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Effect of biofumigation for the management of nematodes in banana

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Abstract
The study was conducted to find out the comparative effect of biofumigation, mulching, bioagent and organic manures for the management of nematodes in banana. The results revealed that application of all the non-chemical treatments gave significant reduction in nematode population. The lowest nematode population (231 nematodes in 200 cc soil) was recorded in paring + biofumigation using crop residues of cabbage @ 5 kg pit^{-1} at harvesting stage giving 80 percent reduction over untreated. The banana yield was higher in paring+ biofumigation with crop residues of cabbage (9.97 / plant), paring + mulching with green leaves of *Gliricidia maculata* (9.63 kg/ plant) and chemical, carbosulfan (10.30 kg/ plant). The population of free living and predatory nematodes were significantly higher in all treatments than untreated and chemical check, paring+ carbosulfan treatment. Paring+ biofumigation using crop residues of cabbage resulted 6 and 8 fold increase in population of free living and predatory nematodes respectively.

Keywords: Nematodes, biofumigation, paring, carbosulfan, mulching

1. Introduction
Banana (*Musa* Sp.) is one of the most important fruit crop in the tropics as well as subtropics. It has been grown in 107 countries and the fourth most important food crop after rice, wheat and maize in the world. India is the leading producer of banana in the world with an annual production of 300 lakh tonnes. It contributes 22 per cent to global production.

Phytoparasitic nematodes are one of the major factors negatively affecting the banana production throughout the world. More than 151 nematode species of 51 genera have been documented worldwide [1]. The nematode of major concern in banana production throughout the world is burrowing nematode, *Radopholus similis* (Cobb.) Thorne which cause yield loss of 30 to 60 per cent. Other nematode species *viz*., lesion nematode, *Pratylenchus coffeae* (Zimmermann.), spiral nematode, *Helicotylenchus multicinctus* (Cobb.) and root-knot nematode, *Meloidogyne incognita* (Kofoid and White.) co-habit with *R. similis* resulting substantial yield loss. Incidentally, the first report of occurrence of *R. similis* on banana from India was made from Kerala where the entire crop was damaged and lost due to its attack [2]. Besides direct damage, they serve as predisposing agents in development of disease complexes with fungi and bacteria. Infection by burrowing nematode causes toppling of banana at harvesting stage.

Previously farmers used chemical soil fumigants to control plant parasitic nematodes. Among pesticides, nematicides are most problematic because these chemicals are highly toxic to humans and also contaminate ground water. During the past decade several agriculturally important nematicides have been restricted in use because of concern about ground water contamination or adverse effect on human health. Nowadays organic farming is gaining momentum and Kerala will be converted to a fully organic state in the near future. In the context of safe to eat concept bio intensive management strategy giving emphasis to non-chemical methods have to be undertaken. Bio fumigation is a traditional agronomic practice that makes use of biocidal activity of glucosinolate containing plants when they are incorporated in to the soil [3]. Hence the present study is undertaken to study the comparative effect of biofumigation, mulching, bioagent and physical treatment in combination with organic amendment for the management of nematodes in the rhizhosphere of banana.

2. Materials and Methods

Field trials were conducted at highly nematode infested sick plots in the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram having an initial inoculum of 420 *Meloidogyne incognita*, 142 *Radopholus similis*, 314 *Pratylenchus coffeae* and 410 *Helicotylenchus dihystera* per 200cc soil. The comparative effect of biofumigation using crop residues of cabbage, mulching with green leaves of *Chromolaena odorata* and *Gliricidia maculata* @5kg/ pit, bioagent (*Purpureocillium lilacinum*) and physical treatment (hot water treatment of suckers) in combination with organic amendment (neem cake) was compared with chemical.

Sword suckers of Nendran variety were pared upto a depth of 1cm and planted at a spacing of 2m X 2m. Experiment was laid out in RBD with seven treatments and three replications. The plants were maintained with uniform cultural operations during cropping period as per the package of practices recommendations of Kerala Agricultural University.

Details of various treatments imposed were

T1-Paring (P) + Biofumigation using crop residues of cabbage (5kg/ pit)

T2-P + Mulching with green leaves of *C. odorata* (5kg/ pit)

T3-P + Mulching with green leaves of *G. maculata* (5kg/ pit)

T4-P + *P. lilacinum* sucker treatment (5g) + pit application (20g) 45 DAP

T5-P + Hot water treatment of suckers at 55 ^{0}C for 20 minutes+ neem cake (1kg /plant)

T6-P + Carbosulfan 6G @1g a.i/ plant (Check)

T7-Untreated

Biofumigation was done in pits 15 days prior to planting. Fresh leaf mass of cabbage were chopped into small pieces and applied @ 5kg / pit follwed by covering with a polythene sheet to prevent the lose of gases produced by biodegradation. Polythene mulch was removed after 15 days and planting of banana was done in the pit.

Nematode population in soil was estimated by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique [4] before planting, three months after planting (vegetative stage), six months after planting (flowering stage), nine months after planting (harvesting stage). Extracted live nematodes were counted under a stereo zoom microscope. The population of plant parasites, free living and predatory nematodes were recorded separately using a tally counter.

Observations on biometric characters (root weight), nematode population characteristics (no. of galls (5g root), no. of lesions (5g root), no. of females (5g root), nematode population in root (5g) and rhizome (10g) and yield (bunch weight) were recorded at the time of harvest. Number of female root-knot nematodes present in 5g root was counted after differential staining [5].

2.1. Effect of biofumigation on beneficial fauna

To evaluate the efficacy of biofumigation on beneficial fauna, soil incorporation of chopped cabbage residues @ 5 kg/ pit was done in the field with three replications and the observations were recorded at 15 days interval. To estimate the population of saprophytic nematodes, fungi and bacteria, soil samples were collected before treatment, 15 days after treatment (DAT), 30 DAT, 45 DAT and 60 DAT. Population of plant parasitic nematodes and saprophytes were estimated by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique. Isolation of fungi and bacteria in the soil sample was done using the serial dilution plate technique [6]. Observation was recorded on colony counts in the plates with 10^{-4} dilution was expressed as number of colony forming units (cfu)/gram (g) of soil for fungi. Instead of Potato Dextrose Agar (PDA), Nutrient agar was used for the isolation of bacteria and the colony counts were recorded at 10^{-6} dilution.

2.2. Statistical analysis

The data generated from the field experiment was subjected to analysis of variance (ANOVA) technique [7]. The variables which did not satisfy the basic assumptions of ANOVA were subjected to square root transformation before analysis.

3. Results and Discussion

The results related to the effect of different treatments on the population characteristics of plant parasites, free living and predatory nematodes were presented in Table 1. The initial nematode population ranged from 899 to 1286 in 200cc soil. Data on population of nematodes showed statistically significant variation at three, six and nine months after planting (MAP). Effect of P+ biofumigation using crop residues of cabbage @5 kg/ pit was statistically on par with the chemical check, P+ carbosulfan 6G @1g a.i/plant in reducing the population of plant parasites in banana rhizhosphere at vegetative, flowering and harvesting stage. Percentage reduction in population of plant parasitic nematodes ranged from 62 to 86 during vegetative stage (three MAP), 68 to 88 during flowering stage (six MAP) and 73 to 86 during harvesting stage (nine MAP) (Fig. 1). The percentage reduction in nematode population recorded in plants treated with P + mulching with green leaves of *C. odorata*@ 5 kg/ pit, P + mulching with green leaves of *G. maculata*@ 5 kg/ pit, P + *P. lilacinum* @ 5g /sucker + pit application @ 20g 45 DAP and P+ HWT of suckers @ 55 ^{0}C for 20 minutes+ neem cake @ 1 kg/ plant were 77.93, 76.47, 73.80 and 76.15 per cent respectively.

The maximum suppression of plant parasitic nematode population in P + biofumigation using crop residues of cabbage @ 5 kg/pit was due to the presence of phytochemical glucosinolates. These glucosinolates present in cruciferous plants get converted in to various degradation products such as isothiocyanates, thiocyanates and indoles after cutting or chewing of the plant parts. Through this process, they come in contact with the vacuolar enzyme myrosinase [8]. Members of family Brassicaceae contain more than 350 genera which contain glucosinolates having nematicidal property [9]. Effectiveness of *C. odorata* as a primary nematicide against larvae and adults of *R. similis* was also reported [10]. In the present study, it was found that P+ HWT of suckers @ 55 ^{0}C for 20 minutes+ neem cake @ 1 kg/ plant also effective in reducing the soil nematode population. This finding was in agreement with Akhtar and Mahmood [11] who reported that paring of banana suckers and hot water treatment at 55 ^{0}C for 20 minutes followed by application of neem seed cake @ 1kg/ plant reduced the soil and root population of *R. similis* in banana.

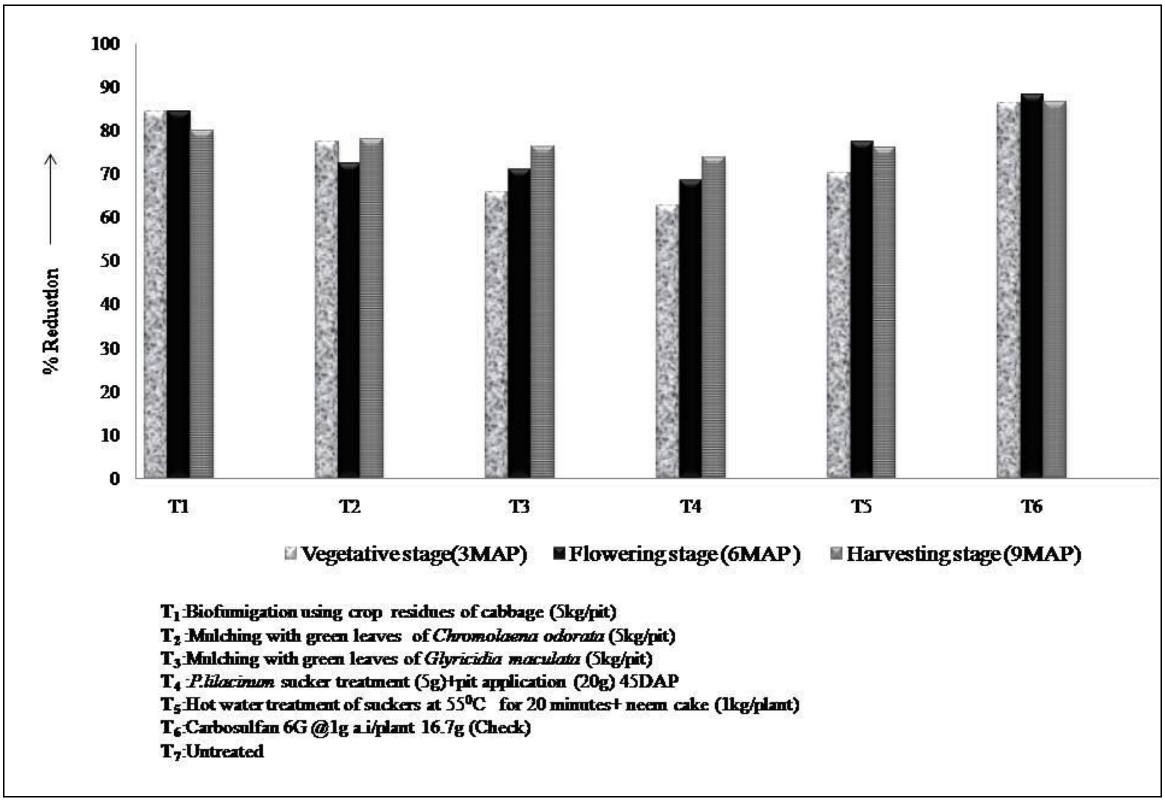

Fig 1: Effect of different treatments on plant parasitic nematode population over untreated

It's very evident that P+ biofumigation using crop residues of cabbage @ 5 kg/ pit recorded the highest number of free living (Dorylaimids) and predatory nematodes (Mononchids) in all stages of the crop (Table 1).The increased multiplication of free living and predatory nematodes in the biofumigation treatment exert antagonism by means of competition for space resulting in reduction in population of plant parasitic nematodes. Soil incorporation of chopped neem leaves resulted in increasing the population of the predatory nematode and thereby reducing the development of root-knot nematode populations by 67.5 per cent [12]. Antagonistic competition effect of free living bacterial feeding nematode against plant parasitic nematodes was reported [13].

Table 1: Effect of treatments on free living and predatory nematode population in the rhizosphere of banana

Treatment	Plant parasitic nematodes				Free living nematodes				Predatory nematodes			
	PT	VS	FS	HS	PT	VS	FS	HS	PT	VS	FS	HS
T_1	1180.00 (34.17)	142.33 (11.91)^{de}	148.66 (12.17)^{d}	231.33 (15.20)^{bc}	267.00 (16.33)	995.00 (31.54)^{a}	1194.66 (34.53)^{a}	1413.33 (37.59)^{a}	32.33 (5.63)	195.00 (13.96)^{a}	248.00 (15.74)^{a}	278.66 (16.69)^{a}
T_2	1286.00 (35.71)	205.00 (14.32)^{cd}	262.00 (16.17)^{bc}	256.33 (16.00)^{b}	242.33 (15.54)	446.00 (21.11)^{d}	833.66 (28.72)^{c}	942.00 (30.69)^{c}	51.33 (7.12)	135.00 (11.61)^{b}	171.66 (13.1)^{c}	185.00 (13.6)^{c}
T_3	1097.00 (33.07)	313.33 (17.69)^{b}	275.33 (16.59)^{b}	273.33 (16.51)^{b}	226.66 (15.05)	431.00 (20.76)^{e}	967.33 (31.1)^{b}	971.00 (31.16)^{b}	33.00 (5.64)	94.66 (9.72)^{e}	105.66 (10.27)^{e}	115.00 (10.72)^{e}
T_4	923.00 (30.21)	341.33 (18.44)^{b}	299.66 (17.31)^{b}	304.33 (17.44)^{b}	222.33 (14.9)	624.00 (24.97)^{c}	677.00 (26.01)^{d}	712.33 (26.68)^{e}	68.33 (8.22)	121.00 (10.99)^{d}	138.00 (11.74)^{d}	143.00 (11.95)^{d}
T_5	1115.33 (33.06)	272.33 (16.46)^{bc}	213.33 (14.59)^{c}	277.00 (16.59)^{b}	261.00 (16.14)	732.33 (27.06)^{b}	858.00 (29.29)^{c}	917.00 (30.28)^{d}	36.00 (5.82)	129.00 (11.35)^{c}	183.66 (13.55)^{b}	207.33 (14.39)^{b}
T_6	950.33 (30.81)	124.33 (11.10)^{e}	110.66 (10.45)^{d}	156.00 (12.31)^{c}	216.66 (14.71)	191.00 (13.82)^{g}	211.33 (14.53)^{f}	218.00 (14.76)^{g}	67.66 (8.13)	22.00 (4.68)^{g}	28.00 (5.29)^{g}	36.66 (6.05)^{g}
T_7	889.66 (29.71)	916.33 (30.16)^{a}	954.66 (30.66)^{a}	1161.66 (33.55)^{a}	246.33 (15.68)	315.00 (17.42)^{f}	348.00 (18.65)^{e}	395.00 (19.87)^{f}	54.00 (7.23)	80.66 (8.98)^{f}	97.66 (9.88)^{f}	88.00 (9.38)^{f}
CD(0.05)	N.S	(2.853)	(1.951)	(3.622)	N.S	(0.042)	(0.571)	(0.042)	N.S	(0.126)	(0.117)	(0.573)

PT: Pre-treatment VS : Vegetative stage (3MAP) FS: Flowering stage (6MAP) HS : Harvesting stage(9MAP) *Values in the parenthesis are square root transformed values *MAP: Months After Planting * P: Paring T_1:Biofumigation using crop residues of cabbage (5kg/pit) T_2 :Mulching with green leaves of *Chromolaena odorata* (5kg/pit) T_3:Mulching with green leaves of *Gliricidia maculata* (5kg/pit) T_4 :*P.lilacinum* sucker treatment (5g)+pit application (20g) 45DAP T_5:Hot water treatment of suckers at 55 ^{0}C for 20 minutes+ neem cake (1kg/plant) T_6:Carbosulfan 6G @1g a.i/plant (Check)

Here in this study also incorporation of cabbage residues under anaerobic condition with sufficient moisture facilitated degradation resulting in increased multiplication and heavy population of free living and predatory nematodes. This finding is in agreement with Bapista *et al.* [14] who reported effect of biofumigation using cabbage and cauliflower leaves @2.5 kg/m^2 in reducing root knot nematode population and increasing yield of okra. The result obtained during entire crop growth period of banana revealed that paring+biofumigation using crop residues of cabbage resulted

6 and 8 fold increase in population of free living and predatory nematodes respectively.

The nematode population estimated from roots varied significantly in different treatments compared to untreated. The mean population of nematodes in the root samples ranged from 12.33 to 29.33 per 5g root in treated plants as against 76.66 in untreated (Table 2). Lowest number of nematodes was observed in P+biofumigation using crop residues of cabbage @5kg/pit (18.00 nematodes per 5g root) and it was statistically on par with Chemical check, P+carbosulfan @1g a.i/plant (12.33 nematodes/5g root). Among the non-chemical methods, nematode population in rhizome was found to be lowest in P+ biofumigation using crop residues of cabbage @ 5 kg/ pit (14.60 nematodes 10 g/ rhizome) which was statistically on par with P + mulching with green leaves of *C. odorata*@ 5 kg/ pit (24.00 nematodes/10g rhizome) and chemical check, P+ carbosulfan 6G @ 1 g a.i/ plant (12.33 nematodes/10 g rhizome). Lowest mean number of galls (9.66/5g root) was observed in P + biofumigation using crop residues of cabbage @ 5 kg/ pit which was statistically on par with chemical check, P+ carbosulfan 6G @ 1 g a.i/ plant (12.66 per 5g root) and P + mulching with green leaves of *G. maculata* @ 5 kg /pit (14 per 5g root). In the case of number of lesions biofumigation treatment was statistically on par with chemical check carbosulfan giving 20.66 and 16.00 lesions per 5g root respectively. Among non-chemical methods, number of females was lowest in P+ biofumigation using crop residues of cabbage @ 5 kg/ pit (13.00 females /5g root) which was statistically on par with P + *P. lilacinum* @ 5g/ sucker + pit application @ 20g 45 DAP (14.33/5g root), P+ HWT of suckers @ 55 ^{0}C for 20 minutes+ neem cake @ 1 kg/ plant (15.33/5g root) and chemical check, P+ carbosulfan 6G @ 1 g a.i/ plant (12.00 females per 5g root).

The significant nematicidal efficacy of cruciferous crop residues observed in the present study could be inferred due to release of methyl isothiocyanate under anaerobic condition. Similar findings were reported in okra [15] and celery [16] respectively. In this study, mulching with green leaves of *C. odorata* also found effective in reducing nematode population in banana rhizome which is in agreement with Goswami *et al*. [17] who reported nematicidal property of green leaves of *C. odorata* and *Azadiracta indica* for managing nematodes associated with bhindi and cowpea. Present study revealed the potential of *P. lilacinum* as sucker treatment and pit application in reducing the number of females. The efficacy of *P. lilacinum* in reducing the nematode population was reported in vegetable coleus [18] and brinjal [19]. It was reported that the nematicidal property of *P. lilacinum* may be due to the action of antibiotics like leucinostatin and lilacinin together with other proteolytic and chitinolytic fungal metabolic products [20].

Table 2: Effect of different treatments on yield and nematode population characters of banana root

Treatment	Population of nematodes		Root knot count (5g root)	No. of lesions (5g root)	No. of females (5g root)	Root weight (g)	Bunch weight (Kg)	% increase over untreated	Yield (t ha^{-1})
	Root (5g)	Rhizome (10g)							
T_1	18.00 (3.59)^c	14.60 (3.33)^d	9.66^d	20.66 (3.41)^d	13.00^{cd}	1120.33^b	9.96^a	72.92	24.9
T_2	21.33 (4.56)^b	24.00 (4.11)^{cd}	16.66^{bc}	35.00 (5.86)^{bc}	15.66^c	973.66^c	7.26^b	26.04	18.15
T_3	25.33 (4.97)^b	21.33 (4.61)^{bc}	14.00^{cd}	36.33 (5.86)^{bc}	17.66^b	985.00^{bc}	9.63^a	67.19	24.07
T_4	24.66 (4.94)^b	23.66 (4.84)^{bc}	22.00^b	43.33 (6.57)^b	14.33^{bcd}	809.33^{de}	7.18^b	24.65	17.95
T_5	29.33 (5.41)^b	25.33 (5.00)^b	22.00^b	91.00 (10.00)^a	15.33^{bcd}	923.00^{cd}	6.85^b	18.92	17.13
T_6	12.33 (3.49)^c	12.33 (3.49)^d	12.66^{cd}	16.00 (4.54)^{cd}	12.00^d	1276.33^a	10.30^a	78.82	25.75
T_7	76.66 (8.75)^a	75.33 (8.67)^a	34.00^a	113.60 (10.46)^a	44.66^a	728.33^e	5.76^b		14.4
CD(0.05)	(0.892)	(0.843)	6.805	(0.886)	4.232	146.223	2.05		-

*Values in the parenthesis are square root transformed values * P: Paring *DAP: Days After Planting T1:Biofumigation using crop residues of cabbage (5kg/pit) T2 :Mulching with green leaves of *Chromolaena odorata*(5kg/pit) T3:Mulching with green leaves of *Gliricidia maculata* (5kg/pit) T4 :*P.lilacinum* sucker treatment (5g)+pit application (20g)45DAP T5:Hot water treatment of suckers at 55 ^{0}C for 20 minutes+ neem cake (1kg/plant) T6: Carbosulfan 6G @1g a.i/plant (Check) T7:Untreated

In this study, data on the population of nematodes in banana revealed that biofumigation using crop residues of cabbage significantly reduced nematode population in soil and root and it was reflected on improvement in growth and yield of banana plants. Among non-chemical methods, P + biofumigation with crop residues of cabbage @5kg/pit showed maximum root weight (1120.33g) which was statistically on par with P + mulching with green leaves of *G. maculata* @5kg/pit (985.00g). Here in this study, incorporation of chopped cabbage residues under anaerobic condition resulted in liberation of methyl isothiocyanate which inhibited the activity of plant parasitic nematodes in banana rhizosphere. Incorporation of crop residues and green leaves resulted in an increase in organic matter, water holding capacity and nutrient status of soil which was in conformity with findings of Nisha and Sheela [21] who reported the antihelminthic property of *G. maculata* in kacholam. Moreover, due to exothermic reaction in rhizosphere under anaerobic condition organic acids are released which are likely to improve physical and chemical properties of soil. All the treatments showed significant superiority in improving the yield over untreated. The bunch yield was significantly higher in P + biofumigation using crop residues of cabbage @ 5 kg /pit (9.97 kg /plant), P + mulching with green leaves of *G. maculata*@ 5 kg/ pit (9.63 kg /plant) and chemical check, P + carbosulfan 6G @ 1 g a.i /plant (10.30 kg/ plant). These treatments increased the banana yield by 72.91, 67.18 and 78.82 per cent than the untreated plants respectively (Table 2).

Data presented in Table 3 on effect of biofumigation on saprophytic nematodes, fungi and bacteria in the rhizosphere of banana revealed that population of both plant parasites and saprophytes were decreased up to 95 per cent 15 days after biofumigation. There was a drastic reduction in population of plant parasites (79per cent reduction when compared to pre-treatment population) and sustainable increase in the population of saprophytic nematodes (36 per cent when compared to pre-treatment population). Similar trend was observed in the number of colonies of fungi and bacteria. Addition of organic matter has shown to increase soil microbial activity that may have a direct effect on plant parasitic nematode population [22]. The faster multiplication of saprophytes might be due to the addition of organic matter in to soil after decomposition of cabbage residue. Plant parasites were reduced because of the increased competition between saprophytes and plant parasites. Incorporation of crop residues into the soil plays a very important role in promoting the microbial diversity of soil. Hence for the management of phytonematodes of banana methyl isothiocyanate containing cabbage residues can be effectively used without polluting the environment.

Table 3: Population of saprophytic nematodes, fungi and bacteria in the rhizosphere of banana

	Plant parasitic nematodes (200cc soil)	**Saprophytic nematodes(200cc soil)**	**Fungi (cfu $(g \times 10^4)^{-1}$)**	**Bacteria (cfu$(g \times 10^6)^{-1}$)**
Pre-treatment population	1029.17	937.54	20.64	17.38
15 DAT	63.51	46.14	16.56	10.68
30 DAT	111.07	1184.48	45.90	42.08
45 DAT	191.35	1248.78	74.236	57.92
60 DAT	214.8	1283.17	78.26	64.32

*DAT: Days After Treatment

4. Conclusion

Biofumigation resulted significant reduction in plant parasitic nematode population along with a considerable increase in the population of saprophytic nematodes. P+ biofumigation using crop residues of cabbage @ 5 kg/ pit can be recommended for managing nematodes in banana.

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