Integrated Management Modules for Root-Knot Nematode (*Meloidogyne incognita*) in Tuberose

ABSTRACT

Aims: Identification of effective management for managing *Meloidogyne incognita* in tuberose.

Methodology: The *invitro* experiment was conducted in nematode lab and field study experiment performed in sick field of Field No. 10, Botanical Garden, Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The experiment was conducted in tuberose cv. Prajwal under drip as well as surface irrigated conditions with the spacing of 30 X 20 cm following randomized block design (RBD) with ten treatment modules and replicated thrice as mentioned below.

Result: The *invitro* studies revealed that *Acorus calamus* and *Swietenia mahogany* culture filtrates showed spectacular ovicidal and juvenile mortality effects against *Meloidogyne incognita*, with 100% juvenile mortality and more than 93% egg hatching inhibition after 72 hours. Biocontrol agents among them, *Purpureocillium lilacinum*, recorded the highest percentage of ovicidal (91.35%) and juvenile mortality (95.67%).Among the management modules, the highest yield attributes such as early emergence of spike (100 days), spike length (78.30 cm), number of florets per spike (48.00) and flower yield (66.82g) was recorded in the module of bulb treatment with *P. fluorescens* @ 10g/kg bulbs + soil application of Carbofuran @ 1kg a.i./ha+ post plant application of *P. fluorescens* @ 2.5 kg/ha mixed with FYM @ 1 t/ha, while the lowest nematode population in soil (145.00) and root (52.33), least gall index (1.66) was recorded in the management module consisting of bulb treatment with carbosulfan 25 ST at 3 per cent concentration + soil application of Carbofuran @ 1kg a.i./ha+ post plant application of *P. lilacinum* @ 2.5 kg/ha mixed with FYM @ 1 t/ha under field conditions.

Conclusion: According to the findings integrated management module reduced the nematode population compared to individual management methods.

Keywords: Tuberose, *Meloidogyne incognita*, Management module, biological control methods.

1. INTRODUCTION

Tuberose, *Polianthes tuberosa* L., is a commercially important flower crop used as cut and loose flowers for its aesthetic value. It plays a major role in the perfumery industry, as it fetches a high market value. It is popularly known as '**Rajanigandha**' in India. The

production of loose and cut flowers is estimated to be 27.71 MT and 1560.70 lakh numbers, respectively. In Tamil Nadu, it is widely cultivated in the Coimbatore and Madurai districts. The area under tuberose cultivation in Tamil Nadu is 2166 ha, with a production of 36,389 tonnes and a productivity of 16.80 t/ha (FloralDaily, 2016). The area under tuberose cultivation and its production have increased from 2011 to 2023, although productivity has declined during the same period, as indicated by the compound annual growth rate (CAGR) (Rahini, 2022). Root-knot nematodes, alongside foliar nematodes (*Aphelenchoides besseyi*), are among the most devastating pests of tuberose (Khan *et al.,* 2020). The commercial cultivation of tuberose is seriously limited by root-knot nematodes, *Meloidogyne* spp. (Sundararababu and Vadivelu, 1988). These nematodes cause yield losses in tuberose up to 13-14% (Ravichandra, 2008) and 14-15% (Grace *et al.,* 2019), with infected plants showing yellowing, stunted growth, and moderate to severe galls on roots. In recent years, *M. incognita* has become a major threat to tuberose cultivation. Managing nematodes in tuberose is challenging, but practices such as using nematode-free planting material, maintaining field cleanliness, and applying nematicides can help reduce their populations (Khan *et al.,* 2020). Hot water treatment of planting materials is an effective method for eliminating nematode infections in vegetatively propagated crops (Khan *et al.,* 2024). Therefore, cost-effective and eco-friendly management modules for *M. incognita* need to be developed, which can help reduce the nematode population and sustainably improve yield.

2. MATERIAL AND METHODS

Maintenance of pure culture

Root samples were collected systematically from nematode-infested tuberose fields of the Botanical Garden of TNAU and Velliankadu village, Coimbatore. The identification of *Meloidogyne incognita* at the species level through morphological examination of perineal patterns in mature females, as Eisenback *et al.* (1981) described. Prior to the experiments, soil was sterilized at 121°C for 30 minutes to eradicate native nematodes and microbial contaminants.Tuberose seedlings (cv. Prajwal) were transplanted into sterilized earthen pots filled with a standardized potting mixture comprising red soil, sand, and farmyard manure in a 1:1:2 ratio. Two weeks after transplantation, juveniles of *M. incognita* (J_2) were inoculated in the rhizosphere at 2 J/g soil Pure cultures of the nematode were grown and maintained under glasshouse conditions in the Department of Nematology, TNAU, and are used for experimental studies.

Invitro studies against *Meloidogyne incognita*

The *in vitro* activity of filtrates from both the biocontrol agents and the botanical formulations has been studied regarding egg hatching inhibition and mortality to the juvenile for *Meloidogyne incognita*. *Pseudomonas fluorescens* and *Trichoderma asperellum* are selected for biocontrol studies, including *Purpureocillium lilacinum* and *Pochonia chlamydosporia*- egg-parasitic fungi- besides the plant extract formulation namely, *Swietenia mahogany* 60 EC, and extract from *Acorus calamus*. For the egg hatching study, 2 ml of fungal or bacterial suspension (100% concentration) or botanical formulation (2000 ppm) was poured into 5.0 cm Petri dishes containing a single egg mass of *M. incognita*.

$$Hatching\ inhibition(\%) = \frac{(Total\ no.of\ eggs - no.of\ hatched\ eggs)}{Total\ no.of\ eggs\ in\ treatment} x100$$

Similarly, for juvenile mortality, 100 second-stage juveniles (J2) were added to Petri dishes containing 2 ml of each treatment. Egg masses or juveniles placed in distilled water served as controls. The dishes were incubated at 26 ± 2°C. Hatching inhibition and

anesthetized juveniles were recorded for 24, 48, and 72 hours. Confirmation of juvenile mortality was made using a revival test where nematodes were moved to distilled water and kept under observation for 12–24 hours. Mortality per cent was calculated and all the experiments were performed under laboratory conditions in completely randomized design, with three replicates per treatment. Treatments include,

T1 – *Pseudomonas fluorescens* (TNAU- Pf1)

T2 – *Trichoderma asperellum* (TRI1)

T3 – *Paecilomyces lilacinum* (TNAU-Pl-001)

T4 – *Pochonia chlamydosporia* (TNAU-PC-001)

T5 – *Acorus calamus* 60 EC

T6- *Swietenia mahogany*

T7- Control (distilled water)

$$Mortality\ (\%) \frac{(No.\ of\ dead\ juveniles\ in\ treatment)}{Total\ no.\ of\ juveniles\ in\ treatment} x100$$

Management module on Tuberose field

An integrated management module involving hot water treatment, chemical nematicides, biocontrol agents, and botanicals was tested for nematode management in tuberose (*Polianthes tuberosa* L.) through a field experiment. The study was carried out on tuberose cv. Prajwal under both drip and surface irrigation systems. The experiment utilized a randomized block design (RBD) with a planting spacing of 30 x 20 cm. Ten treatment modules were evaluated with three replicates.The bulbs of tuberose were subjected to dormancy-breaking treatment with 3% thiourea for 12 hours and then disinfected with 0.1% bavistin to prevent fungal rot. The bulbs were divided into ten treatments with 9 different treatment groups, including untreated controls. Treated bulbs were shade-dried before planting. Soil application of Carbofuran 3G at 1 kg a.i./ha was done one week before planting. Biocontrol agents consisting of *P. lilacinum, P. chlamydosporia, P. fluorescens, T. asperellum*, and AM fungi were enriched with 1 t/ha of FYM, moistened for one week to ensure microbial proliferation, and incorporated at 2.5 kg/ha, 15 days after planting. The treatment include,

List 1 : Treatment module for the field experiment

Module no.	Bulb Treatments	Soil application	Post plant application
T_1	Hot water treatment @ 50ºC for 15 minutes	Carbofuran 1kg a.i./ha	*P. lilacinum* @ 2.5 kg/ ha mixed with FYM @ 1t/ha
T_2	Carbosulfan 25 ST @ 3%	Carbofuran 1kg a.i./ha	*P. lilacinum* @ 2.5kg/ha mixed with FYM @ 1t/ha

	w/w		
T_3	*Acorus calamus* @ 2000 ppm	Carbofuran 1kg a.i./ha[1]	Consortium of *P. lilacinum* and *P. chlamydosporia* @ 1.25 kg/ha each mixed with FYM @ 1t/ha
T_4	*Sweitenia mahogany* @ 2000 ppm	Carbofuran 1kg a.i./ha	Consortium of *P. lilacinum* and *P. chlamydosporia* @ 1.25 kg/ha each mixed with FYM @ 1t/ha
T_5	*Purpureocillium lilacinum* @ 10g/kg bulbs	Carbofuran 1kg a.i./ha[1]	*P. lilacinum* @ 2.5 kg/ha mixed with FYM @ 1t/ha
T_6	*Pochonia chlamydosporia* @ 10g/kg bulbs	Carbofuran 1kg a.i./ha	*P. chlamydosporia* @ 2.5 kg/ha mixed with FYM @ 1t/ha
T_7	*Pseudomonas fluorescens* @ 10g/kg bulbs	Carbofuran 1kg a.i./ha	*P. fluorescens* @ 2.5kg/ha mixed with FYM @ 1t/ha
T_8	*Trichoderma asperellum* @ 10g/kg bulbs	Carbofuran 1kg a.i./ha	*T. asperellum* @ 2.5 kg/hamixed with FYM @ 1t/ha
T_9	Arbuscular Mycorrhizal fungi root powder @ 10g/kg bulbs	Carbofuran 1kg a.i./ha	AM fungi @ 6.25 kg/ha mixed with FYM @ 1t/ha
T_{10}	Untreated control		

Nematode populations in soil were quantified by collecting soil samples, which were processed using Cobb's decanting and sieving method (Cobb, 1918), followed by the modified Baermann's funnel technique (Schindler, 1961). The nematode population density was then estimated based on these methods. Root samples were washed, cut into 2–3 cm segments, and stained with 0.1% acid fuchsin-lactophenol, followed by destaining with lactophenol to remove excess stain. After 24 hours, stained roots were examined under a stereozoom microscope to count the number of adult females per 5 g of root tissue and to assess the root knot index. The root knot index was calculated according to the percentage of galls present on the root system, with scores ranging from 0 (no galls) to 5 (more than 75% galls) (Headle *et al.,* 1989).Plant growth parameters, including days to spike emergence, spike length, number of florets per spike, and flower yield, were recorded. Flower yield was quantified by weighing 10 flowers per plant from each treatment, expressed in grams per plant, and compared across treatments under both drip and surface irrigation systems.

Statistical analysis

The experimental design was a randomized block design (RBD) with ten different treatment modules. Each treatment of both trials (drip and surface irrigated condition) was replicated three times. The original data on plant yield attributes and nematode multiplication were analyzed by ANOVA using SPSS (Ver-9.01). The critical difference was worked out for 5 per cent (0.05) probability (Gomez and Gomez, 1985).

3. RESULTS AND DISCUSSION

Effect of Bioagents and Botanicals on Egg Hatching

In egg hatching study, the lowest egg hatching was recorded with *A. calamus* (20.66 eggs), followed by *S. mahogany* (21.00 eggs) with inhibition rates of 94.28% and 93.44% respectively, as compared to the control (150.66 eggs) at a 100% concentration of culture filtrate after a 72-hour exposure period. Among the biocontrol agents, *P. lilacinum* showed the highest ovicidal effect with a 91.35% inhibition of egg hatching, followed by *P. chlamydosporia* with 90.97% inhibition after 72 hours. The highest number of hatched eggs (350.66) was recorded in the distilled water (Table 1). Our study is consistent with the findings of Vanitha (2016), where botanical formulations of *A. calamus* and *S. mahogany* exhibited 100% inhibition of *M. incognita* egg hatching. Furthermore, it aligns with the findings of Khan *et al*. (2023), which show that *P. lilacinum* effectively inhibited *M. incognita* egg hatching, achieving a maximum reduction of 62.8% at the standard concentration, with peak hatching observed after 48 hours. This highlights *P. lilacinum*'s ovicidal potential, likely due to bioactive compounds that disrupt egg viability. Culture filtrates of *P. chlamydosporia* isolates at varying concentrations (20–100%) showed significant nematicidal activity, with maximum egg hatch inhibition of 58.17% (Uddin *et al.,* 2019).

Effect of Bioagents and Botanicals on Juvenile mortality

The study demonstrated a gradual increase in the mortality of *M. incognita* juveniles with extended exposure periods, with complete juvenile mortality (100%) observed for *A. calamus* and *S. mahogany* after 72 hours. Among the tested cell-free culture filtrates, *P. lilacinum* exhibited the highest juvenile mortality (95.67%), followed by *P. chlamydosporia* with 93.33% mortality after 72 hours. In contrast, no mortality was recorded in the distilled water control (Table 2). This supports the toxic effects of *A. calamus* and *S. mahogany* aligns with the observations of Vanitha (2016) and Mohana (2005), who reported their effectiveness against nematode juveniles. Additionally, *P. chlamydosporia* has been reported to induce juvenile mortality ranging from 11.3% to 76.3% (Uddin *et al.,* 2019). The efficacy of *Trichoderma* spp. in juvenile mortality was highlighted by Khan *et al*. (2020), while Cannayane *et al*. (2007) demonstrated that *P. chlamydosporia* and *T. viride* at a 75% concentration caused juvenile mortality of up to 90%. Further supporting evidence comes from Khan *et al.* (2023), who observed the highest mortality (61%) with the standard suspension of *P. lilacinum*.

Field assessment of integrated management module

The findings of the present study demonstrate the efficacy of integrated management modules in reducing nematode populations and improving plant health and yield in tuberose cultivation. The nematode population in soil (145.00) and roots (52.33), along with the gall index (1.66), showed significant reductions when a management module comprising bulb treatment with carbosulfan 25 ST at 3% concentration, soil application of carbofuran at 1 kg a.i./ha, and post-plant application of *P. lilacinum* (2.5 kg/ha) mixed with FYM (1 t/ha) was implemented. Similarly, a module including bulb treatment with *A. calamus* at 2000 ppm, soil application of carbofuran, and post-plant consortial application of *P. lilacinum* and *P. chlamydosporia* (1.25 kg/ha each) mixed with FYM significantly reduced nematode populations. These management modules not only controlled nematode infestations but also enhanced yield attributes such as early spike emergence (100 days), spike length (78.30 cm), number of florets per spike (48.00), and flower yield (66.82 g). Field trials have consistently validated the synergistic effects of biocontrol agents, botanicals, and

organic amendments in managing nematodes (Fig 1,2,3,4). Phani Kumar (1997) demonstrated the efficacy of *P. lilacinum* (10 g/plant) and nematicides like carbofuran and phorate in reducing the pathogenic impact of *M. incognita* in tuberose, leading to improved growth and reduced gall formation. Similarly, Nagesh *et al.* (1997) highlighted the advantages of combining biocontrol agents with botanicals, such as neem leaf extracts, in significantly lowering root gall indices in tuberose. Supporting these results, Kavitha (2012) observed effective control of M. incognita in carnations through post-plant application of *P. lilacinum*, which enhanced plant growth and reduced gall formation. Botanicals have also proven effective in integrated management. Jothi *et al.* (2017) and Mishra *et al.* (2017) reported that neem oilcake at 1.5 t/ha, either alone or in combination with carbofuran, effectively suppressed nematode populations and promoted plant growth. Biocontrol agents, including *P. lilacinum*, *T. harzianum, P. fluorescens*, and *P. chlamydosporia*, have shown significant nematicidal effects, as validated by Saha and Khan (2016). Among these, *P. lilacinum* enriched with FYM (5 kg) was the most effective and cost-efficient treatment, achieving enhanced flower yield and reduced nematode infestations. These findings align with earlier reports by Ravichandra *et al.* (2007), Jonathan *et al.* (2009), and Muthulakshmi *et al.* (2010), which demonstrated improved growth and yield across various crops, such as tuberose, tomato, and mulberry, following the application of biocontrol agents. Kavitha (2012) further highlighted the benefits of *P. lilacinum* in reducing root-knot nematode populations and promoting better root development, spike emergence, and flower yield in carnations (Grace *et al.,* 2019). Field trials showcasing a 20.97% yield increase and a 55.8% reduction in nematode incidence emphasize the synergistic role of botanicals, biocontrol agents, and organic amendments in sustainable nematode management strategies for tuberose cultivation (Rajamanickam *et al.,* 2019).Bioformulations such as Neem cake, Neem leaf powder, Biover, Biozium, Biomonas, and AAU-Bioguard shows the efficacy to manage root knot nematodes as well as increased the floral characters of Tuberose (Neog *et al.,* 2024).

Table 1. Effect of Treatments on Root-Knot Nematode Egg Hatching

Treatments	No. of egg hatching		
	24 hr	**48hr**	**72hr**
T1- *P.fluorescens* (TNAU-Pf1)	24.26[c]	41.66[d]	53.66[c]
	(5.00)	(6.45)	(7.32)
T2 – *T. asperellum* (TRI1)	24[c]	41[d]	53[c]
	(5.00)	(6.40)	(7.28)
T3 – *P.lilacinum* (TNAU- Pl-001)	12.66[b]	22.64[b]	30.23[ab]
	(3.60)	(4.70)	(5.82)
T4 – *P.chlamydosporia* (TNAU- Pl-001)	16[b]	25[c]	31.65[a]
	(4)	(5)	(5.6)
T5- *A. calamus*	10.33[a]	14[a]	20.66[a]
	(3.30)	(3.68)	(4.53)
T6 – *S. mahogany*	10.66[a]	17.33[b]	23[a]
	(3.30)	(4.21)	(4.80)
T7 – Control	71[d]	180[e]	350.66[d]
	(8.42)	(13.40)	(18.70)
Sed	0.18	0.45	0.49
CD(P=0.05)	0.37	0.99	1.07

(Figures in parentheses are arc sine transformed values. The column followed by alphabet are significantly different from each other at 1% level by DMRT)

Table 2 Effect of Treatments on juvenile mortality study

Treatments	No. of juveniles mortality		
	24 hr	**48hr**	**72hr**

T1- *P.fluorescens* (TNAU-Pf1)	56[bc]	71.67[c]	89[ab]
	(7.52)	(8.48)	(9.46)
T2 – *T. asperellum* (TRI1)	54.67[c]	73.23[c]	86.65[c]
	(7.43)	(8.57)	(9.33)
T3 – *P.lilacinum* (TNAU- Pl-001)	67.33[b]	83.33[b]	95.67a
	(8.24)	(9.16)	(9.81)
T4 – *P.chlamydosporia* (TNAU- Pl-001)	65[b]	80.33[b]	93.33[a]
	(8.09)	(8.99)	(9.67)
T5- *A. calamus*	88[a]	96.33a	100[a]
	(9.41)	(9.84)	(10)
T6 – *S. mahogany*	83.33[a]	94.67[a]	100[a]
	(9.16)	(9.71)	(10)
T7 – Control	0[d]	0[d]	0[d]
	(0.71)	(0.71)	(0.71)
Sed	0.34	0.22	0.19
CD(P=0.05)	0.73	0.47	0.41

Table 3: Effect of different modules on yield attributes and *M. incognita* multiplication in tuberose under surface irrigation

Treatment modules	No. of days taken for spike emergence	Spike length (cm)	No. of flowers spike^{-1}	Flower yield (g. plant^{-1})	Final nematode population (200 cc soil)	No. of females in 5 g roots	Gall Index
T_1	111.00	68.24 (36.01)	35.33 (64.33)	51.08 (43.80)	180.33 (63.22)	74.00 (67.20)	3.00
T_2	107.33	74.32 (48.13)	40.66 (89.11)	58.55 (64.84)	145.00 (70.29)	52.33 (76.81)	2.00

T_3	100.33	77.20 (53.87)	45.50 (111.63)	64.30 (81.02)	150.33 (69.34)	56.66 (74.89)	2.00
T_4	102.00	76.45 (52.38)	44.50 (106.98)	62.90 (77.08)	158.00 (67.77)	58.00 (74.30)	2.00
T_5	103.66	75.80 (51.08)	42.67 (98.46)	61.43 (72.94)	165.00 (66.34)	60.33 (73.26)	2.33
T_6	105.00	74.68 (48.85)	41.33 (92.23)	60.75 (71.03)	166.33 (66.07)	62.00 (72.52)	2.33
T_7	100.00	78.30 (56.06)	48.00 (123.26)	66.82 (88.11)	168.66 (65.60)	64.66 (71.34)	2.50
T_8	109.00	72.00 (43.51)	38.00 (76.74)	56.72 (59.69)	173.66 (64.71)	70.00 (69.00)	2.66
T_9	110.66	70.55 (40.62)	37.66 (75.16)	54.88 (54.51)	170.66 (65.19)	66.33 (70.60)	2.50
T_{10}	132.00	50.17	21.5	35.52	490.33	225.66	5.00
S.Ed	1.34	1.63	1.16	0.081	0.71	0.92	-
CD (P=0.05)	4.68	3.43	3.55	0.235	1.94	2.85	-

Table 4: Effect of different modules on yield attributes and *M. incognita* multiplication in tuberose under drip irrigation

Treatment modules	No. of days taken for spike emergence	Spike length (cm)	No. of flowers spike^{-1}	Flower yield (g. plant^{-1})	Final nematode population (200 cc soil)	No. of females 5g roots^{-1}	Gall Index
T_1	117.33	62.50 (21.83)	30.66 (53.30)	48.50 (43.70)	187.33 (62.88)	88.33 (61.93)	3.66
T_2	113.00	65.00 (26.70)	36.50 (82.50)	55.82 (65.39)	153.66 (69.55)	60.00 (74.14)	2.00
T_3	105.33	72.66 (41.64)	42.66 (113.30)	61.33 (81.71)	160.00 (68.30)	62.66 (73.00)	2.33
T_4	106.00	71.50 (39.38)	41.00 (105.00)	60.50 (79.25)	162.66 (67.77)	65.00 (72.00)	2.33
T_5	109.40	69.33 (35.14)	40.33 (101.65)	59.30 (75.70)	168.00 (66.71)	71.66 (69.11)	2.66
T_6	111.00	67.66 (32.00)	38.66 (93.30)	57.00 (69.00)	170.33 (66.25)	73.00 (68.53)	2.66
T_7	103.50	74.30 (44.83)	44.33 (121.65)	62.40 (85.00)	174.66 (65.39)	75.66 (67.40)	3.00
T_8	115.30	64.50 (25.73)	33.66 (68.30)	53.00 (57.03)	180.33 (64.26)	85.33 (63.22)	3.33
T_9	118.66	63.33 (23.45)	31.50 (57.50)	50.75 (50.37)	178.00 (64.73)	80.00 (65.51)	3.33
T_{10}	137.00	51.30	20	33.75	504.66	232.00	5.00
S.Ed	2.17	1.78	0.69	1.36	4.90	1.87	-
CD (P=0.05)	4.56	3.74	1.44	2.87	10.30	3.93	-

Fig 1 Infested root of Tuberose

Fig 2 Comparing the plant growth parameters of Management modules

b. Spike length b. Root galls

Fig. 3 Influence of different management modules on the yield attributes of tuberose under field conditions

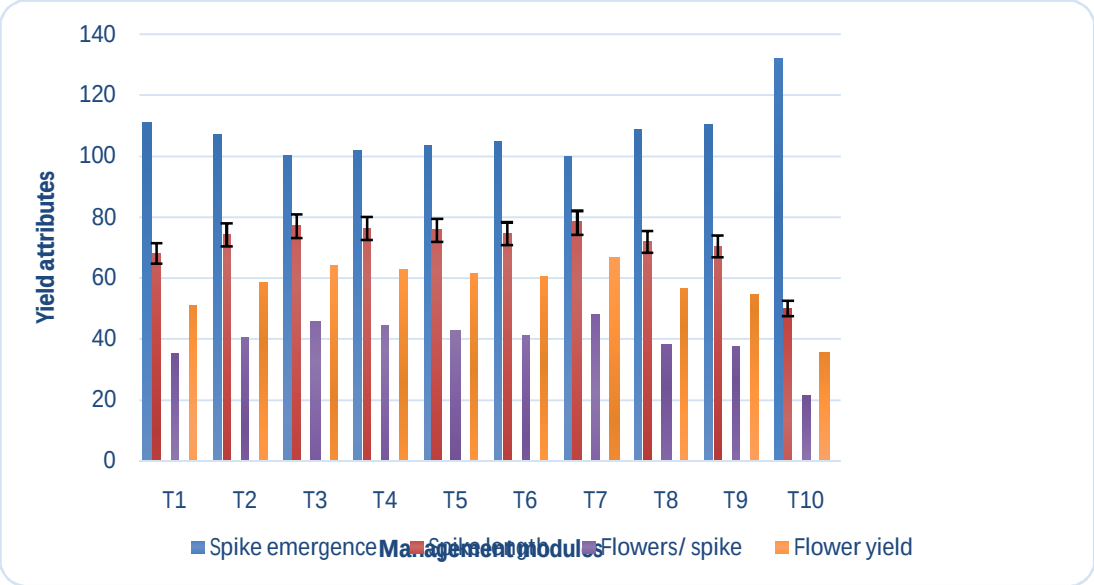
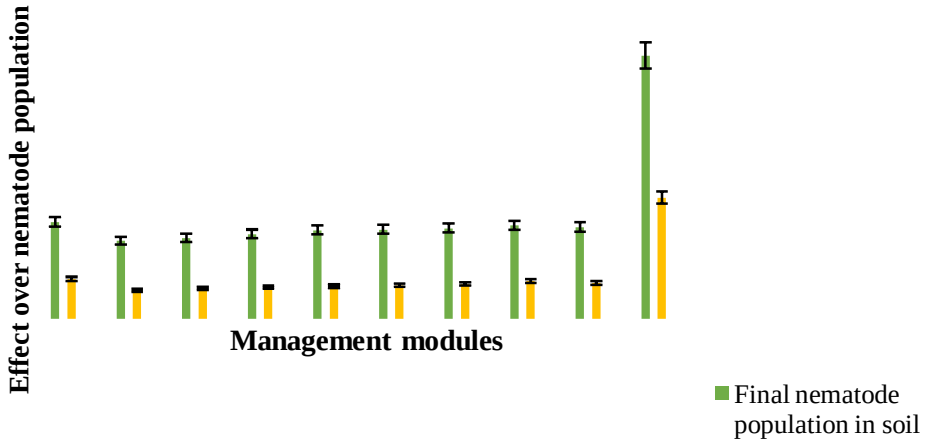


Fig. 4 Effect of different management modules against M. incognita population in tuberose under field conditions



4. CONCLUSION

The study underscores the effectiveness of integrating chemical, botanical, and biological approaches for managing nematodes in tuberose cultivation. Modules combining chemical nematicides like carbofuran with botanicals (Acorus calamus, Swietenia mahogany) or biocontrol agents (P. lilacinum, P. chlamydosporia) showed synergistic effects, significantly reducing nematode populations and improving plant growth parameters. Enhanced plant health and flower yield were attributed to the combined effects of reduced nematode activity, secondary metabolite production, and hormone secretion by biocontrol agents. Initial treatments with bulb treatment, botanicals or biocontrol agents proved effective, reducing the reliance on repeated chemical applications. The study also highlighted the challenges posed by drip irrigation, which fosters nematode reproduction due to favorable soil moisture conditions. Farmers can adopt these integrated modules for

sustainable and cost-effective nematode management, ensuring high yields with minimal environmental impact.

CONSENT (WHEREEVER APPLICABLE)

All authors declare that they have obtained appropriate consent from all relevant parties for the publication of this research.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

This study does not involve any experiments with humans or animals that require ethical approval.Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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