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PLANT-DERIVED LIQUID BIODIGESTER FOR THE SUPPRESSION OF *RADOPHOLUS SIMILIS* COBB. IN BANANA THROUGH GREEN MANAGEMENT STRATEGY

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ABSTRACT

Banana (*Musa* sp.) is one of the most important tropical fruit crops, valued for both its nutritional and economic significance. However, its productivity is severely constrained by the burrowing nematode, *Radopholus similis* (Cobb), which causes root damage, corm necrosis, and plant toppling. The present study evaluated the bio-efficacy of liquid biodigester against *R. similis* under pot culture conditions. Different concentrations of liquid biodigester (from undiluted to 1:10 dilution) were applied at planting, and subsequently at 45, 90, and 150 days after planting (200ml/plant), along with a chemical check (Cartap hydrochloride 50% SP @ 10 g/plant) and an untreated control. Results indicated that the undiluted liquid biodigester treatment significantly reduced nematode populations to 42.66 juveniles per 250 cc of soil and 160.34 juveniles per 10 g of roots, compared to 983.08 and 277.07 juveniles, respectively, in the untreated control. Correspondingly, root lesion number (15.11), lesion index (0.75), and corm necrosis grade (1) were markedly lower than in the control (116.73 lesions, lesion index 4.00, and necrosis grade 4). While the chemical check recorded the lowest nematode populations (28.70 juveniles/250 cc soil, 121.88 juveniles/10 g roots, 12.02 lesions, lesion index 0.51, and necrosis grade 1) as compared to liquid biodigester treatments. Importantly, no phytotoxic symptoms were observed in any of the treatments. These findings suggest that liquid biodigester is a promising, eco-friendly alternative to chemical nematicides for sustainable banana cultivation, effectively suppressing *R. similis* while promoting overall plant health.

***Key words*:** Banana, *Radopholus similis*, *Musa* sp., liquid biodigester, root lesion index, corm necrosis

Introduction

Banana (*Musa* sp.) is one of the most important fruit crops worldwide, is cultivated extensively in tropical and subtropical regions for its nutritional, economic and cultural significance. Globally, bananas contribute substantially to food security and livelihood with India being the largest producer, accounting for approximately 26 per cent of global production (FAOSTAT, 2022). The crop is grown predominantly in Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh and Karnataka. However, the productivity of banana is seriously constrained by various biotic stresses, among which plant-parasitic nematodes (PPNs) play a major role in limiting yield potential.

The burrowing nematode, *Radopholus similis* Cobb, is regarded as the most economically significant nematode pest affecting banana cultivation (Gowen *et al*., 2005). This migratory endoparasitic nematode invades and tunnels through root cortical tissues, creating necrotic lesions that impair water and nutrient uptake. The resultant damage leads to poor plant growth, reduced

fruit size, susceptibility to toppling and overall yield decline. Field infestations of *R. similis* can cause yield losses ranging from 30 per cent to over 60 per cent in severe cases (Secretariat *et al.*, 2021), threatening the sustainability of banana production.

Traditionally, the management of *R. similis* has relied on chemical nematicides. However, growing concerns over environmental pollution, pesticide residues in food, human health risks and the development of nematode resistance have led to increased interest in safer, eco-friendly alternatives (Dixit, 2019). Biological management and organic soil amendments have emerged as potential components of Integrated Nematode Management (INM). Among these, plant-derived liquid biodigesters-fermented extracts from nematode-suppressive crops—are gaining attention due to their dual role in pest suppression and soil health improvement.

Several plants possess natural nematicidal properties, either through allelochemicals, secondary metabolites, or bioactive compounds. For instance, *Tagetes erecta* (marigold) is known to produce thiophenes, which exhibit toxic effects on nematodes. *Azadirachta indica* (neem) yields azadirachtin and other limonoids that inhibit nematode development and reproduction. *Ricinus communis* (castor) contains ricin and other phytochemicals that adversely affect nematodes, while *Crotalaria juncea* (sun hemp) is rich in monocrotaline alkaloids that exhibit nematotoxic activity (Abd-Elgawad, 2021). When these plants are processed into liquid biodigesters via anaerobic fermentation, their bioactive principles are concentrated, enhancing their nematicidal efficacy.

Moreover, liquid biodigesters improve soil microbial diversity and activity promoting antagonistic organisms that naturally suppress nematode population (Nasr, 2015). This holistic approach not only targets nematode suppression but also enhances soil fertility and plant resilience, aligning with the goals of sustainable agriculture. In this context, the present investigation was aimed at evaluating the *in vitro* bio-efficacy of plant-derived, nematode-suppressive plant-based liquid biodigesters for the management of the banana burrowing nematode, *R. similis*. The study investigates the development of a green, sustainable and farmer-friendly management strategy that can reduce reliance on chemical nematicides while ensuring environmental safety and soil health.

Material and methods

Study location and period

The present investigation was conducted during 2023–24 at the Department of Plant Pathology, Kittur Rani Channamma College of Horticulture (KRCCH), Arabhavi, Belagavi district, Karnataka (India).

Table 1: Experimental treatments applied to potted banana (cv. Grand Nain) under greenhouse conditions.

Treatment No.	Treatment Details
T_1	Liquid biodigester (no dilution) @ 200 ml/plant
T_2	Liquid biodigester diluted with water (1:1) @ 200 ml/plant
T_3	Liquid biodigester diluted with water (1:2) @ 200 ml/plant
T_4	Liquid biodigester diluted with water (1:3) @ 200 ml/plant
T_5	Liquid biodigester diluted with water (1:4) @ 200 ml/plant
T_6	Liquid biodigester diluted with water (1:5) @ 200 ml/plant
T_7	Liquid biodigester diluted with water (1:6) @ 200 ml/plant
T_8	Liquid biodigester diluted with water (1:7) @ 200 ml/plant
T_9	Liquid biodigester diluted with water (1:8) @ 200 ml/plant
T_{10}	Liquid biodigester diluted with water (1:9) @ 200 ml/plant
T_{11}	Liquid biodigester diluted with water (1:10) @ 200 ml/plant
T_{12}	Chemical control (Cartap hydrochloride @ 10 g/plant)
T_{13}	Untreated control (distilled water @ 200 ml/plant)

Collection, extraction and identification of *Radopholus similis*

Soil (250 cc) and root (10 g) samples were collected from banana plants showing typical burrowing nematode symptoms from ICAR-All India Co-ordinated Research Projects (AICRP) on Fruits, KRCCH, Arabhavi, at 15–20 cm soil depth. Samples were stored at 4°C until further processing. Nematodes from soil were extracted using Cobb's sieving and decanting method followed by the modified Baermann funnel technique (Ayoub, 1977). Root-associated nematodes were extracted by the root incubation method after grinding root samples in a blender. Nematodes were identified morphologically using features such as stylet, oesophagus, vulva and tail terminus under a compound microscope. Permanent slides were prepared as per Seinhorst's method (1966), involving fixation with FA 4:1 solution and gradual dehydration in ethanol-glycerol series (Dinesh, 2014).

Maintenance and multiplication of *R. similis*

Pure cultures of *R. similis* were established on

banana cv. Grand Nain in pots filled with sterilized soil, sand and FYM, it was further multiplied *in vitro* on carrot discs, following the method of O'Bannon and Taylor (1968). Nematodes were periodically extracted from the carrot discs, counted under a stereomicroscope using a counting dish and standardized for experimental use (Dinesh, 2014).

Preparation of plant-based liquid biodigester

The liquid biodigester was prepared using a 200 L plastic drum, by fermenting desi cow dung (5 kg), cow urine (5 L) and equal parts (1 kg each) of antagonist plant materials including *Azadirachta indica*, *Tagetes erecta*, *Ricinus communis*, *Millettia pinnata*, *Brassica oleracea var. capitata*, *Lantana camara* and *Melia dubia*. The mixture was diluted with 100 L of water and allowed to ferment anaerobically for one month (Akhtar and Mahmood, 1996) (Fig. 1).

Pot culture experiment

Pathogenicity was confirmed by inoculating 1000 nematodes per 1000 g soil into pots planted with tissue-cultured banana cv. Grand Nain. Post 30 days, roots were assessed for lesion formation, necrosis and nematode re-isolation to satisfy Koch's postulates (Satyanarayana, 1982). A pot experiment was conducted under greenhouse conditions following a completely randomized design (CRD) comprising 13 treatments (Table 1), each replicated three times with six plants per replication. Banana cultivar Grand Nain was inoculated with 1000 nematodes per 1000 g of sterilized potting mixture (soil, sand and FYM). Liquid biodigester was applied at the time of planting, and subsequently at 45 and 90 days after planting.

Observations on nematode parameters were recorded at initial, 45, 90 and 150 days after inoculation. The nematode population in 250 cc of soil and 10 g of roots was extracted using Cobb's sieving and the Modified Baermann funnel method and counted under a stereoscopic microscope. The percent decrease over control (PDC) in nematode population was calculated using the formula:

$$PDC = \frac{C - T}{C} \times 100$$

Where, C is the nematode population (number) in

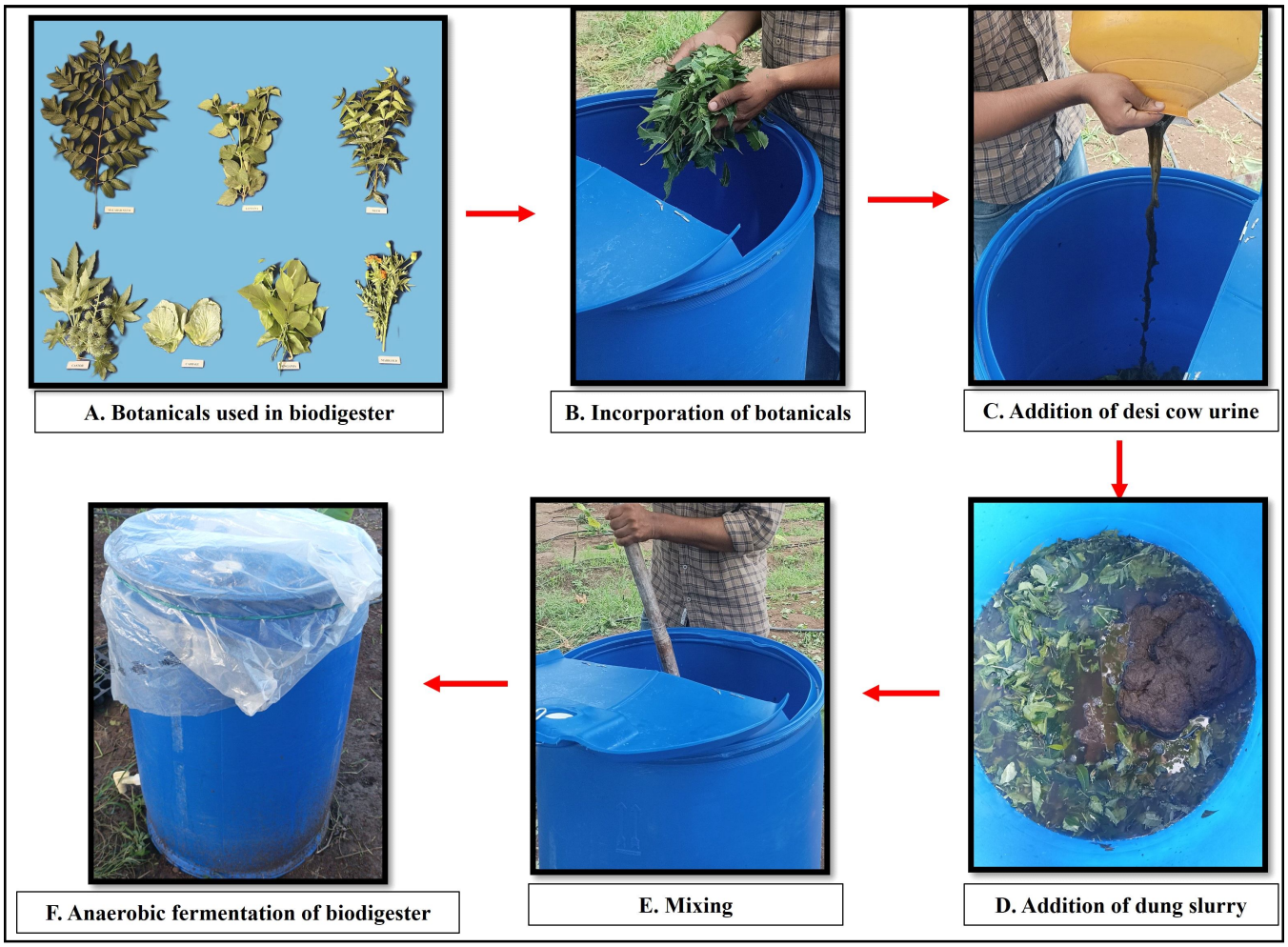

Fig. 1: Preparation of plant based liquid biodigester.

untreated control and T is nematode population (number) in the treatment.

The root lesion index was assessed at 150 days after planting using a 0–4 scale as described by Ravichandra and Krishnappa (1985):

0: No lesions

1: Few lesions up to 1 mm diameter (1–20 lesions)

2: Many lesions up to 1 mm diameter (21–50 lesions)

3: Many lesions up to 1 cm diameter (51–100 lesions)

4: Very severe lesions ≥1 cm diameter (>100 lesions)

Corm necrosis grading was also performed on a 0–4 visual scale, based on the extent of internal damage. The phytotoxicity effect of the liquid biodigester was assessed using a 0–10 visual scale (Tiyagi and Ajaz, 2004). Parameters observed included chlorosis, necrosis, wilting, scorching, hyponasty and epinasty.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) and treatment means were compared using Critical Difference (CD) at 5 per cent significance level.

Results and Discussion

Collection and Isolation of Burrowing Nematode

The burrowing nematode (*R. similis*) was successfully isolated from symptomatic banana plants collected from AICRP on Fruits, KRCCH, Arabhavi, Gokak taluk, Belagavi district, Karnataka. The diseased plants exhibited typical symptoms associated with *R. similis* infestation, such as root lesions, stunted growth and yellowing of leaves. Isolation was performed following standard procedures of soil and root processing, using Cobb's sieving and Baermann funnel techniques for nematode extraction. The isolates were purified and maintained for subsequent morphological and pathogenic analyses (Bartholomew *et al.*, 2014).

Morphological identification of *Radopholus similis*

Morphological examination under a stereoscopic microscope confirmed the identity of the nematode as *R. similis*. The diagnostic features of the females included a nearly straight to slightly ventrally curved body, a low and rounded lip region slightly offset from the body contour, and a robust stylet with well-developed dorsal and subventral knobs. The dorsal gland opening was positioned near the stylet base, a characteristic of *R. similis* (Brooks, 2008). The excretory pore was located just posterior to the hemizonid and the oesophageal gland overlapped the intestine dorsally. The vulva was post-median with a didelphic reproductive system, containing a single row of oocytes and the spermatheca was round or oval, filled with rod-shaped sperm (Bartholomew *et al.*, 2014).

Table 2: Effect of liquid biodigester on *R. similis* population (No./250 cc of soil) at 45, 90, and 150 days after nematode inoculation (DAI) in banana (cv. Grand Nain).

Treatment No.	45 DAI	90 DAI	150 DAI
T_1	170.98i	110.57i	42.66i
T_2	178.19hi	123.24h	51.17h
T_3	182.31gh	130.09gh	56.83g
T_4	186.77fg	136.93fg	59.91g
T_5	190.21ef	142.07ef	64.36f
T_6	195.36de	146.86de	68.12f
T_7	199.48cd	151.65cd	74.29e
T_8	201.19c	156.79bc	79.08d
T_9	205.66c	159.19bc	80.45cd
T_{10}	213.55b	162.27b	83.87bc
T_{11}	219.73b	165.69b	87.98b
T_{12}	132.28j	80.45j	28.70j
T_{13}	615.71a	827.93a	983.08a
S.E m ±	2.40	2.98	1.43
CD @ 5%	6.98	8.66	4.15

Means followed by the same letter(s) within columns are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level of probability

Male nematodes displayed distinctly offset, high, rounded lip regions with 3-5 annuli. Their stylet was weakly developed, with either absent or slightly expanded basal knobs, while the median bulb and oesophageal gland were degenerate - features consistent with previous descriptions of *R. similis* males (Agrios, 2005). The excretory pore was located near the hemizonid and the bursa extended across 47-90 per cent of the tail length. The tail morphology, being subconoid and exceeding 70 µm with a clear hyaline region averaging over 5.6 µm, further corroborated the species identity.

These morphological observations align well with classical taxonomic descriptions of *R. similis* provided by previous researchers (Gowen *et al*., 2005; Haegeman *et al*., 2010). The distinct sexual dimorphism, especially in the male's degenerate feeding structures and the female's robust stylet and reproductive features, facilitated clear identification. Such precise identification is critical, as *R. similis* is recognized globally as a destructive nematode causing "toppling disease" in banana due to extensive root damage (Gowen *et al*., 2005).

Bio efficacy of liquid biodigester against burrowing nematode under pot conditions

Effect of Liquid Biodigester on Nematode Population in Soil

The application of liquid biodigester at different

Table 3: Bio-efficacy of liquid biodigester on R. similis population present in 10 g of roots of banana (cv. Grand Nain) at 150 days after nematode inoculation.

Treatment No.	*R.similis* population (No. per 10g root)	% Decrease over control
T_1	160.34j	42.13
T_2	165.83j	40.15
T_3	170.64hu	38.41
T_4	173.38h	37.42
T_5	177.16gh	36.06
T_6	182.31fg	34.20
T_7	187.46ef	32.34
T_8	193.30de	30.23
T_9	197.76d	28.62
T_{10}	205.31c	25.90
T_{11}	213.55b	22.93
T_{12}	121.88k	56.01
T_{13}	277.07a	0.00
S.E m ±	2.34	-
CD @ 5%	6.80	-

Means followed by the same letter(s) within columns are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level of probability

concentrations significantly reduced *R. similis* population in banana rhizosphere soils. At 150 days after planting, nematode populations in 250 cc of soil decreased markedly from 983.08 in the untreated control to 42.66 juveniles in undiluted liquid biodigester (T_1) and 51.17 juveniles in the 1:1 dilution (T_2). Even at the weakest dilution of 1:10 (T_{11}), populations were reduced to 87.98 juveniles, showcasing the dose-dependent suppression of nematodes. The chemical check (Cartap hydrochloride 50% SP) recorded the lowest count of 28.70 juveniles, demonstrating high nematicide activity (Table 2).

The reduction in nematode population with increased digestate concentration is consistent with prior studies. Buskov *et al*., (2002) and Taba *et al*., (2006) reported that glucosinolate derivatives and organic acids in organic amendments exhibit nematicidal effects. Similarly, Oka and Pivonia (2003) highlighted the role of nitrogenous compounds in nematode suppression. The digestion process enhances the formation of bioactive fermentation products, including ammonia, volatile fatty acids and phenolics, which inhibit nematodes (Bulluck *et al*., 2002; Xiao *et al*., 2007).

Effect on Nematode Population in Roots

The application of liquid biodigester at varying dilutions demonstrated significant reductions in the *Radopholus similis* population per 10 g of roots compared to the untreated control (Table 3). The control recorded the highest nematode population of 277.07 juveniles per 10 g of roots. In contrast, the undiluted liquid biodigester (T_1) treatment reduced the nematode population to 160.34 juveniles, representing a 42.13% reduction over control. The 1:1 dilution (T_2) was nearly as effective, reducing the population to 165.83 juveniles with a 40.15% decrease.

Table 4: Effect of liquid biodigester on number of lesions, root lesion index and corm necrosis grade in banana (cv. Grand Nain) at 150 days after nematode inoculation.

Treatment No.	Number of Lesions	Root lesion Index (0-4 scale)	Corm necrosis grade
T_1	15.11a	0.75a	1
T_2	19.23b	0.97b	1
T_3	25.41bc	1.18bc	1
T_4	34.68c	1.48c	1
T_5	41.20d	1.71cd	2
T_6	44.63d	1.83d	2
T_7	47.72de	1.97de	2
T_8	50.13f	2.02f	2
T_9	56.99ef	2.19ef	2
T_{10}	64.55g	2.29g	2
T_{11}	75.56h	2.51h	3
T_{12}	12.02a	0.51a	1
T_{13}	116.73i	4.00i	4
S.E m ±	0.90	0.02	-
CD @ 5%	2.63	0.07	-

Means followed by the same letter(s) within columns are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level of probability

As the dilution of liquid biodigester increased, a gradual decline in efficacy was observed. The most diluted treatment (T11; 1:10 dilution) still achieved a significant reduction, recording 213.55 juveniles (22.93% reduction over control). The standard chemical check, Cartap hydrochloride 50% SP (T_{12}), exhibited the highest suppression, reducing the nematode populations to 121.88 juveniles, corresponding to a 56.01% decrease over control. These findings align with earlier studies where organic amendments and digestates effectively reduced nematode populations due to the release of nematicidal compounds during microbial digestion (Oka, 2010; He *et al*., 2020).

Effect of liquid biodigester on Root Lesions, Lesion Index and Corm Necrosis

Liquid biodigester treatments also significantly impacted root lesions, lesion index and corm necrosis grades (Table 4). The untreated control plants exhibited the highest number of lesions (116.73), the maximum root lesion index of 4.00 (on a 0–4 scale) and a corm necrosis grade of 4, indicating severe damage.

The application of undiluted liquid biodigester (T_1)

resulted in the lowest lesion number (15.11), a root lesion index of 0.75, and a corm necrosis grade of 1, showing considerable protection of the root system. Similarly, the 1:1 dilution (T_2) recorded 19.23 lesions with a lesion index of 0.97 and a necrosis grade of 1. Higher dilutions corresponded to increased lesion numbers and severity. The T_{11} treatment (1:10 dilution) still showed improvement over control, with 75.56 lesions, a lesion index of 2.51 and a necrosis grade of 3.

The chemical check (T_{12}) remained superior, with only 12.02 lesions, a lesion index of 0.51, and a corm necrosis grade of 1. This corroborates findings by Natarajan *et al*., (2006) and Eberlein *et al*., (2020), who reported that bio-organic treatments, including digestates and biofumigants, effectively suppress nematode-induced root damage and promote healthier root systems.

All the treatments significantly decreased root lesion counts, lesion index and corm necrosis grades, with the most effective reductions observed in T_1 and T_2. This aligns with findings by Natarajan *et al*., (2006), where aqueous extracts of *Tagetes erecta* effectively reduced *Meloidogyne incognita* in tomato and improved plant growth parameters. Additionally, Habriantono *et al*., (2023) showed that biofumigants, including cabbage leaves, significantly suppressed nematode populations in soil. Further, ammonia released during biodigester decomposition is known to suppress nematodes (Alam, 1992) and its efficacy against *M. incognita* was confirmed by Khan and Khan (1995). The organic matter from digestates enhances soil microbiota and produces compounds that may disrupt nematode survival and reproduction (Hassan *et al*., 2010; Youssef and Lashein, 2013; Eberlein *et al*., 2020).

Effect of liquid biodigester application on phytotoxicity in banana plants

The application of liquid biodigester at different concentrations, including undiluted forms, showed no phytotoxic effects on banana plants under pot conditions up to 150 days after planting. Visual observations confirmed the absence of symptoms such as chlorosis, necrosis, wilting, scorching, hyponasty or epinasty across all treatments and observation intervals. This indicates that the biodigester was safe for repeated application at planting, 45 and 90 days after planting, with no detrimental impact on plant health. The safety of biodigesters regarding phytotoxicity has been corroborated by several studies. Oldani *et al*., (2023) reported that digestate applications at varying concentrations did not induce phytotoxicity in tomato plants; instead, treated plants exhibited enhanced shoot height and biomass. Similarly, Li *et al*., (2024) demonstrated that soil flooded with up to 100% biogas slurry did not show any phytotoxic symptoms in watermelon, confirming the biocompatibility of digestate-based treatments. Alburquerque *et al*., (2012) explained that the anaerobic digestion process effectively stabilizes organic matter and reduces harmful compounds like volatile fatty acids and free ammonia, converting them into plant-available nutrients and safer forms like ammonium (Bartholomew *et al.*, 2014). This stabilization process prevents phytotoxic effects while enriching the soil with humic substances, micronutrients and bioactive compounds that support plant growth and soil health. Furthermore, liquid digestates can improve soil pH, cation exchange capacity and microbial diversity, fostering a rhizosphere environment that mitigates stress and supports healthy plant development. Overall, the absence of phytotoxicity in banana plants treated with liquid biodigester reinforces its potential as a sustainable, safe and effective component in nematode management and plant nutrition strategies.

Conclusion

The present study confirmed that liquid biodigester, especially at higher concentrations is highly effective in suppressing *R. similis* populations and mitigating associated root and corm damage in banana under controlled conditions. The undiluted liquid biodigester significantly reduced nematode populations in both soil and roots, which decreased the lesion formation, minimized corm necrosis and simultaneously enhanced plant growth parameters without any phytotoxic effects. Although the chemical standard Cartap hydrochloride 50% SP @ 10g /plant exhibited the highest nematode suppression, biodigester treatments provided an eco-friendly, sustainable alternative by combining nematode management with soil health enhancement and plant growth promotion. The bioactivity of digestate is attributed to the release of nematicidal compounds, improved soil nutrition and stimulation of beneficial microorganisms. These findings highlight the potential of integrating liquid biodigesters into banana nematode management strategies as part of sustainable agriculture practices, reducing reliance on synthetic nematicides. Future research under field conditions and with varied cropping systems would further validate the scalability and long-term benefits of this approach.

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