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Integrated nematode management: A comprehensive approach against root-knot nematode, *Meloidogyne incognita* infecting cucumber under protected cultivation

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Abstract
Root-knot nematode, in particular has come out as the sole problematic reason under protected cultivation. Integration of chemicals, organic amendments and bio-agents was done and applied as soil treatment one after the other for the management of root-knot nematode infecting cucumber under polyhouse conditions. Every treatment was significant over the untreated control in increasing the plant growth and yield of cucumber along with containing the population of root-knot nematode in soil and roots. However, metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2 was the best treatment among all treatments. Suppression of nematode population per 200 cc soil was 73.89, 73.22 and 73.56 per cent over untreated control in 2017, 2018 and pooled average of both years, respectively.

Keywords: Nematode management, *Meloidogyne incognita*, root-knot nematode

Introduction
Protected cultivation technology has been adopted by a number of farmers in India. Farmers are commencing cultivation of cucumber, tomato and capsicum under protected cultivation. Under protected conditions root-knot nematode is creating a significant threat to successful cultivation of crops. Root-knot nematode problem in polyhouses is stimulated by environmental conditions congenial for their survival (Sabir and Walia, 2017) [21]. The population build-up is rapid in polyhouse and the nematode population reaches five to six times the threshold levels within 18-24 months, making protected cultivation a wasteful exercise. The root-knot nematodes are sedentary endoparasitic nematodes which complete their life cycle inside the plant tissues. Otherwise, the common symptoms of the infestation with root-knot nematode are stunting, yellowing and wilting, but the major symptom is the gall formation in plant roots (Khalil, 2013, Khalil *et al.*, 2017) [11, 10]. Implementation of integrated nematode management approach can give good results in keeping nematode population below economic threshold level. Therefore, integrated management approach was evaluated under protected conditions for the management of root-knot nematode, *Meloidogyne incognita* infecting cucumber.

Materials and Methods
Experimental site: The trial was executed in year 2017 and 2018 consecutively in a polyhouse of 28 m x 32 m (896 m^2) size at Horticulture Farm of College of Agriculture, MPUAT, Udaipur, Rajasthan. Udaipur lies on the south slope of Aravalli range in Rajasthan, between altitudes 23.49' and 25.28' N and longitudes 73.01' and 75.49' E at an average altitude of about 579.4 m above sea level. Soil in this place is sandy loam to clay loam in texture.
Treatments: There were nine treatments *viz.*, metham sodium @ 30 ml/m^2, STTC @ 30 ml/m^2, neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2, poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2, metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2, metham sodium @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2, STTC @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2, STTC @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2 (Table 1). An Untreated control was also maintained for comparison purpose. Every treatment was replicated thrice in a completely randomized design. Soil was covered with 25 micron transparent polythene film after the application of metham sodium and STTC and edges were sealed. Polythene sheet was removed after two weeks and pulverization of soil was done for removal of fumes. Cucumber seeds were sown after one week of removal of polythene sheet. Organic amendments fortified

with bio-agents were applied at the time of sowing. The target of this experiment was to compare the efficacy of chemicals alone and in combination in an integrated approach to figure out the best combination and also to develop absolute tactic for the management of root-knot nematode in cucumber under protected cultivation.

Fortification of organic amendments with bio-agents: Organic amendments and bio-agents were fortified together thoroughly and watered regularly and kept for 15 days for build-up of inoculums and applied at the time of sowing.

Irrigation and fertigation: Drip irrigation was provided daily to supply 2 to 3 liters of water/m^2/day depending on crop requirement and weather conditions. Water soluble fertilizer 19:19:19 was provided to the plants through drip fertigation. The fertigation was provided twice a week.

Pruning and Training: The cucumber plants were trained upwards retaining two branches for better interception of light. The main stem was pruned to 25 cm and two strong laterals were allowed to grow. Weak and unproductive lateral branches were removed.

Disease and pest management: Blitox (2.5 g per litre of water) was used to prevent damping off at the seedling stage. Fenzaquin (1.0 ml per litre of water) was sprayed to protect the crop from red spider mite. Yellow sticky traps were installed @ 1 trap per 10 m^2 for trapping of whitefly adults.

Results

Every treatment was significant in improving the vine length, root length, vine weight, root weight and yield along with reducing the number of galls, egg masses, eggs & J2 per egg mass and final nematode population over untreated control in year 2017, 2018 and pooled average of both years (Tables 1, 2 and 3). The order of effectiveness of treatments in suppression of nematode reproduction characters and proliferation of yield on the basis of data was: T5 (metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2) > T6 (metham sodium @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2) > T7 (STTC @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2) > T8 (STTC @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50g/m^2) > T1 (metham sodium @ 30 ml/m^2 > T2 (STTC @ 30 ml/m^2) > T3 (neem cake @ 500 g + *P. lilacinum* @ 50g/m^2) > T4 (poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2) in 2017, 2018 and pooled average of both years.

Among all treatments, the best treatment was T5 *i.e.,* metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2 during both years. Yield of cucumber was 318.00, 494.73 and 390.34% higher in T5 than untreated control in 2017, 2018 and pooled average of both years, respectively. Maximum vine weight (206.33, 188.25 and 197.29 g) and root weight ((27.50, 22.73 and 25.16 g) was noticed in T5, followed by T6 *i.e.,* metham sodium @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2 (203.00, 183.66 and 193.33 g) and (25.10, 21.13 and 23.91 g) in 2017, 2018 and pooled average of both years, respectively. However reduction in number of galls per plant was 54.61, 59.18 and 56.83 percent and nematode population was 73.89, 73.22 and 73.56 per cent with the application of metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2 (T5) over untreated control in individual years and pooled average of both years, respectively. Minimum number of egg masses per plant (43.00, 38.51 and 40.75) as well as eggs and J2 per egg mass (155.76, 165.20 and160.48) was found in T5 *i.e.,* metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2, followed by T6 *i.e.,* metham sodium @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2 (47.88, 39.10 and 43.49), (178.25, 170.00 and 174.12) respectively in 2017, 2018 and pooled average of both years, respectively (Tables 1, 2 and 3).

Integrated treatments were the best in enhancement of plant growth parameters and suppression of nematode growth parameters over other treatments including application of fumigants alone and combination of organic amendments + bio-agents only as well as untreated control. However, integrated treatments and fumigants only were at par with each other during both years. Combination of organic amendments + bio-agents *i.e.,* T3 (neem cake @ 500 g + *P. lilacinum* @ 50g/m^2) and T4 (poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2) were significant over untreated control and at par with each other in 2017 and 2018 but non significant in improving the yield as well as reducing the galls, egg masses and final nematode population when compared to fumigants only and integrated treatments (Tables 1, 2 and 3).

Discussion

In the course of present study, metham sodium @ 30 ml/m^2 + neem cake @ 500 g + *P. lilacinum* @ 50 g/m^2 was recorded foremost in reducing the nematode population in soil as well as in roots and enhancing the plant vigor and yield, followed by metham sodium @ 30 ml/m^2 + poultry manure @ 500 g + *P. lilacinum* @ 50 g/m^2. Fumigants alone and in combination with organic amendments + bio-agents were effective against root-knot nematode**.** Fumigants are broad-spectrum and effectively reduce nematode populations and increase crop yields, particularly when compared with non-fumigant nematicides. Since these products diffuse through soil as gaseous and decompose into products that penetrate directly into the cuticle of the nematode, reacting rapidly with amino acids, oxidases and proteins, causing metabolic

Table 1: Effect of integrated management against root-knot nematode, *Meloidogyne incognita* infecting cucumber under protected cultivation in 2017

S. No	Detail of Treatments	Vine length (cm)	Root length (cm)	Vine weight (g)	Root weight (g)	No. of galls per plant	No. of egg masses per plant	No. of eggs and J2 per egg mass	Nematode population per 200 cc soil	Yield (kg per plant)
T_1	Metham Sodium @ 30 ml/m^2	191.59	31.50	152.81	21.00	74.00	60.00	198.00	432.00	3.08
T_2	STTC @ 30 ml/m^2	189.77	29.95	149.66	20.13	78.50	63.16	205.45	460.20	2.86
T_3	Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	198.50	34.75	194.00	21.75	92.16	73.50	219.00	1,173.25	2.13
T_4	Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	192.00	31.96	189.00	20.90	100.00	82.00	228.40	1,185.00	1.93
T_5	Metham Sodium @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinms* @ 50 g/m^2	212.57	42.03	206.33	27.50	59.00	43.00	155.76	373.33	4.30
T_6	Metham Sodium @ 30 ml/m^2 + Poultry	207.60	39.25	203.00	25.10	65.15	47.88	178.25	392.00	4.18

	manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2									
T_7	STTC @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	206.25	38.75	200.33	24.10	70.50	49.99	185.10	375.00	4.00
T_8	STTC @ 30 ml/m^2 + Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	200.12	36.00	197.00	22.32	69.97	48.72	192.40	399.20	3.87
T_9	Untreated Control	129.00	20.75	120.40	10.75	130.00	101.10	260.00	1,430.00	1.00
	C.D. (P = 0.05)	27.41	5.43	27.25	3.04	19.78	16.65	32.58	105.89	0.79
	SE(m)	9.15	1.81	9.10	1.01	6.60	10.88	10.88	35.36	0.26

Table 2: Effect of integrated management against root-knot nematode, *Meloidogyne incognita* infecting cucumber under protected cultivation in 2018

S. No	Detail of Treatments	Vine length (cm)	Root length (cm)	Vine weight (g)	Root weight (g)	No. of galls per plant	No. of egg masses per plant	No. of eggs and J2 per egg mass	Nematode population per 200 cc soil	Yield (kg per plant)
T_1	Metham Sodium @ 30 ml/m^2	199.31	38.00	179.00	19.00	60.12	47.00	186.99	402.00	3.25
T_2	STTC @ 30 ml/m^2	192.26	32.74	175.15	18.00	67.66	52.33	200.33	425.25	3.00
T_3	Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	207.33	39.12	179.38	19.12	98.66	75.10	209.33	1,120.00	2.33
T_4	Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	200.00	37.45	176.38	18.75	107.06	82.16	218.00	1,146.66	2.00
T_5	Metham Sodium @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	219.98	46.25	188.25	22.73	50.25	38.51	165.20	368.10	4.52
T_6	Metham Sodium @ 30 ml/m^2 + Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	214.00	43.69	183.66	21.13	54.00	39.10	170.00	375.00	4.45
T_7	STTC @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	210.24	42.03	185.55	21.79	56.63	41.03	179.30	383.00	4.25
T_8	STTC @ 30 ml/m^2 + Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	206.77	40.17	181.55	20.11	62.99	43.00	174.60	390.00	4.00
T_9	Untreated Control	138.50	23.00	127.00	12.13	123.11	92.05	236.31	1,375.00	0.76
	C.D. (P = 0.05)	22.35	3.63	16.29	2.86	12.63	8.61	23.54	95.66	0.47
	SE(m)	7.46	1.21	5.44	0.95	4.22	2.87	7.86	31.95	0.15

Initial nematode population: 570 J2 per 200 cc soil Replications: 3;

Table 3: Pooled effect of integrated management against root-knot nematode, *Meloidogyne incognita* infecting cucumber under protected cultivation in 2017 and 2018

S. No	Detail of Treatments	Vine length (cm)	Root length (cm)	Vine weight (g)	Root weight (g)	No. of galls per plant	No. of egg masses per plant	No. of eggs and J2 per egg mass	Nematode population per 200 cc soil	Yield (kg per plant)
T_1	Metham Sodium @ 30 ml/m^2	195.45	34.75	165.90	20.00	67.06	53.50	192.49	417.00	3.16
T_2	STTC @ 30 ml/m^2	191.01	31.34	162.40	19.06	73.08	57.74	202.87	442.72	2.93
T_3	Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	202.91	36.93	186.69	20.43	95.41	74.30	214.16	1,146.62	2.23
T_4	Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	196.00	34.70	182.69	19.82	103.53	82.00	223.20	1,165.83	1.96
T_5	Metham Sodium @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	216.27	44.14	197.29	25.16	54.62	40.75	160.48	370.71	4.41
T_6	Metham Sodium @ 30 ml/m^2 + Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	210.80	40.47	193.33	23.91	59.57	44.45	174.12	383.50	4.31
T_7	STTC @ 30 ml/m^2 + Neem cake @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	208.24	40.34	192.94	22.94	63.56	44.54	182.25	379.00	4.12
T_8	STTC @ 30 ml/m^2 + Poultry manure @ 500 g + *Purpureocillium lilacinum* @ 50 g/m^2	203.44	38.08	189.27	21.21	66.48	45.86	183.50	394.60	3.93
T_9	Untreated Control	133.75	21.87	123.70	11.44	126.55	96.57	248.15	1,402.50	0.88
	CD (P = 0.05)	15.76	3.94	17.76	2.56	11.86	9.52	20.42	64.53	0.41
	SE(m)	5.51	1.38	6.21	0.89	4.15	3.33	7.14	22.58	0.14

dysfunctions and are most efficacious in management of nematodes (Galbieri and Belot, 2016) [7]. Results revealed that fumigants are compatible with organic amendments and bio-agents and yield was more in integrated treatments as compared to application of fumigants alone and combination of organic amendments + bio-agents only. Perhaps, fumigants, organic amendments and bio-agents in integration gave additive results due to their interaction. Neem has been widely studied for its nematicidal properties and has been used as plant extracts, oil cakes or whole plant materials in a large number of studies against root-knot nematode, particularly in India (Akhtar and Alam, 1993; Ferraz and De-Freitas, 2004; Oka, 2010) [1, 6, 17]. Nemato-toxic compounds of the neem plant, especially the azadirachtins, are released through volatilization, exudation, leaching and decomposition (Akhtar, 2000) [2]. Neem extract contains various limonoids, such as salannin, nimbin and azadirachtin, as well as minute amounts of by-products such as ammonia, formaldehyde, phenols, fatty acids and tannins (Khan *et al.*, 1974; Alam *et al.*, 1980) [13, 3]. Soil treatment with a neem based formulation (Neemaplus) greatly improved the growth of cucumber plants in nematode-infested pots (Lynn *et al.*, 2010) [14]. Neem extracts also enhanced the performance of other organic amendments when used in combination. Soil analysis indicated that neem extract inhibited the nitrification of the ammonium released from the amendments and extended the persistence of the ammonium concentrations in the soil. The extended exposure of nematodes to ammonia as a result of nitrification inhibition by the neem extracts appeared to be the cause of the enhanced nematicidal activity of the ammonia-releasing amendments (Oka *et al.*, 2007) [16].

P. lilacinum is an opportunistic fungus has been established as a potential bio-agent for controlling *Meloidogyne* spp., *Globodera rostochiensis*, *G. pallida*, *Tylenchulus semipenetrans* and *Rotylenchulus reniformis* etc. in wide range of crops (Jatala *et al.*, 1979; Rao *et al.*, 2002) [9, 20]. Egg shell of nematode become weak to enable a narrow infection peg to push through it due to action of proteases and chitinase enzymes produced by this fungi (Khan *et al.*, 2004) [12]. Trials in both pot and field conditions for two consecutive seasons indicated that *P. lilacinum* formulations (25% SC/25% WP) were effective at different doses to reduce population of *M. incognita* infesting tomato (Hano and Khan, 2015) [8]. The talc-based formulation (8×10^6 spores/g) of *P. lilacinum* when applied as seed treatment, seedling-dip and soil application was found to be highly effective against *M. incognita* on tomato (Priya and Kumar, 2006) [19].

Similar results were observed by Chandel *et al.*, 2014 [5] that soil fumigation with metham sodium alone and in combination with neem cake enriched with bio-agents such as *P. lilacinum* or *Pseudomonas fluorescens* has been found effective against root-knot nematode infecting tomato, capsicum and carnation under polyhouse conditions. Application of dazomet 83.3 g/m^2, metham sodium 0.12 g/l, *Brassica napus* and *B. juncea* 2, 3 and 4 kg/m^2 (as biofumigants) in rose indicated reduction in root-knot nematode in the range of 25-62%. Dazomet and metham sodium were best in reducing the number of galls in two seasons (Oloo *et al.*, 2009) [18]. Pre-plant application of dazomet 40 g/m^2 alone and repeated at 6 months interval with bio-agent *P. lilacinum* and *Pochonia chlamydosporia* 1 l/m^2 (2 x 10^{12} cfu) with neem cake 1 kg/m^2 in a microplot study found that dazomet, neem cake and bio-agent diminished root-knot nematode by 50% for two years (Nagesh and Reddy, 2005) [15]. As stated by annual report of IARI (Anonymous, 2011) [4] combined treatment of FYM and fumigants significantly reduced root gall index caused by root-knot nematode.

Conclusion

Fumigation with metham sodium followed by application of neem cake/poultry manure fortified with *P. lilacinum* was the best option in attenuating the adverse effect of root-knot nematode under polyhouse conditions. It resulted in additive effect on the yield of cucumber. Therefore, it is determined through current research that deployment of integrated nematode management practice is a comprehensive approach to keep population of root-knot nematode below economic threshold level and improve the yield of cucumber by utilizing chemical and eco-friendly approaches in integration rather than using alone.

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