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: 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.

Comment [H1]: Add some points on best treatment on egg inhibition and mortality.

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

1. INTRODUCTION

Tuberose, *Polianthestuberosa* L. is the commercially important flower crop used as cut and loose flowers for its aesthetic value. It plays a major role in perfumery industries, since it fetches high market value. It is popularly known as '**Rajanigandha**' in India. As per area and production statistics of National Horticulture Board (2015-16), the total area under tuberose cultivation in the country is about 7.95 lakh ha. 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 widespread in areas of Coimbatore and Madurai districts. The area under cultivation of tuberose in Tamil Nadu is 2166 ha with production of 36,389 tonnes and productivity of

Comment [H2]: Put the recent data from recent year

16.80 t/ha (FloralDaily, 2016). The commercial cultivation of tuberose is seriously limited by root knot nematodes; *Meloidogyne spp.* (Sundarababu and Vadivelu, 1988). It causes yield loss in tuberose up to 13-14 percent and the infected plants show yellowing, stunted growth and moderate to severe galls on roots (Ravichandra, 2008). In recent years, *M. incognita* has become a major threat for tuberose cultivation. Therefore, cost effective and eco-friendly management modules for *M. incognita* are to be developed which can aid in reducing the nematode population thereby improving the yield in a beneficial manner.

Comment [H3]: Please refer to recent work as more important nematode pests eg. Aphelenchoides can be added though root knot nematode being one of the important pest

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 nematodes were identified as Meloidogyne incognita based on posterior cuticular pattern (PCP) variations, which formed the diagnostic criterion for species confirmation. 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.

Comment [H4]: Can add more details like perineal pattern for identification purpose

Comment [H5]: Sterilization methods can be more specific with 1 0r 2 lines by adding how the sterilization of pot and potting mixture was done

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 *Trichodermaasperellum* are selected for biocontrol studies, including Purpureocilliumlilacinum and *Pochoniachlamydosporia*- egg-parasitic fungi- besides the plant extract formulation namely, *Swietenia mahogany* 60 EC, and extract from *Acoruscalamus*. 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 an egg mass of *M. incognita*.

Comment [H6]: Specify the no. Of egg masses

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

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 – *P. fluorescens* (TNAU-Pf1), T2 – *T. asperellum* (TRI1), T3 – *P. lilacinum* (TNAU-Pl-001), T4 – *P.chlamydosporia* (TNAU-PC-001), T5 – *A. calamus*, T6- *S.mahogany*, T7- Control (distilled water)

Comment [H7]: Please arrange the treatment in table form for more clarity along with scientific name

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

Management module on Tuberose field

Comment [H8]: Mentioned here the design of the experiment CRD OR RBD

An integrated management module involving hot water treatment, chemical nematicides, biocontrol agents, and botanicals was tested for nematode management in tuberose (*Polianthestuberosa* L.) through a field experiment. 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 nine 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,

Comment [H9]: Nine or ten

List 1 :Different treatments for the field experiment

Comment [H10]: Mentioned here the treatment module

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% w/w	Carbofuran 1kg a.i./ha	*P. lilacinum* @ 2.5kg/ha mixed with FYM @ 1t/ha
T_3	*Acoruscalamus* @ 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	*Purpureocilliumlilacinum*@ 10g/kg bulbs	Carbofuran 1kg a.i./ha[1]	*P. lilacinum* @ 2.5 kg/ha mixed with FYM @ 1t/ha
T_6	*Pochoniachlamydosporia*@ 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	*Trichodermaasperellum* @ 10g/kg bulbs	Carbofuran 1kg a.i./ha	*T. asperellum* @ 2.5 kg/hamixed with FYM @ 1t/ha
T_9	ArbuscularMycorrhizal 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

Comment [H11]: Write all the results and discussion for different methods separately in separate para

In egg hatching study, the lowest egg hatching was recorded with *Acoruscalamus* (20.66 eggs), followed by *Swietenia mahogany* (21.00 eggs) and *P. lilacinum* (30.33 eggs), with inhibition rates of 94.28%, 93.44%, and 91.35%, 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).The results demonstrated a gradual increase in the mortality of *M. incognita* juveniles with extended exposure periods. Complete juvenile mortality (100%) was observed with *Acoruscalamus* and *Swietenia mahogany*. Among the cell-free culture filtrates of the four biocontrol agents, *P. lilacinum* exhibited the highest juvenile mortality (95.67%), followed by *P. chlamydosporia* with 93.33% mortality after 72 hours of exposure. No juvenile mortality was recorded in the distilled water control at any of the three exposure periods (Table 2).The present experimental findings indicated that the nematode population in soil (145.00) and roots (52.33), along with the gall index (1.66), were significantly reduced by the management module, which included 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* at 2.5 kg/ha mixed with farmyard manure (FYM) at 1 t/ha. Additionally, bulb treatment with *Acoruscalamus* at 2000 ppm, soil application of carbofuran at 1 kg a.i./ha, and post-plant consortial application of *P. lilacinum* and *P.chlamydosporia* at 1.25 kg/ha each, mixed with FYM, was also effective in controlling the nematode population (Table 3 and 4). Furthermore, the management module comprising bulb treatment with Pseudomonas fluorescens at 10 g/kg, soil application of carbofuran at 1 kg a.i. ha^{-1}, and post-plant application of P. fluorescens at 2.5 kg/ha mixed with FYM significantly enhanced yield attributes, including early spike emergence (100 days), spike length (78.30 cm), number of florets per spike (48.00), and flower yield (66.82 g) (Fig 1, 2).

Comment [H12]: Mentioned the hour.

Our study is consistent with the findings of Vanitha (2016), where botanical formulations of *Acoruscalamus* and *Swietenia mahogany* exhibited 100% inhibition of *M. incognita* egg hatching, showing complete inhibition by these formulations. Regarding P.

lilacinum, Zaki (1994) reported a 62% reduction in egg hatching at a 100% concentration of the culture filtrate after 72 hours of exposure, which aligns with our results, where *P. lilacinum* similarly significantly affected egg hatching. Additionally, the toxic effects of *A. calamus* and *S. mahogany* on nematode juveniles observed in this study further support the findings of Vanitha (2016) and Mohana (2005). Furthermore, the results of Cannayane*et al.* (2007) reinforce our findings, as they reported that *P. chlamydosporia* and *T. viride* at 75% concentration induced juvenile mortality of up to 90% against *M. incognita*.

Comment [H13]: Include recent findings for discussion

The findings of field study are consistent with the work of Phani Kumar (1997), who demonstrated the effectiveness of *P. lilacinum* (10 g/plant) and nematicides such as carbofuran and phorate (2 kg a.i/ha) in mitigating the pathogenic effects of *M. incognita* in tuberose. These treatments resulted in enhanced plant growth, reduced gall index, and decreased nematode populations in the soil. In a similar vein, Nagesh*et al.* (1997) emphasized the synergistic effects of biocontrol agents when combined with botanicals, noting that the application of *P. lilacinum* in conjunction with neem leaf extracts as bulb treatments and soil drenches significantly reduced the root gall index in tuberose. Kavitha (2012) further corroborated these findings, demonstrating that post-plant application of *P. lilacinum* effectively controlled M. incognita in carnations, leading to reduced nematode populations and diminished gall formation.

Moreover, Saha and Khan (2016) confirmed the efficacy of *P. lilacinum, T. harzianum, P. fluorescens, and P. chlamydosporia* in managing *M. incognita* in tuberose. Among these biocontrol agents, *P. lilacinum* at a dose of 5 kg enriched with farmyard manure (FYM) was found to be the most effective and cost-efficient, significantly enhancing flower yield while reducing nematode infestation. These results align with earlier findings by Phani Kumar (1997), Rao *et al.* (2004), Saha and Khan (2016), and Preethi*et al*. (2011), who observed significant improvements in floral characteristics and increased flower yield in biocontrol agent-treated plots.

Furthermore, the present study corroborates the results of Ravichandra*et al.* (2007), Jonathan *et al*. (2009), and Muthulakshmi*et al.* (2010), who reported enhanced growth and yield in a range of crops, including tuberose, tomato, and mulberry, following inoculation with *P. fluorescens*. Additionally, Kavitha (2012) demonstrated that *P. lilacinum* treatment led to a reduction in root knot nematode populations, along with improved plant growth parameters, such as better root development and earlier flower bud emergence in carnations.

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 (40.00)
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 (60.00)

Comment [H14]: Gall index need no transformation as it is index

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 (60.00)
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 (60.00)
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 (53.40)
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 (53.40)
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 (50.00)
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 (46.80)
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 (50.00)
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 moduleson 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 (26.80)
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 (60.00)
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 (53.40)
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 (53.40)
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 (46.80)
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 (46.80)
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 (40.00)
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 (33.40)
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 (33.40)
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 Influence of different management modules on the yield attributes of tuberose under field conditions

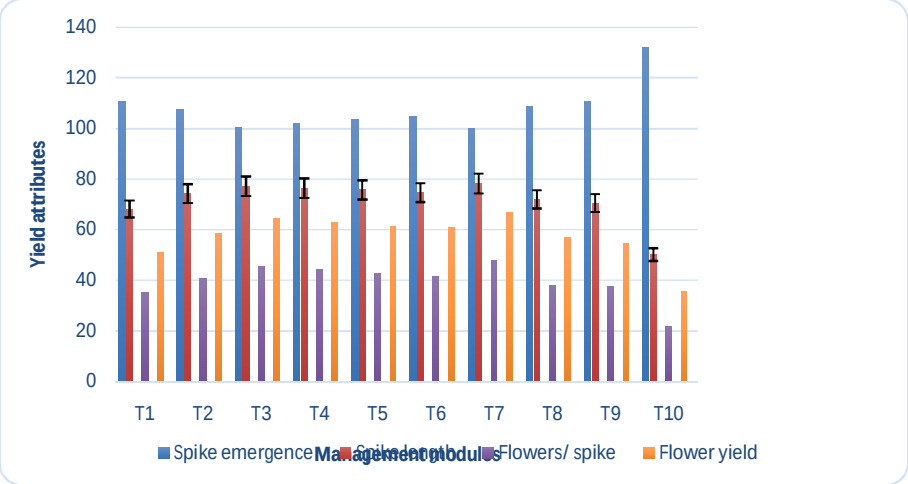
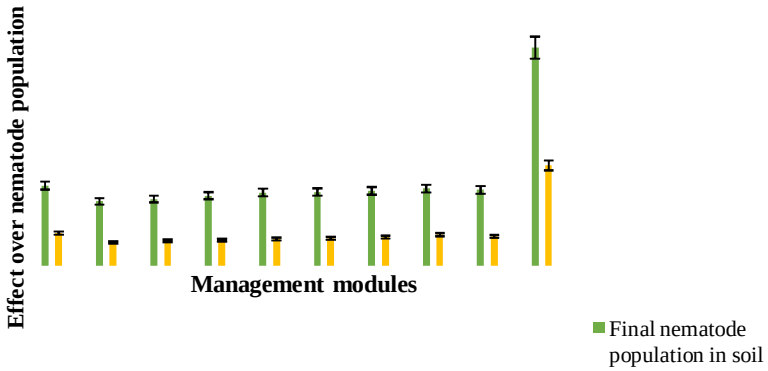


Fig. 2 Effect of different management modules against M. incognita population in tuberose under field conditions



4. CONCLUSION

Mostly the biocontrol agents and botanicals were reported effective in inhibiting the activities of deleterious microorganisms by competing with them for space and nutrients, causing lysis of their body contents. Asarones (2,4,5-trimethoxypropenyl-benzenes) produced by A. calamus, exhibited growth inhibitory and anti-feedant effects on nematodes. The increase in plant growth may be associated with the secretion of auxins, gibberellins and cytokines. Reduction in nematode population in roots might be due to the premature egg hatching and production of secondary metabolites and lytic enzymes especially in case of P.

Comment [H15]: Along with this figure, please include 1 or 2 photos for showing symptoms

Comment [H16]: Add more about the modules which reflect the work like chemical along with botanical is better or not. Initial botanicals treatment or physical or chemicals is effective or can be recommended for farmers field or not?

fluorescens. Also, drip irrigated system favours the growth, development and reproductive potential of M. incognita due to the conducive soil moisture conditions which promotes more new root development thereby harbouring more number of nematodes as compared to surface irrigated system.

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.

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