Compatibility of *Trichoderma asperellum* (Tr-9) with Carbofuran and Cassava-based biopesticide, Nanma under *in-vitro* conditions

Abstract

Root-knot nematodes (*Meloidogyne* spp.) pose a major threat to tuber crops, necessitating the development of effective and environmentally sustainable management strategies. Although chemical nematicides have been successful in controlling nematodes, but their adverse environmental impact has urged to explore eco-friendly alternatives such as bioformulations. *Trichoderma asperellum* (Tr-9), a promising biocontrol agent, can be integrated with chemical nematicides to enhance efficacy while reducing environmental risks. However, assessing the compatibility of *Trichoderma* with nematicides is essential before integrating both strategies for nematode management in tuber crops. In this context, the present study evaluated the compatibility of *T. asperellum* (Tr-9) with different concentrations of Carbofuran 3G (50, 100, 200, 400, 800 and 1000 ppm) and the Cassava-based biopesticide, Nanma (5, 10, 25, 50, 75 and 100 ppm) using the poisoned food technique after a five-day incubation period under *in-vitro* conditions. Results revealed that Carbofuran 3G exhibited complete compatibility at 50 ppm, and with minimal inhibition (6.98%) observed at 100-200 ppm. Even at higher concentrations (400-800 ppm), only slight mycelial growth inhibition (15.25% - 19.75%) was recorded, while at 1000 ppm, inhibition increased to 36.2%. These findings indicate that all concentrations up to 800 ppm are highly compatible, while 1000 ppm remains moderately compatible with Carbofuran. In contrast, Nanma exhibited high incompatibility, completely inhibiting *Trichoderma* growth at concentrations up to 400 ppm, likely due to the antifungal properties of neem oil. At lower concentrations, inhibition remained high (70.32% - 78.55%). These findings indicated that Carbofuran 3G can be effectively integrated with *T. asperellum* (Tr-9) for nematode management in tuber crops, offering a balanced approach that combines chemical and biological methods while reducing environmental impact. Further research is required to assess the compatibility of novel nematicides such as Fluopyram, Fluensulfone, and Fluazaindolizine with *Trichoderma*, along with pot and field studies, to develop an effective integrated nematode management module for 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 (Mohandas & Siji, 2012; Kolombia et al., 2017; Reddy, 2021; Wendimu, 2021; Singh et al., 2024). 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 (Sathyarajan et al., 1966; Mohandas, 1994). 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-knot 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 (Kolombia et al., 2017; Kolombia & Fabiyi, 2023). In addition to lowering tuber yield and quality, the damage caused by these nematodes predisposes plants to secondary infections by fungal and bacterial pathogens, increasing yield 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, and 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., 2004). 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*, (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 depending only 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

Biocontrol agent, *T. asperellum* (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 *T. asperellum*, (Tr-9) was evaluated using the poisoned food technique (Nene and Thapliyal, 1993). Carbofuran 3G were tested at six concentrations (50, 100, 200, 400, 800 and 1000 ppm), while Nanma was screened at six concentrations (5, 10, 25, 50, 75, and 100 ppm) against *T. asperellum* (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 amount 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).

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

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 *T. asperellum* and the percentage of inhibition was analysed. 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 *T. asperellum* (Tr-9)

The study assessed the compatibility of various concentrations of Carbofuran 3G (50, 100, 200, 400, 800 and 1000 ppm) with *Trichoderma* (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 50 ppm, no inhibition of *Trichoderma* growth was observed, indicating full compatibility. A slight increase in inhibition (6.98%) was observed at 100-200 ppm, indicating 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. At 1000 ppm, moderate inhibition (36.20%) of mycelial growth was observed, attributed to the higher concentration of the chemical. (Table 1).

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*. 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 and Phorate for the management of root-knot nematodes in rice.

Several researchers have studied the compatibility of insecticides, fungicides, and herbicides with bioformulations, as they play a crucial role in integrated disease management, which involves a combination of cultural, physical, chemical, and biological methods (Desai & Kulkarni, 2004; Ranganathswamy et al., 2011; Singh et al., 2012; Thiruchchelvan et al., 2013; Vasundara et al., 2015; Dhanya et al., 2016). Compatibility of *T. pseudokoningii* with selective insecticides, fungicides, herbicides, fertilizers and organic stickers was studied by Dutta et al. (2017). They found that all the tested pesticides inhibited the growth of *T. pseudokoningii*, with the exception of thiamethonaus 25% WG at 0.125% and ritha at the highest test dose found compatible. In case of fertilizers, Urea and MOP were found to be compatible, whereas SSP and CAN inhibited the growth of *Trichoderma*. In another study, *T. viride* also showed compatibility with insecticide (imidacloprid), fungicides (mancozeb, tebuconazole, pencycuron, and propineb), and herbicides (imazathafir, 2, 4-D sodium salt, and oxyfluorfen) (Madhavi et al., 2011). These differences in inhibitory potential developed from inherent variations in the chemical constituents of the fungus's cellular components.

At present study, *T. asperellum* isolate (Tr-9) was found to be highly compatible with *Trichoderma* at lower concentrations, higher dose moderately impacts its growth. These findings clearly indicate that *Trichoderma* can be effectively integrated with Carbofuran for managing root-knot nematodes in tuber crops.

3.2. Compatibility Studies of Cassava-Based Biopesticide, Nanma with with *T. asperellum* (Tr-9)

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 nematode infestation. Additionally, the indigenous isolate *T. 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 (5, 10, 25, 50, 75 and 100 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 (upto 400

ppm), there was complete inhibition (100 %) of *Trchoderma* mycelial growth. At lower concentrations, the growth inhibition percentage ranged between 70.32% and 78.55%, 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). Integrating *Trichoderma* with lower concentrations of Carbofuran offers a more sustainable approach to nematode management, ensuring effective control with minimal environmental impact. This study demonstrated the high compatibility of *T. asperellum* (Tr-9) with Carbofuran, highlighting the potential of combining biological and chemical control methods for improved nematode management. However, while Nanma is incompatible, 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* (Tr-9) after 5 days incubation period

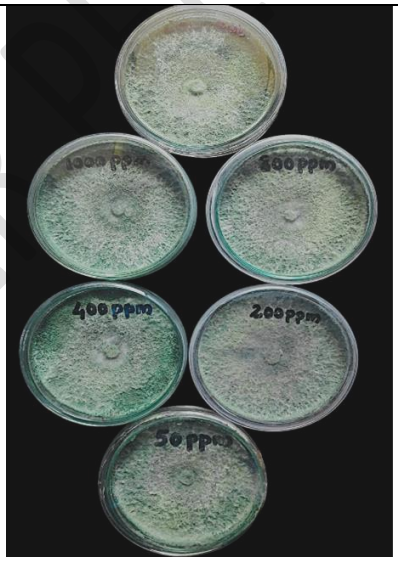
Carbofuran 3G (PPM)	**Radial growth (cm)**	**Mycelial growth inhibition (%)**
50	6.16	0.00
100	5.73	6.98
200	5.73	6.98
400	5.22	15.25
800	4.94	19.75
1000	3.92	36.30
Control	6.16	-
CD at 5% level	0.28	5.06
CV	2.97	19.82
SE(m)	0.09	1.62

*Mean of three replications

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

Cassava based biopesticide (PPM)	Radial growth (cm)	Mycelial growth inhibition (%)
5	1.83	70.32
10	1.66	72.35
25	1.30	78.55
50	0.00	100
75	0.00	100
100	0.00	100
Control	6.16	-
CD at 5% level	0.33	5.40
CV	12.06	3.45
SE(m)	0.10	1.73

*Mean of three replications



Carbofuran

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

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

Nanma

Nanma 5 PPM

Nanma 10 PPM

Nanma 25 PPM

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

4. Conclusion:

T. 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 *T. asperellum* (Tr-9) for effective management of root-knot nematode in tuber crops. Further research is required to assess the compatibility of novel nematicides such as Fluopyram, Fluensulfone, and Fluazaindolizine with *T. asperellum* (Tr-9), along with pot and field studies, to develop an effective integrated nematode management module for tuber crops.

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Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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