Meyer et al., 2001). Fungal bioagents like *Trichoderma* spp. have been shown to effectively manage root-knot nematodes (*Meloidogyne* species) by reducing nematode damage and promoting plant growth (Meyer et al., 2004). They emerged as potential biocontrol agent, successfully utilised in many crops to manage root-knot nematode (Poveda et al., 2020; Forghani and Hajihassani, 2020; Harman 2011; and Yao et al., 2023). They combat plant-parasitic nematodes through parasitism, competition, production of toxic metabolites, induction of plant systemic resistance, and enhancement of plant growth (Ibrahim et al., 2020). Indigenous fungal bioagent, *Trichoderma asperellum*, isolate Tr-9 isolated from elephant foot yam at ICAR-CTCRI was effective against root-knot nematode under *in-vitro* conditions (Tadigiri et al., 2020). Besides, Nanma, a biopesticide developed by ICAR-CTCRI exhibits nematicidal properties and is primarily used for treating elephant foot yam tubers before planting to manage diseases and root-knot nematode infestation.

Instead of relying solely on a biopesticide-based strategy, incorporating chemical methods along with biological approaches is more effective for managing nematodes under field conditions. Therefore, the compatibility of *Trichoderma* with nematicide carbofuran and cassava based biopesticide, Nanma must be confirmed before incorporating it into an integrated management system. To address this, an *in-vitro* evaluation of nematicide Carbofuran and Cassava based biopesticide, Nanma was assessed to understand its compatibility on *T. asperellum,* Tr-9.

2. Materials and methods

2.1. Test bioagent cultures

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Biocontrol agent, *Trichoderma asperellum* isolate Tr 9 used for the study was native isolate maintained at Microbial repository, Division of Crop Protection, ICAR-CTCRI, Sreekariyam, Thiruvananthapuram. It was maintained in potato dextrose agar (PDA) medium.

2.2. Test agro-chemical and Biopesticide

Nematicides *viz*., carbofuran (Furadan 3G) and Cassava biopesticide (Nanma) were tested in this study.

2.3. *In-vitro* compatibility test

The *in-vitro* compatibility of nematicide, Carbofuran 3G and cassava-based biopesticide, Nanma with *Trichoderma asperellum*, Tr-9 was evaluated using the poisoned food technique (Nene and Thapliyal, 1993). Carbofuran 3G was tested at five concentrations (50, 100, 200, 400, 800 and 1000 ppm), while Nanma was screened at six concentrations (5, 10, 25, 75, and 100 ppm) against *T. asperellum* isolate Tr-9 to assess their inhibitory effects on mycelial growth.

Stock solutions of nematicide and cassava-based biopesticide (10,000 ppm) were prepared by dissolving the required amounts of each chemical in sterile distilled water. For the test concentrations, the appropriate quantities of the stock solution were mixed into molten potato dextrose agar (PDA) medium to achieve the desired concentration. The medium was thoroughly mixed by gentle shaking, and 20 ml of the molten medium was poured into sterilized 90-mm petri plates, which were allowed to solidify. Once solidified, the plates were inoculated with 7-mm discs of fresh fungal culture. Each chemical treatment was replicated three times, and the experimental setup followed a completely randomized design. PDA plates without chemicals served as controls, and all plates were incubated at 28 ± 2°C. Radial growth of the fungal colony was measured five days after inoculation. Percentage inhibition of Trichoderma isolate was calculated based on the diameter of growth of the colony by using the formula given by Vincent (1947)

$I = (C\text{-}T/C) \times 100$

Where, I = Per cent inhibition C = Growth of Trichoderma isolate in control T = Growth of Trichoderma isolate in chemicals

2.4. Data analysis:

The statistical analysis of mycelial growth diameters of *Trichoderma asperellum* and the percentage of inhibition was conducted. Mean comparisons of different parameters were performed using the WASP 2.0 software. Duncan's multiple range test ($P<0.05$) was applied for mean separation.

3. Results and Discussion

3.1. Compatibility Studies of Carbofuran with Trichoderma

The study assessed the compatibility of various concentrations of Carbofuran 3G (100, 200, 400, 800 and 1000 ppm) with *Trichoderma* isolate Tr-9 to explore its potential for integrated nematode management. The compatibility of carbofuran with *T. asperellum* Tr-9 was assessed based on radial growth (mean in cm) and the corresponding percentage reduction compared to the control after a five-day incubation period

At a concentration of 100 ppm, no inhibition of *Trichoderma* growth was observed, indicating full compatibility. A slight increase in inhibition (6.98%) was observed at 200 ppm, indicting a minor inhibitory effect. Inhibition percentages ranged from 15.25% to 19.75% at concentrations of 400 and 800 ppm, demonstrating that *Trichoderma* remained highly compatible with Carbofuran up to 800 ppm (Table 1).

At 1000 ppm, moderate inhibition (36.20%) of mycelial growth was observed, attributed to the higher concentration of the chemical. After a 10-day incubation period, complete radial growth of *Trichoderma* was observed at all concentrations of Carbofuran, indicating overall compatibility (Fig 1). However, the density of the mat formation was slightly reduced at 1000 ppm compared to the control, suggesting a minor effect of Carbofuran on the growth vigour of *Trichoderma*. These findings suggest that Carbofuran 3G is highly compatible with *Trichoderma* at lower concentrations, higher dose moderately impact its growth. These findings clearly indicate that *Trichoderma* can be effectively integrated with Carbofuran for managing root-knot nematodes in tuber crops. The results are consistent with the findings of Sushir et al., (2008), who demonstrated that *T. harzianum* tolerated higher concentrations of Carbofuran, up to 2000 mg/ml. Similarly, the results align with earlier studies by Singh et al. (2019), who conducted *in-vitro* experiments and reported that *Trichoderma* isolates were compatible with various concentrations of Carbofuran for the management of root-knot nematodes in rice.

3.2. Compatibility Studies of Cassava-Based Biopesticide Nanma with Trichoderma

Nanma, a biopesticide developed by ICAR-CTCRI, is derived from cassava and neem oil. It exhibits nematicidal properties and is primarily used for treating elephant foot yam tubers before planting to manage diseases and root-knot nematodes. Additionally, the indigenous isolate *Trichoderma asperellum* Tr-9 has shown effectiveness in managing root-knot nematodes under *in-vitro* conditions (Tadigiri et al., 2020).

To evaluate the compatibility of *Trichoderma* with Nanma, studies were conducted using different concentrations of the biopesticide (50, 100, 200, 400, 800, and 1000 ppm). The compatibility of Nanma with *T. asperellum* Tr-9 was assessed based on radial growth (mean in cm) and the corresponding percentage reduction compared to the control after a five-day incubation period The results revealed that at higher concentrations of Nanma (up to 400 ppm), there was complete inhibition of *Trichoderma* mycelial growth. At lower concentrations, the growth inhibition percentage ranged between 78.55% and 70.32%, indicating that Nanma is highly incompatible with *Trichoderma*. (Table 2; Fig 2). This incompatibility is attributed to the antifungal properties of neem oil present in Nanma, which suppresses the growth of *Trichoderma* (Kumar et al., 2017).

T. asperellum is an emerging and highly effective biocontrol agent, well-known for its ability to manage plant-parasitic nematodes and disease complexes caused by secondary pathogens (Tadigiri et al., 2020; Idowu et al., 2016; Sayed et al., 2019). Instead of solely relying on nematicides, it is more environmentally sustainable to use *Trichoderma* in combination with Carbofuran at lower concentrations for effective nematode management, as both have minimal environmental side effects. The results of this study demonstrate the compatibility of *Trichoderma* with Carbofuran, supporting the integration of chemical and biological control methods for efficient nematode management. However, while Nanma is compatible, it is not recommended for integration with *Trichoderma* in the management of nematodes in tuber crops

Table 1: Effect of nematicide, Carbofuran 3G on growth of *Trichoderma asperellum* isolate Tr 9 after 5 days incubation period

Carbofuran 3G (PPM)	Radial growth (cm)	Mycelial growth inhibition (%)
50	6.16[a]	0.00
100	5.73[b]	6.98
200	5.73[b]	6.98
400	5.22[c]	15.25
800	4.94[c]	19.75
1000	3.92[d]	36.30
Control	6.16[a]	
	C.D (0.05) = 0.28	C.D (0.05) = 5.06
	CV = 2.97	CV = 19.82
	SE(m) = 0.09	SE(m) = 1.62

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Table 2: Effect of nematicide, Cassava based biopesticide, Nanma on growth of *Trichoderma asperellum* isolate Tr 9 after 5 days incubation period

Cassava based biopesticide (PPM)	Radial growth (cm)	Mycelial growth inhibition (%)
50	1.83[b]	70.32
100	1.66[b]	72.35
200	1.30[c]	78.55
400	0.00[d]	100
800	0.00[d]	100
1000	0.00[d]	100
Control	6.16[a]	
	CD (0.05) = 0.33	CD (0.05) = 5.40

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	CV = 12.06	CV = 3.45
	SE(m) = 0.10	SE(m) = 1.73

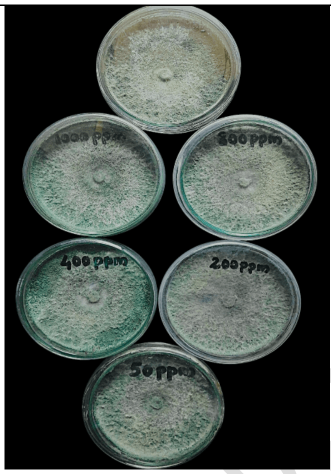


Fig 1: Compatibility of *T. asperellum* Tr-9 with different concentrations of nematicide, Carbofuran

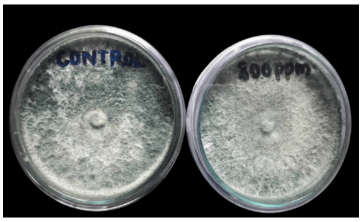
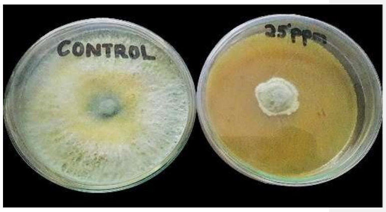
		
Carbofuran 50 PPM	**Carbofuran 100 PPM**	**Carbofuran 200 PPM**
		
Carbofuran 400 PPM	**Carbofuran 800 PPM**	**Carbofuran 1000 PPM**

Fig 2: Compatibility of *T. asperellum* Tr-9 with different concentrations of Cassava based biopesticide, Nanma



Nanma 5 PPM	Nanma 10 PPM	Nanma 25 PPM

4. Conclusion:

Trichoderma asperellum Tr-9 was found to be compatible with Carbofuran 3G but incompatible with the cassava-based biopesticide, Nanma. This compatibility highlights the potential of integrating Carbofuran with *Trichoderma* isolate Tr-9 for effective management of root-knot nematodes in tuber crops. Further research is recommended to evaluate the compatibility of new nematicides such as Fluopyram and Fluensulfone with *Trichoderma*, along with pot and field studies, to develop an efficient integrated nematode management module for tuber crops.

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