



Compatibility of *Trichoderma asperellum* (Tr-9) with Carbofuran and Cassava-based Biopesticide, Nanma under *In-vitro* Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) pose a major threat to tuber crops, challenging the development of effective and environmentally sustainable management strategies. Although chemical nematicides have been effective in controlling nematodes, but their adverse environmental impact has urged to explore eco-friendly alternatives such as bioformulations. *Trichoderma asperellum* (Tr-9), an indigenous fungal bioagent isolated from elephant foot yam was effective against root-knot nematode under *in-vitro* conditions. Additionally, Nanma, a cassava-based biopesticide developed by ICAR-CTCRI, is commonly used to treat elephant foot yam tubers before planting to control diseases and nematode infestation. They can be combined with nematicides for effective nematode management while minimizing environmental risks. However,

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evaluating the compatibility of *Trichoderma* with nematicides is crucial 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 cent percent 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 percentage increased to 36.30%. 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, inhibiting *Trichoderma* growth completely at concentrations ranging from 50 to 100 ppm. At even lower concentrations, inhibition percentage still 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 evaluate the compatibility of novel nematicides such as Fluopyram, Fluensulfone, and Fluzaindolizine with *Trichoderma*, along with pot and field studies, to develop an effective integrated nematode management module for tuber crops.

Keywords: *Meloidogyne incognita*, *T. 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 nematode 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; however, its usage must be restricted due to its environmental and health hazards. As a result, there is a need to adopt economically viable and environmentally benign approaches for combating these challenges, such as biopesticides. Biopesticides derived from living microorganisms, plant extracts, and other natural compounds offer eco-friendly, non-chemical alternatives for pest management (Kumar et al., 2018). Among these, soil-dwelling microorganisms such as bacteria and fungi have been effectively employed as bioagents against plant-parasitic nematodes, suggesting a promising strategy for root-knot nematode management (Hussain et al., 2017). Various bacterial and fungal agents have been utilized to suppress a wide range of plant-parasitic nematodes in different crops (Hallmann et al., 2001; Meyer et al., 2004). Fungal bioagents, particularly *Trichoderma* spp., have proven effective in controlling root-knot nematodes (*Meloidogyne* species) by reducing nematode-induced damage and promoting plant growth (Meyer et al., 2004). They have emerged as potent biocontrol agents, widely applied in various crops for root-knot nematode management (Harman, 2011; Poveda et al., 2020; Forghani and Hajihassani, 2020; 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, *T. 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 evaluated to understand its compatibility with *T. asperellum*, (Tr-9).

2. MATERIALS AND METHODS

2.1 Test Bioagent Culture

In the present study, the biocontrol agent *T. asperellum* (Tr-9), a native isolate from the microbial repository of the Division of Crop Protection, ICAR-CTCRI, Sreekariyam, Thiruvananthapuram, was maintained on potato dextrose agar (PDA) medium.

2.2 Test Agro-Chemical and Biopesticide

Nematicide, Carbofuran 3G and the cassava-based biopesticide, Nanma were evaluated in the present 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 $28 \pm 2^\circ\text{C}$. 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 = Percent 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 statistical software.

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 50 ppm, no suppression of *Trichoderma* growth was observed, indicating cent percent compatibility. A minimal increase in inhibition (6.98%) was observed between 100 and 200 ppm, indicating a minor inhibitory effect. At concentrations of 400 and 800 ppm, inhibition percentages varied from 15.25% to 19.75%,

suggesting that *Trichoderma* still remained highly compatible with Carbofuran upto 800 ppm. At 1000 ppm, mycelial development was moderately inhibited (36.30%), which can be attributed to the higher chemical concentration. (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 because they play an important role in integrated disease management, which integrates 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*, except for Thiamethoxam 25% WG at 0.125%, which was found to be 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* showed compatibility with insecticide (Imidacloprid), fungicides (Mancozeb, Pencycuron, and Propineb), and herbicides (Imazathafir, 2, 4-D Sodium salt, and oxyfluorefen). Highly incompatible with systemic fungicides like Carbendazim, Hexaconazole, Tebuconazole and Propiconazole (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 Carbofuran at

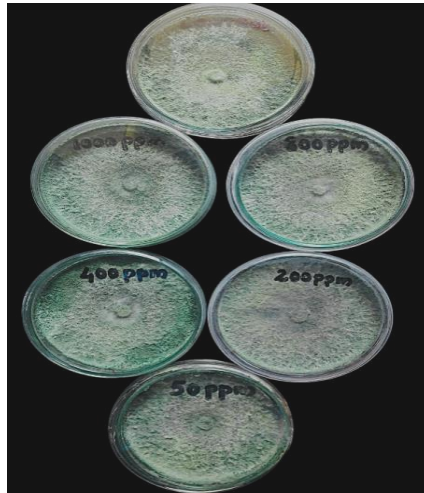
lower concentrations, higher dose moderately impacts its growth. These findings clearly indicate that *Trichoderma* can be effectively integrated with Carbofuran for managing root-knot nematode in tuber crops.

3.2 Compatibility Studies of Cassava-Based Biopesticide, Nanma 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 nematode 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 higher concentrations of Nanma ranging between 50 and 100 ppm completely inhibited *Trichoderma* mycelial growth. Even at lower dosages, the growth inhibition percentage ranged from 70.32% to 78.55%, indicating that Nanma is highly incompatible with *Trichoderma*. (Table 2, Fig. 2). This incompatibility is due to the antifungal effects of neem oil in Nanma, which inhibits 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 revealed that *T. asperellum* (Tr-9) exhibited high compatibility with Carbofuran, emphasizing the potential of integrating biological and chemical control methods for enhanced nematode management. However, while Nanma is incompatible, it is not recommended for integration with *Trichoderma* in the management of nematodes in tuber crops.



T. asperellum (Tr-9) compatibility with Carbofuran

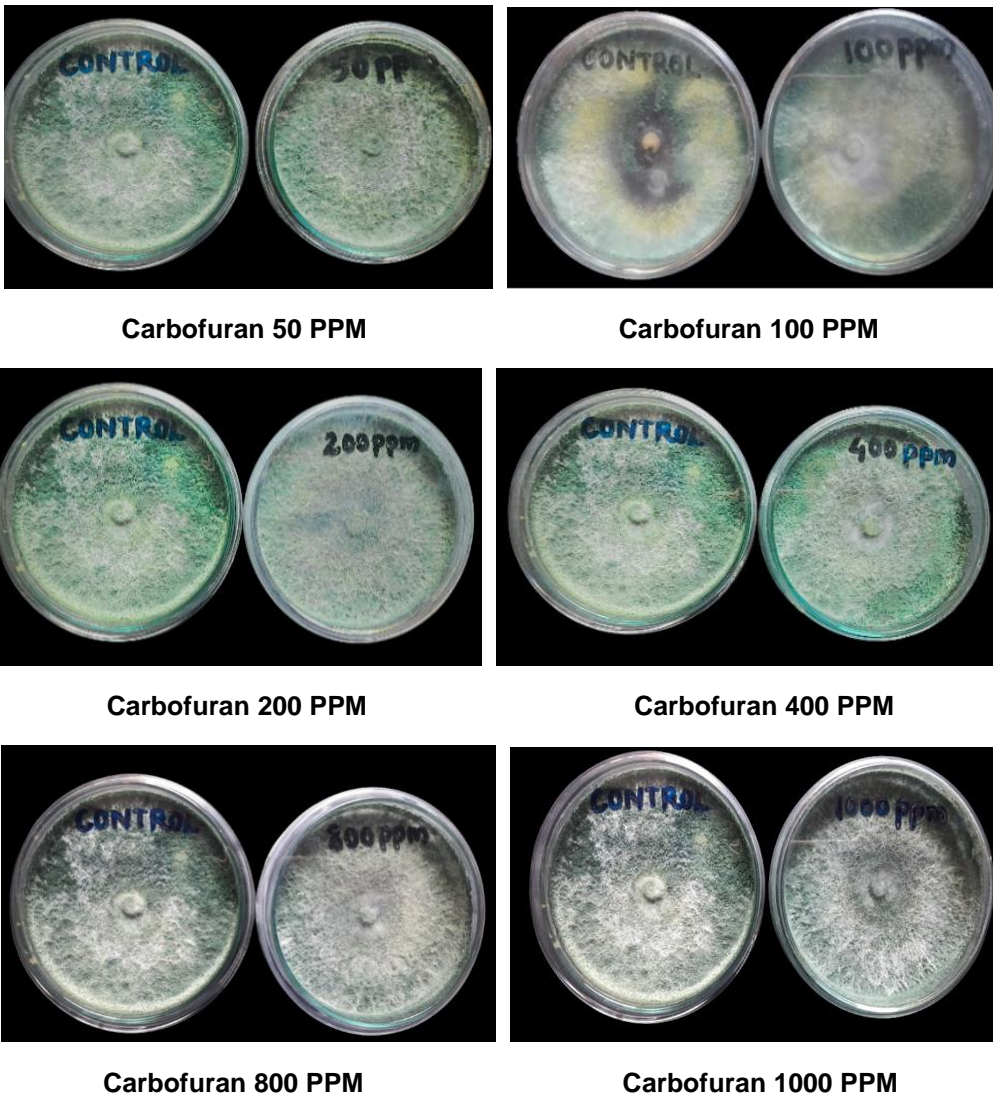
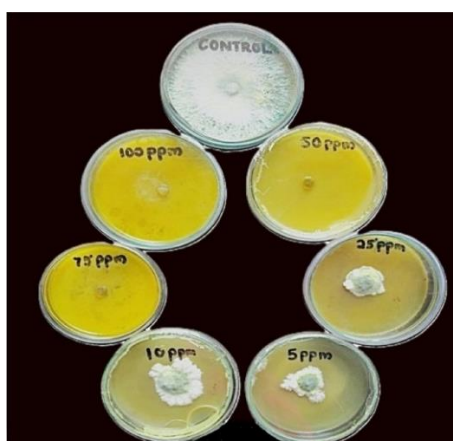


Fig. 1. Compatibility of *T. asperellum* (Tr-9) with different concentrations of nematicide, Carbofuran



***T. asperellum* (Tr-9) compatibility with Cassava based biopesticide, Nanma**

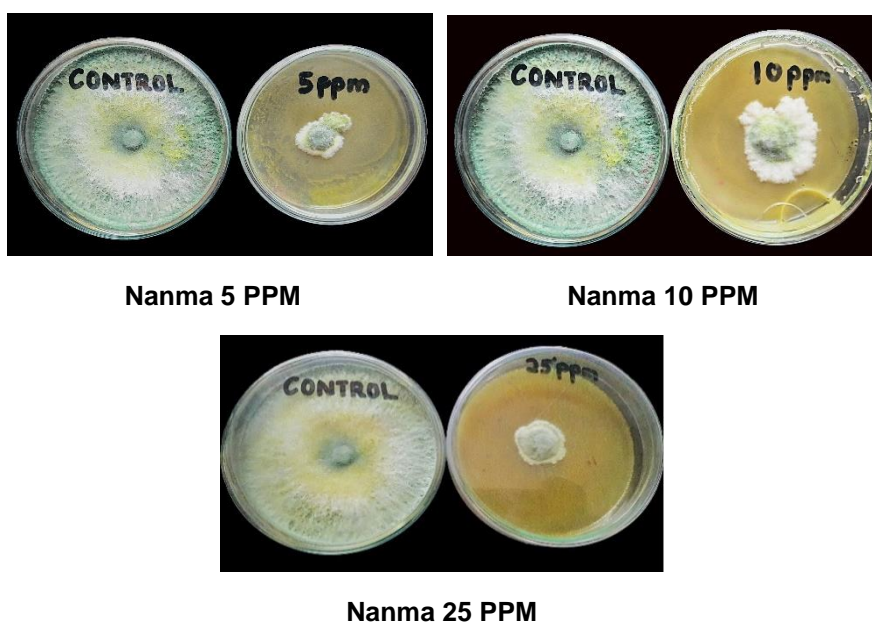


Fig. 2. Compatibility of *T. asperellum* (Tr-9) with different concentrations of Cassava based biopesticide, Nanma

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 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

4. CONCLUSION

T. asperellum (Tr-9) was found to be compatible with Carbofuran 3G but incompatible with the cassava-based biopesticide, Nanma. This compatibility suggests the possibility of integrating Carbofuran with *T. asperellum* (Tr-9) for effective management of root-knot nematode in tuber crops. Further research is needed to develop an effective integrated nematode management module for tuber crops by evaluating the compatibility of novel nematicides like Fluopyram, Fluensulfone and Fluazaindolizine with *T. asperellum* (Tr-9), along with comprehensive pot and field trials.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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