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Co-application of bio-organic and fly ash: strategies for managing root-knot nematode, *Meloidogyne incognita* in papaya (*Carica papaya*) crop

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***Abstract*— *This study investigates the effects of various bio-organic amendments on the growth, physiological parameters, and nematode infestation in papaya crop. Treatments included fly ash (FA) at different concentrations (10%, 20% and 30%) inoculated with 2000 juveniles of Meloidogyne incognita (MI), neem cake (NC) at 50g and 100g inoculated with MI, and Trichoderma viride (Tv) at 1g and 2g inoculated with MI juveniles. Results showed significant improvements in shoot length, root length, fresh and dry weights, and chlorophyll content in plants treated with bio-organic and FA amendments compared to the untreated inoculated control (UIC). The highest shoot length was observed in the untreated un-inoculated control (UUC) at 55 cm, while UIC had the lowest at 32 cm. Treatments with 100g NC + MI and 2g Tv + MI resulted in shoot lengths of 52 cm and 54 cm, respectively, and increases of 62.5% and 68.8% compared to UIC. Root length was significantly improved with 100g NC + MI and 2g Tv + MI, reaching 43 cm and 47 cm, respectively, representing increases of 230.8% and 261.5% compared to UIC. Chlorophyll-a content increased to 2.04 mg/g and 2.113 mg/g, while chlorophyll-b content reached 1.06 mg/g and 1.15 mg/g in treatments with 100g NC + MI and 2g Tv + MI, respectively. Total chlorophyll content was highest at 3.1 mg/g with 100g NC + MI. Nematode infestation was markedly reduced, with 100g NC + MI and 2g Tv + MI showed no egg masses and root-knot indices (GI) of 2 and 2, respectively. Overall, bio-organic amendments, particularly higher concentrations of neem cake and T. viride, significantly enhance plant growth and reduce nematode infestation, suggesting their potential as sustainable agricultural practices for managing plant parasitic nematodes.*

Keywords*— Bio-organic amendments, Fly ash, Neem cake, Nematode suppression, Plant growth and yield enhancement, Trichoderma viide

I. INTRODUCTION

Nematodes, particularly plant parasite nematodes (PPNs) like root-knot nematodes (RKNs), pose a severe economic ($157 billion) threat to global agriculture [1]. Every year, plant parasitic nematodes (PPNs) result in agricultural loss (21.3%), which amounts to Rs. 102,039.79 million. Anticipated losses in 19 horticultural crops were estimated to be Rs. 50,224.98 million, while anticipated losses in 11 field crops were estimated to be Rs. 51,814.81 [2]. RKNs, including *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*, are predominant in agriculture, inflicting damage by establishing feeding sites on plant roots, causing galling, stunted growth, and reduced yields [3]. Their ability to attack underground plant parts, combined with a broad host range and rapid reproduction, complicates effective control measures [4,5]. RKN's are sedentary endo-parasites infesting over 3,000 plant species, including major crops like cotton, bananas, and tomatoes [6], and chemical nematicides pose health risks [7]. Eco-friendly alternatives like bio-control agents (*Trichoderma* spp.) and organic amendments (oil cakes and fly ash) have proven effective against nematode infestations [8,9,10,11,12]. With approximately 10,000 species, *Trichoderma* strains exhibit diverse morphologies from white to green compact tufts, crucial for bio-control against plant [13,14,15,16]. Azadirachtin, a key insecticidal compound found in neem, offers biopesticidal properties [17,18].

Fly ash (FA), applications have proven effective in acid lateritic soils, benefiting field crops [19,20,21]. Recent studies underscore FA potential in remedying degraded soils and exploring sustainable nematode management practices [22].

Papaya (*Carica papaya* L.) is a versatile tropical crop known globally for its culinary uses and medicinal benefits. Its fast-growing, semi-woody trees yield latex containing papain, used for meat tenderization and rich in nutrients, papaya's edible portion includes 88.83% water, carbohydrates, and essential vitamins (A, B1, and B2) [23]. India ranks papaya as its fourth highest fruit crop by yield, with 138.4 thousand hectares cultivated producing 5988.8 thousand metric tons annually [24]. Papaya is crucial for its immune-boosting properties and anticancer effects, particularly against hormone-related cancers [25]. It's significance in India underscores its economic and health benefits. Our study aims to investigate the synergistic effects of fly ash, *Trichoderma*, and neem cake on papaya plants infected with *Meloidogyne incognita.*

We hypothesize that the application of different concentrations of these bio-organic amendments will positively influence papaya growth, photosynthetic pigment content, and mitigate disease intensity compared to untreated plants. Specifically, our hypothesis suggests that FA will enhance growth parameters, *Trichoderma* will increase photosynthetic pigments such as chlorophylls and carotenoids, and NC will reduce nematode-induced disease symptoms and root damage. Our objectives include evaluating root, shoot growth, measuring chlorophyll, and carotenoid levels.

II. MATERIALS AND METHODS

2.1 Experimental site

The experiment was conducted at the Department of Botany (27°52' N and 78°05' E) Aligarh Muslim University, Uttar Pradesh, India. The area features diverse soil types, including loamy, sandy, clay loam, and sandy loam, suitable for various agricultural experiments. Aligarh experiences a tropical semi-arid climate, with temperatures ranging from 4.0°C in winter to 45°C in summer. The district receives an average annual rainfall of 800 mm, with a relative humidity of 70%, mainly during July to September.

2.2 Soil sterilization

Soil was placed in gunny bags, steam sterilized and autoclaved at 15 psi and 121°C for 15 minutes. The autoclaved soil was then dried and mixed with various concentrations of chopped fly ash, neem oil cake, and *T. viride* to create different ratios.

2.3 Experimental materials

The fungal bio-agent, *T. viride*, was cultured on Potato Dextrose Agar (PDA) medium. Petri dishes with *T. viride* were incubated at 25°C for 15 days. For mass culturing, *T. viride* was inoculated into PDA flasks and incubated at s25±1°C for 15 days. After sufficient growth, the fungal mycelia were filtered, dried, and ground with distilled water to prepare inoculum suspensions containing 1g/10 mL and 2g/10 mL of distilled water. Neem cake was purchased from a seed shop in Aligarh. It was weighed to 50g and 100g as needed and applied to the soil in pots. Water was added, and the soil was left to decompose for 10-14 days. Fly ash was collected from the thermal power plant in Kasimpur. It was weighed to create 10%, 20%, and 30% mixtures. For a 10% mixture, 400g of fly ash was combined with 3600g of soil in a 4 kg pot. The same procedure was followed for other percentages (Fig. 1).

2.4 Collection and preparation of nematode inoculum

J2s of *M. incognita* were cultured on egg plants and housed in a greenhouse at university. Egg plants were carefully uprooted to prevent detachment of egg masses from the roots, followed by thorough washing in distilled water to remove all soil debris. Egg masses were carefully removed from infected roots using sterilized forceps, washed with distilled water and transferred to 25 µm pore-size mesh sieves lined with tissue layers. The sieves were placed in petri dishes with water just enough to cover the egg masses, facilitating the hatching of J2s of *M. incognita*. The petri dishes were then incubated at 25°C for 24 hours. The mesh sieves retained the egg masses while the hatched J2s passed through and settled at the bottom of the dishes. The suspension containing hatched J2s was collected, and the concentration of juveniles per ml was standardized by counting them in a 10ml suspension (Fig. 2).

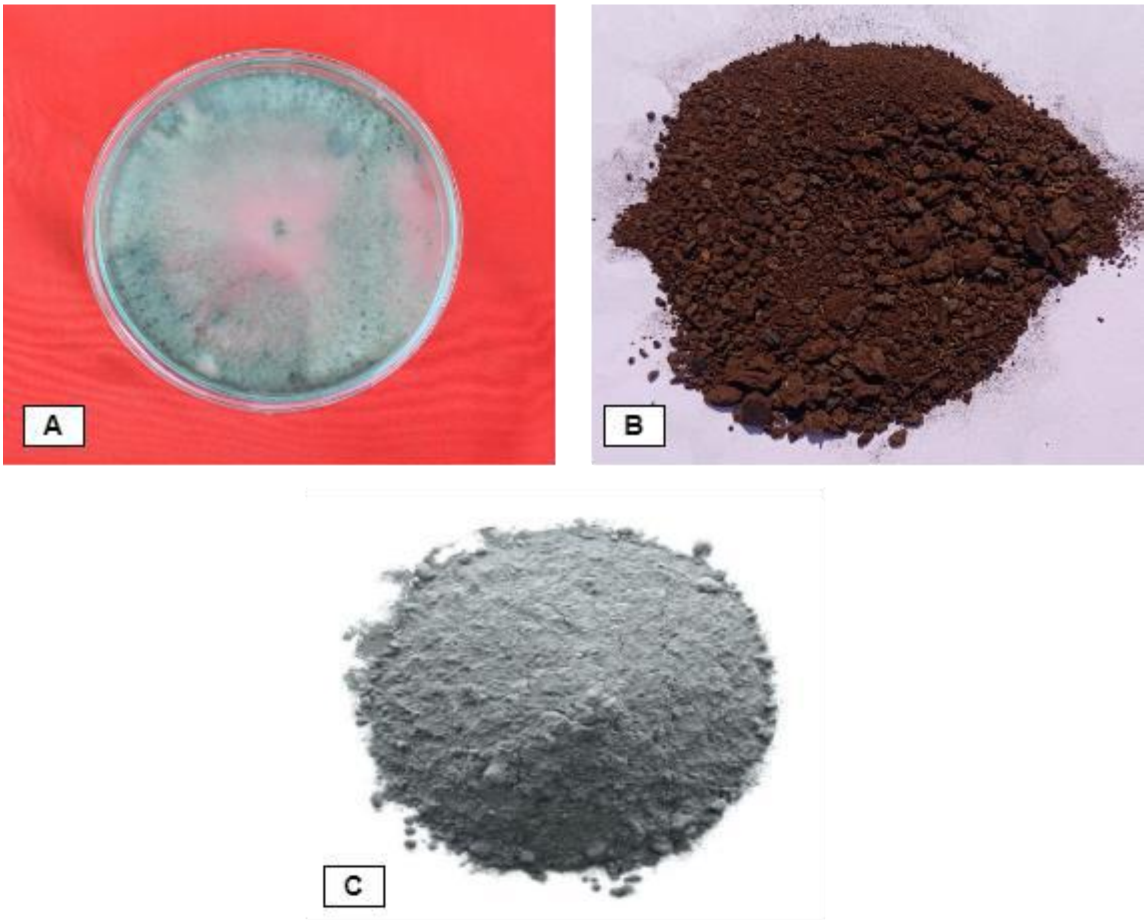

Fig. 1: Figures show the bio-organic materials and fly ash used for the treatments against M. incognita, (A) Trichoderma viride, (B) Neem cake, (C) Fly ash

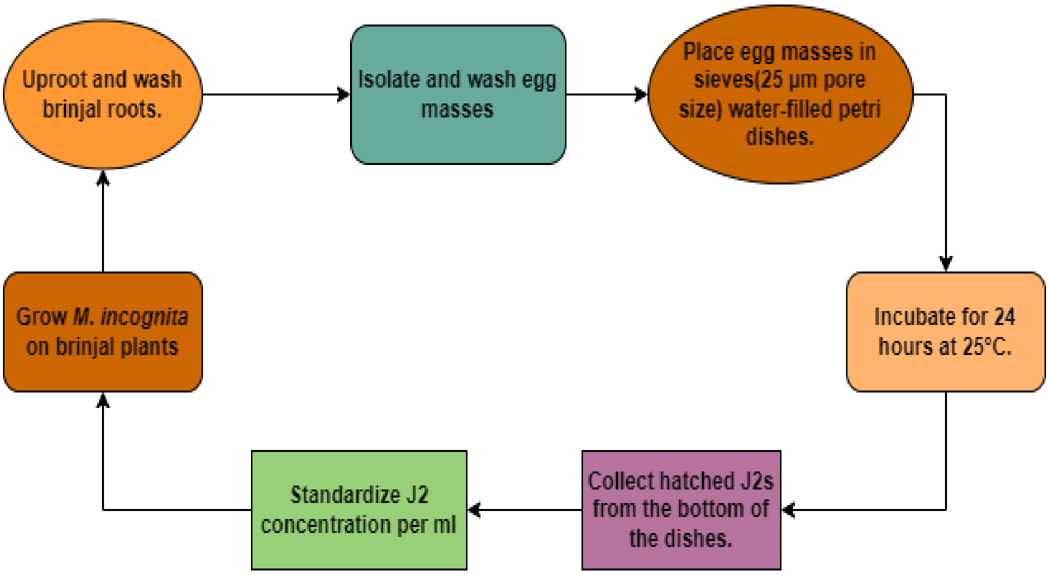

Fig. 2: Steps of collection and preparation of nematode inoculum

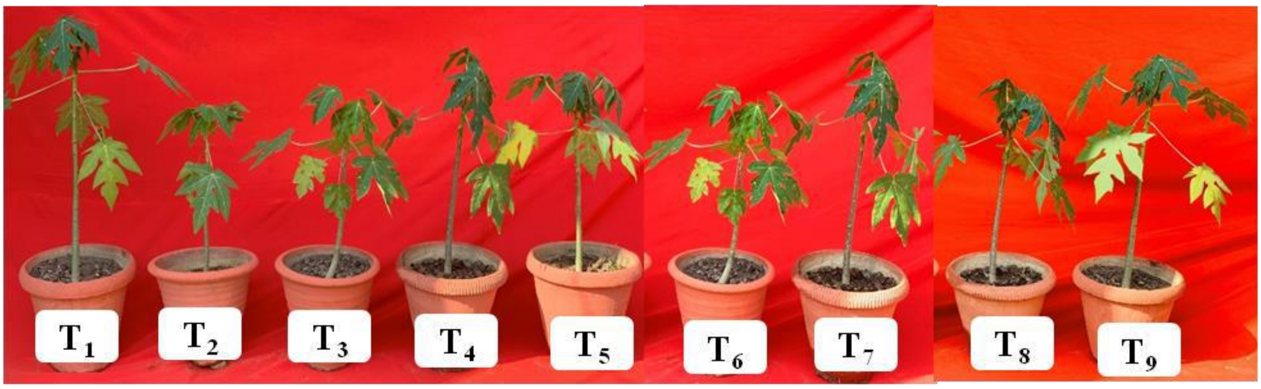

Fig. 3: Effect different treatments on M. incognita and growth of papaya plants (for treatment details Table 1)

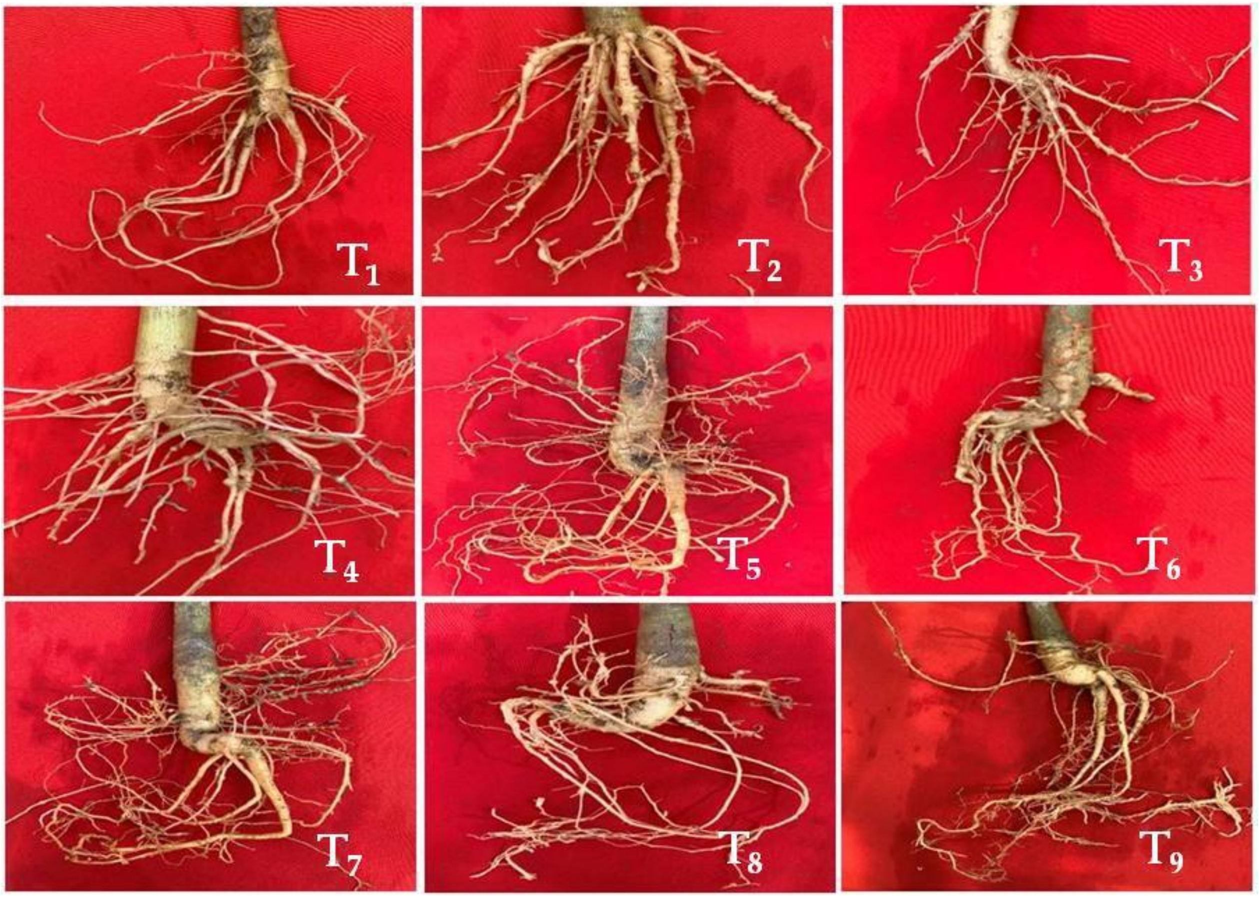

Fig. 4: Effect of different treatments on root-knot disease development caused by M. incognita on papaya roots (for treatment details Table 1)

2.5 Pot experiment and data collection

A pot experiment was carried out in the September, 2023, winter season with papaya and *M. incognita* (Table 1). At maturity plants were uprooted and root and shoot lengths were measured using a meter scale, and fresh weights were recorded with a weighing machine. Subsequently, root and shoot samples were dried in an incubator at 72°C to determine dry weights.

2.6 Estimation of photosynthetic pigments

Fresh leaves (1g) were ground in 100 ml acetone (80%) to extract chlorophyll a, chlorophyll b, and carotenoids. The filtrate was analyzed for percent transmittance at specific wavelengths using a spectrophotometer: 645 nm and 663 nm for chlorophyll [26], and 480 nm and 510 nm for carotenoids [27].

$$\text{Chlorophyll} - \text{a}\ (\text{mg g}^{-1}) = \frac{12.7\ (A663) - 2.69\ (A645) \times V}{1000\ \times W} \dots\dots\dots\dots\dots\dots\dots..\text{(i)}$$

$$\text{Chlorophyll} - \text{b} \,(\text{mg g}^{-1}) = \frac{22.9\,(A645) - 4.68\,(A663) \times V}{1000 \times W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..(\text{ii})$$

$$\text{Chlorophyll} - \text{a} + \text{b} \,(\text{mg g}^{-1}) = \frac{20.2\,(A645) + 8.02\,(A663) \times V}{1000 \times W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..(\text{iii})$$

$$\text{Carotenoid} \,(\text{mg g}^{-1}) = \frac{7.62\,(A480) - 1.49\,(A510) \times V}{1000 \times W} \ldots\ldots\ldots\ldots\ldots\ldots..(\text{iv})$$

Where, A = absorbance of all specific wavelength; V= final volume of chl. extract in 80% acetone(ml), W = weight of the fresh tissues extracted (g)

2.7 Pathological parameters

The number of galls per plant was determined by measuring their dimensions with a micrometer and visually counting them. Egg masses on infected roots were stained with phloxine-B, washed, and counted. The root-knot index, based on [28], assessed gall and egg mass severity on a scale from 0 to 5, ranging from no galls/egg masses to over 100 per root system.

2.8 Data analysis

Three replicates were considered for each treatments. Analysis of variance (ANOVA) was used to assess differences among treatments ($p < 0.05$).

Table 1: Treatment details used during experiment

Treatment[1]	Treatment Description	Fly Ash (%)	*Trichoderma viride* (g)	Neem Cake (g)	J2s (per pot)
T_1	Untreated uninoculated control	-	-	-	-
T_2	Untreated inoculated control	-	-	-	2000
T_3	10% Fly ash + 2000 J2s	10	-	-	2000
T_4	20% Fly ash + 2000 J2s	20	-	-	2000
T_5	30% Fly ash + 2000 J2s	30	-	-	2000
T_6	50 g Neem cake + 2000 J2s	-	-	50	2000
T_7	100 g Neem cake + 2000 J2s	-	-	100	2000
T_8	1 g *Trichoderma viride* + 2000 J2s	-	1	-	2000
T_9	2 g *Trichoderma viride* + 2000 J2s	-	2	-	2000

Table 2 : Effect of different treatments on growth performance of papaya (treatment details in table 1)

Treatments	Length (cm)		Fresh weight (g)		Dry weight(g)	
	Shoot	Root	Shoot	Root	Shoot	Root
T1	55	48	62.2	27.2	12.01	4.39
T2	32	13	27.12	9.3	4.31	1.91
T3	37	21	39.7	12.91	7.13	2.35
T4	47	36	53.69	20.01	9.83	3.36
T5	35	19	31.61	11.1	5.91	2.01
T6	41	24	46.3	15.01	7.49	2.61
T7	52	43	59.71	25.9	11.01	3.99
T8	45	29	49.21	18.01	8.91	2.98
T9	54	47	61.01	26.13	11.93	4.21

[1]Fly Ash (%) - percentage of fly ash used in the treatment; *Trichoderma viride* (g) – amount of *Trichoderma viride* applied in grams. Neem Cake (g) - amount of neem cake applied in grams. J2s (per pot) - number of *M. incognita* juveniles inoculated per pot

Table 3: Effect of different treatments on number of leaves and root circumference of papaya (treatment details in table 1)

Treatments	Number of leaves	Root circumference (cm)
T1	19	6.98
T2	8	3.26
T3	11	3.99
T4	15	5.96
T5	10	3.48
T6	13	4.81
T7	16	6.41
T8	14	5.21
T9	18	6.81

Table 4: Effect of different treatments on photosynthetic pigments of papaya (treatment details in table 1)

Treatments	Photosynthetic pigments (mg/g leaf tissue)			
	Chl.'a'	Chl.'b'	Total Chl. (a+b)	Carotenoids
T1	2.158	1.213	3.371	0.705
T2	1.263	0.313	1.576	0.17
T3	1.595	0.695	2.29	0.31
T4	1.913	0.96	2.873	0.52
T5	1.363	0.516	1.879	0.27
T6	1.7	0.74	2.44	0.39
T7	2.04	1.06	3.1	0.63
T8	1.809	0.896	2.705	0.46
T9	2.113	1.15	3.263	0.69

Table 5: Effect of different treatments on pathological attributes of papaya (treatment details in table 1)

Treatments	Disease	Number of galls	Number of egg masses	GI/EMI
T1	**Nil**	0	0	**0/0**
T2	**Severe**	67	31	4/4
T3	**Moderately severe**	29	19	3/3
T4	**Mild**	17	10	3/2
T5	**Very mild**	10	2	2/1
T6	**Mild**	19	13	3/3
T7	**Nil**	5	0	2/0
T8	**Very mild**	7	1	2/1
T9	**Nil**	3	0	2/0

III. RESULTS

3.1 Shoot length

Pot with UUC showed maximum (55 cm) shoot length, while UIC showed reduced (32 cm) growth. Treatments with 10% FA+MI increased (+15.6%, 37 cm), 20% FA+MI resulted significant improvement (46.9%, 47 cm), 30% FA+MI showed a moderate increase to 35 cm. Notably, treatments with 50g NC+MI and 100g NC+MI increased shoot length to 41 cm and 52 cm, respectively, with increases of 28.1% and 62.5% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI, demonstrated substantial growth, reaching 45 cm and 54 cm, respectively, representing increases of 40.6% and 68.8% compared to UIC.

3.2 Root length

Data showed that UUC treatment exhibited root length (48 cm), while UIC showed decreased root length (13 cm). 10% FA + MI noticed 21 cm root length, representing a 61.5% increase compared to UIC. Treatments with 20% FA+MI led to a significant improvement to 36 cm, marking a 176.9% increased. The treatment with 30% FA+MI showed a moderate increase (19 cm). 50g NC+ MI and 100g NC+ MI increased root length to 24 cm and 43 cm, respectively, with increases of 84.6% and 230.8% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI, demonstrated substantial growth, reaching 29 cm and 47 cm, respectively, representing increases of 123.1% and 261.5% compared to UIC.

3.3 Shoot fresh weight

Results showed that pot with UUC had a fresh weight of shoot of 62.2 g, while UIC showed reduced growth with a fresh weight of shoot of 27.12 g. At 10% FA+MI increased fresh weight of shoot to 39.7 g, representing a 46.5% increase compared to UIC. Treatments with 20% FA+MI resulted in a significant improvement to 53.69 g, marking a 98.1% increase. 30% FA+MI showed a moderate increase to 31.61 g. Treatments with 50g NC+MI and 100g NC+MI increased fresh weight of shoot to 46.3 g and 59.71 g, respectively, with increases of 70.7% and 120.3% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI demonstrated substantial growth, reaching 49.21 g and 61.01 g, respectively, representing increases of 81.3% and 125.6% compared to UIC.

3.4 Shoot dry weight

Data showed that pot with UUC had a dry weight of shoot of 12.01 g, while the UIC had a reduced dry weight of shoot of 4.31 g. Treatments with 10% FA + MI resulted in a dry weight of shoot of 7.13 g, representing a 65.9% increased compared to UIC. At 20% FA+MI showed a significant improvement to 9.83 g, marking a 128.5% increase. 30% FA+MI showed a moderate increase to 5.91 g. Treatments with 50g NC+ MI and 100g NC+ MI increased dry weight of shoot to 7.49 g and 11.01 g, respectively, with increases of 73.5% and 155.7% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI demonstrated substantial growth, reaching 8.91 g and 11.93 g, respectively, representing increases of 106.7% and 177.4% compared to UIC.

3.5 Root fresh weight

Data showed that the pot with UUC had a fresh weight of root of 27.2 g, while the UIC showed reduced growth with a fresh weight of root of 9.3 g. Treatments with 10% FA+MI increased the fresh weight of root to 12.91 g, representing a 38.6% increase compared to UIC. Treatments with 20% FA+MI resulted in a significant improvement to 20.01 g, marking a 115.1% increase. 30% FA+MI showed a moderate increase to 11.1 g. Treatments with 50g NC+ MIand 100g NC+ MI increased fresh weight of root to 15.01 g and 25.9 g, respectively, with increases of 61.3% and 178.5% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI demonstrated substantial growth, reaching 18.01 g and 26.13 g, respectively, representing increases of 93.5% and 181.3% compared to UIC.

3.6 Root dry weight

Results showed that pot with UUC had a dry weight of root of 4.39 g, while the UIC had a reduced dry weight of root of 1.91 g. 10% FA+MI resulted in a dry weight of root of 2.35 g, representing a 23.6% increase compared to UIC. 20% FA+MI showed a significant improvement to 3.36 g, marking a 75.4% increase. The treatment with 30% FA+MI showed a moderate increase to 2.01 g. Treatments with 50g NC+ MI and 100g NC+ MI increased dry weight of root to 2.61 g and 3.99 g, respectively, with increases of 36.6% and 109.4% compared to UIC. Moreover, treatments with 1g Tv + MI and 2g Tv + MI demonstrated substantial growth, reaching 2.98 g and 4.21 g, respectively, represented increases of 56.0% and 120.9% compared to UIC.

3.7 Root circumference

Data showed that UCC had a root circumference of 6.98 cm, while the UIC had a significantly lower circumference of 3.26 cm. Treatments with 10% FA+MI resulted in a root circumference of 3.99 cm, representing a 22.7% increase compared to UIC. At 20% FA+MI showed a substantial increase to 5.96 cm, marking an 82.2% increase. The treatment with 30% FA+MI resulted in a root circumference of 3.48 cm. Treatments with 50g NC + MI and 100g NC + MI increased the root circumference to 4.81 cm and 6.41 cm, respectively, with increases of 47.2% and 96.6% compared to UIC. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated significant growth, reaching 5.21 cm and 6.81 cm, respectively, representing increases of 60.1% and 108.9% compared to UIC.

3.8 Chlorophyll a

Pot with UUC exhibited a chlorophyll a content of 2.158 mg/g. In contrast, UIC showed a significantly lower content of 1.263 mg/g. Treatments with 10% FA+MI resulted in a chlorophyll a content of 1.595 mg/g, representing a 26.3% increase compared to UIC. At 20% FA+MI showed a substantial increase to 1.913 mg/g, marked (+51.6%). Treatment with 30% FA+MI resulted in a chlorophyll a content of 1.363 mg/g. Treatments with 50g NC + MI and 100g NC + MI increased the chlorophyll a content to 1.7 mg/g and 2.04 mg/g, respectively, with increases of 34.5% and 61.5% compared to UIC. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated significant increases, reaching 1.809 mg/g and 2.113 mg/g, respectively, representing increases of 43.3% and 67.4% compared to UIC.

3.9 Chlorophyll b

Pot with UUC exhibited a chlorophyll-b content of 1.213 mg/g. In contrast, the UIC showed a significantly lower content of 0.313 mg/g. Treatments with 10% FA+MI resulted in a chlorophyll b content of 0.695 mg/g, represented (+122.8%) compared to UIC. Treatment with 20% FA+MI showed a substantial increase to 0.96 mg/g, marking a 206.1% increase. 30% FA+MI resulted in a chlorophyll b content of 0.516 mg/g. Treatments with 50g NC + MI and 100g NC + MI increased the chlorophyll b content to 0.74 mg/g (+136.3%) and 1.06 mg/g (+238.7%) compared to UIC. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated significant increases, reaching 0.896 mg/g and 1.15 mg/g, respectively, representing increases of 186.6% and 267.8% compared to UIC.

3.10 Total chlorophyll (a + b)

Pot with UUC exhibited a total chlorophyll content of 3.371 mg/g. In contrast, the UIC showed a significantly lower content of 1.576 mg/g. Treatments with 10% FA+MI resulted in total chlorophyll content of 2.29 mg/g, represented (+45.5%) compared to UIC. At 20% FA+MI showed a substantial increase (2.873 mg/g, +82.6%). At 30% FA+MI resulted in total chlorophyll content of 1.879 mg/g. Treatments with 50g NC + MI and 100g NC + MI increased the total chlorophyll content to 2.44 mg/g (+55.4%) and 3.1 mg/g (+97.5%), than UIC. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated significant increased (2.705 mg/g and 3.263 mg/g) than UIC.

3.11 Carotenoid content

Pot with UUC showed a carotenoid content of 0.705 mg/g. In contrast, the UIC exhibited a significantly lower content of 0.17 mg/g. Treatments with 10% FA+MI resulted in a carotenoid content of 0.31 mg/g, represented (+82.4%) compared to UIC. Treatment with 20% FA+MI showed a substantial increase to 0.52 mg/g (+205.9%). At 30% FA+MI resulted in a carotenoid content of 0.27 mg/g. Treatments with 50g NC + MI and 100g NC + MI increased the carotenoid content to 0.39 mg/g and 0.63 mg/g, respectively, with increases of 129.4% and 270.6% compared to UIC. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated significant increased (0.46 mg/g, +170.6% and 0.69 mg/g +305.9%) than UIC.

3.12 Disease ratings and plants infestation

Pot with UUC showed no disease symptoms. In contrast, UIC exhibited severe disease symptoms. Treatments with 10% FA+MI and 20% FA+MI resulted in moderately severe and mild disease symptoms, respectively. At 30% FA+MI resulted in very mild disease symptoms. Treatments with 50g NC + MI and 100g NC + MI showed mild and no disease symptoms, respectively. Additionally, treatments with 1g Tv + MI and 2g Tv + MI demonstrated very mild and no disease symptoms, respectively.

3.13 Number of galls

Pot with UUC showed no galls, indicated the absence of RKN infestation. In contrast, UIC exhibited a substantial infestation with 67 galls per plant. 10% FA+MI resulted in 29 galls (-56.7%) than UIC. Similarly, 20% FA+MI and 30% FA+MI showed further reductions with 17 galls (-74.6% reduction) and 10 galls (85.1% reduction), respectively. 50g NC + MI and 100g NC + MI resulted in 19 galls (71.6% reduction) and 5 galls (92.5% reduction), respectively.

3.14 Number of egg masses

Pot UUC exhibited no egg masses, confirming the absence of RKN infestation. In contrast, UIC showed 31 egg masses per plant, indicated a severe infestation with *M. incognita*. Treatments with bio-organic amendments demonstrated varying degrees of effectiveness in reduced egg mass formation. At 10% FA+MI resulted in 19 egg masses, represented (38.7%) reduction than UIC. Increased the concentration of 20% FA+MI further reduced the egg masses to 10 (67.7% reduction), and at 30% FA+MI, only 2 egg masses were observed (93.5% reduction). 50g NC + MI showed 13 egg masses (58.1% reduction), while at 100g NC + MI, there were no egg masses observed (100% reduction). Furthermore, treatments incorporating *T. viride* (1g Tv + MI and 2g Tv + MI) also showed significant reductions in egg masses compared to UIC, with 1 egg mass (96.8% reduction) for 1g Tv + MI and no egg masses observed (100% reduction) for 2g Tv + MI.

3.15 Root-knot index (GI) and egg mass index (EMI)

Pot with UUC showed a GI of 0 and an EMI of 0, indicated no infection by M. *incognita*. In contrast, UIC had a GI of 4 and an EMI of 4, indicated a severe infestation with significant gall formation and egg mass production. Treatment with 10% FA+MI resulted in a GI of 3 and an EMI of 3, represented a moderate reduction than UIC. Increased the concentration of 20% FA+MI resulted in a GI of 3 and an EMI of 2, indicated further improvement in nematode suppression. At 30% FA+MI, the GI reduced to 2 and the EMI to 1, showed even better control of nematode infestation. At 50g NC + MI showed a GI of 3 and an EMI of 3, demonstrated moderate effectiveness. Increased the neem cake (100g NC + MI) resulted in a GI of 2 and an EMI of 0, indicated significant suppression of nematode activity, with no observed egg masses. Treatments incorporating *T. viride* (T8 and T9) also showed promised results. 1g Tv + MI had a GI of 2 and an EMI of 1, while 2g Tv + MI showed a GI of 2 and an EMI of 0. These treatments effectively reduced both gall formation and egg mass production than UIC.

IV. DISCUSSION

4.1 Root length and shoot weight

All treatments except 30% FA+MI significantly increased shoot and root length and fresh and dry weight compared to the untreated inoculated control (UIC). The most improved effects were observed with 2g Tv + MI, which substantially increased shoot and root growth parameters compared to UIC. Significant improvements were observed in growth performance, photosynthetic pigments, protein, proline, antioxidant activity, and mineral levels when 10% FA-amended soil was used. On the other hand, all of the previously described metrics were adversely affected by the higher fly ash doses (25%) which caused oxidative stress by raising the levels of lipid peroxidation and electrolytic leakage [29]. Fungi, among tiny creatures, can colonize, trap, and diminish nematode populations. Some *Trichoderma* fungi have been demonstrated to control plant parasitic nematode populations while increased vegetable crop yields [30].

4.2 Root circumference

Similar to root length, all treatments except 30% FA+MI increased root circumference compared to UIC. The most substantial increase was observed with 2g Tv + MI. Several *Trichoderma* species, including *T. harzanium* and *T. viride*, have been previously identified as promising bio-control agents against RKNs [31,32,33]. This finding suggests that Tv+N amendments are particularly effective in promoting overall root development, potentially by increasing root branching and diameter.

4.3 Chlorophyll content

The application of all treatments except 30% FA+MI significantly increased chlorophyll a, chlorophyll b, and total chlorophyll content compared to UIC.*M. incognita* considerably lowered plant growth performance, photosynthetic pigments, and beetroot yield when compared to control plants; however, 15% FA greatly reduced the detrimental effects of *M. incognita*. When compared to a control, confocal laser microscopy demonstrated that 15% FA in soil decreased nematode-juvenile invasion in beetroot [34]. The highest increases were observed with 2g Tv + MI. Many secondary metabolites produced by *Trichoderma* spp., including flavonoids and phenols, provide resistance to biotic stress [35,36]. This indicated that these treatments, particularly Tv + MI, play a role in enhanced chlorophyll biosynthesis, potentially leading to improved photosynthetic activity and plant growth.

4.4 Carotenoid content

All treatments except 30% FA+MI increased carotenoid content compared to UIC. The most significant increase was again observed with 2g Tv + MI. In comparison to the control, the photosynthetic pigments (carotenoid and chlorophyll) were considerably elevated at fly ash concentrations of 10%, 20%, and 30%. But both pigments gradually decreased at 40% and 50% fly ash levels [37]. Many of *Trichoderma* spp. effects are now established as BCA, including competition, antibiosis, resistance, plant tolerance to biotic and abiotic stressors, and activation of pathogen defenses [38]. Results suggested that these treatments may stimulate carotenoid production, which can play a role in photosynthesis and defense against stress.

4.5 Disease ratings and plants infestation

Disease symptoms were less severe in plants treated with amendments compared to UIC. Notably, both concentrations of Tv + MI completely suppressed disease symptoms. *Trichoderma* chemicals, including B-1,3-glucanase, protease, chitinase, lipase, and other metabolites, may contribute to nematode parasitism [39,30,40]. This suggests that these treatments, particularly Tv + MI, may enhance plant defense mechanisms against pathogens.

4.6 Number of galls

All treatments significantly reduced the number of galls compared to UIC, indicating their effectiveness in suppressing RKN infestation. The most significant reduction was observed with 100g NC+MI and 2g Tv + MI. NC reduced root galls (96%) and gall index (62%), outperforming artificial nematicides. The maximum yield was found with the NC treatment. As a result, neem-based solutions, particularly NC, can be recommended for the environmentally friendly management of *M. javanica* in field peas [41]. Applying organic materials to the soil, such as oil cakes, plant extracts, and agricultural wastes, both *in vitro* and *in vivo*, is beneficial. Oil cakes made from botanical extracts destroy nematodes by preventing their juveniles from moving about in the soil and releasing secondary metabolites [42]. These findings suggest that both neem cake (NC) and *T. viride* amendments have nematicidal properties and can be used as control strategies against RKN.

4.7 Number of egg masses

Similar to gall formation, all treatments except 10% FA+MI significantly reduced the number of egg masses compared to UIC. The most effective treatments were 100g NC+MI and 2g Tv + MI, which completely suppressed egg mass production. The number of egg masses and eggs per egg mass on tomato roots were dramatically reduced after applying the neem cake to the soil for protection and cure [43]. Bio-carbon compounds generated from oil cakes have been investigated and used to control nematodes. Oilseed cakes regulate nematodes in vegetables, cereals, and fruit crops [44]. This indicates that these amendments can effectively disrupt the RKN life cycle by reducing egg-laying by females.

4.8 Root-knot index (GI) and egg mass index (EMI)

The root-knot index (GI) and egg mass index (EMI) reflected the overall severity of RKN infestation. All treatments except 10% FA+MI significantly reduced both GI and EMI compared to UIC. The most effective treatments were 100g NC+MI and 2g Tv + MI, achieving the lowest GI and EMI values for RKN infestation. Botanical extracts and oil cakes can kill nematodes by producing secondary metabolites and limiting juvenile mobility in the [42]. Soil application of *T. viride* in conjunction with neem cake at varying doses was found to be more effective against *M. incognita*, with increased plant growth parameters and a decrease in root-knot nematode population in soil than other treatments [45]. Fly ash concentrations significantly and dose-dependently decreased the gal index, egg mass index, and reproduction factor of *M. incognita*. Therefore, at the right dosages, fly ash suppresses root-knot nematodes while simultaneously promoting plant development and yield [46]. These findings show us the effectiveness of these treatments in controlling RKN.

V. CONCLUSION

This study explored the efficacy of various bio-organic amendments in enhancing plant growth and suppressing nematode infestation. Results demonstrated significant improvements in multiple growth parameters and physiological traits of papaya plant treated with fly ash (FA), neem cake (NC), and *Trichoderma viride* (Tv) inoculated with 2000 juveniles of *Meloidogyne incognita* (MI). Shoot and root lengths, fresh and dry weights, and chlorophyll content were notably enhanced compared to untreated inoculated controls (UIC). The highest shoot length of 55 cm and root length of 48 cm were observed in the untreated uninoculated control (UUC), indicated the potential growth under optimal conditions. Treatments with 100g NC + MI and 2g Tv + MI showed substantial increases in shoot and root lengths, with improvements of up to 68.8% and 261.5%, respectively, compared to UIC.

Chlorophyll-a and chlorophyll-b contents also increased significantly in treated plants, with concentrations peaked at 2.113 mg/g and 1.15 mg/g, respectively, in the 2g Tv + MI treatment. Total chlorophyll content reached 3.1 mg/g in plants treated with 100g NC + MI, indicated robust physiological activity and photosynthetic efficiency. These enhancements highlight the role of bio-organic amendments in promoting plant health and vigor. Importantly, bio-organic treatments exhibited strong nematode suppression effects. Plants treated with 100g NC + MI and 2g Tv + MI showed no egg masses and reduced root-knot indices (GI), demonstrating effective control of nematode infestation. This suggests that bio-organic amendments not only support plant growth but also contribute to sustainable pest management strategies in agriculture. Overall, utilize bio-organic amendments, particularly neem cake and *T. viride*, to enhance plant growth and suppress nematode infestation effectively. Optimize application rates and assess long-term impacts on soil health and crop productivity for sustainable agricultural practices.

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