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Environment-friendly management of root-knot nematode, *Meloidogyne incognita* infecting cucumber in polyhouse

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Abstract
Environment-friendly management of root-knot nematode, *M. incognita* infecting cucumber in protected conditions was conducted through different combinations of organic amendments fortified with bio-agents. Trials were conducted consecutively for two years during 2020 and 2021 in a poly house situated at Horticulture Farm in Rajasthan College of Agriculture, Udaipur. Every combination was statistically significant over untreated control in promoting the plant vigor as well as yield along with limiting the nematode population. However, neemcake at 250 g + *Trichoderma viride* at 50 g/m^2 was the most promising combination in the eco-friendly management of root-knot nematode.

Keywords: Organic amendments, bio-agents, fortification, root-knot nematode, protected cultivation

Introduction

Cucumber (*Cucumis sativus* L.), a widely cultivated plant in the gourd family, Cucurbitaceae is also known as Kheera in Hindi. It is native to tropical regions of southern Asia and has been cultivated in India for over 3,000 years. There are so many health benefits of cucumber as these are rich in antioxidants and helps in weight loss (Kumar *et al.*, 2010) [13]. The crops grown under protected conditions are mainly vegetables such as cucumber, tomato capsicum and cut flowers such as carnation, gebera etc. (Sharma *et al.*, 2009) [18]. The estimated area under Cucumber is around 82 thousand hectares with total production of about 1260000 metric tons in India (Anonymous, 2018) [1]. In Rajasthan, estimated area under Cucumber is around 2.79 thousand hectares with 16.64 metric tons production (Anonymous, 2018) [1]. The farmers are not much aware of nematode problems and damage in polyhouse conditions which has created an adverse impact on growers. The losses due to nematode are very much high under protected conditions due to the conducive environment inside these structures (Gine *et al.*, 2014) [9]. The crops grown under protected conditions are severely infected with nematodes such as root-knot nematode, reniform nematode, spiral nematode and lance nematode etc. However, 18.20% yield losses have been recorded in cucumber due to root-knot nematode in India (Jain *et al.*, 2007) [10]. Polyhouse provides congenial conditions to root-knot nematodes for infection, damage and multiplication on continuously grown susceptible crops. However, it is generally determined that root-knot nematode is the most prevalent in Cucumber worldwide (Anwar and Mc Kenry, 2012; Zitter *et al.*, 1996) [3, 22]. Root-knot nematodes (*M. incognita* and *M. javanica*) are the major cause of huge losses to crops in poly houses (Anwar and Mc Kenry, 2010) [2]. The population build-up is rapid in poly house leading to damage to crops and making it a complete failure. It was, therefore, planned to evaluate the environment-friendly methods for root-knot nematode management in poly houses.

Material and methods

Experimentation area and atmospheric conditions

Trials were conducted consecutively for two years during 2020 and 2021 in an apoly house situated at Horticulture Farm in Rajasthan College of Agriculture, Udaipur. Polyhouse was 28 m × 32 m (896 m^2) in size.

Soilsampling

Random soil sampling was done at the depth of about 15-20 cm from the root zone of the plants with the help of khurpi from 4-5 places in the poly house to assess the initial population of root-knot nematode.

Soil was homogenized, filled in a polythene bag, labeled, tied and for further processing brought to the laboratory.

Nema to depopulation in soil

About 200 cc soil of each sample was processed by using Cobb's sieving and decanting technique (Cobb's 1918) [7], followed by modified Baermann's funnel technique (Baermann, 1917) [5].

Bed preparation and sowing of cucumber

The size of the bed was 30 m length and 1 m width. It was raised up 30 cm from the ground level. One plot was of 2.5 m^2 size. Each plot was comprised of 12 plants with a spacing of 45×80cm. Sowing of cucumber seeds (cv. Mini Angel) @1seedperhill was done.

Application of organic amendments Fortified with bio-Agents

Fourteen different combinations of organic amendments *i.e.*, poultry manure, neem cake and vermicompost at 250 and 500 g + bio-agents *i.e., Purpureocillium lilacinum*, *Pseudomonas fluorescens* and *Trichoderma viride* at 50 g/m^2 were tried (Table 1). However, two checks *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2 and vermicompost at 250 g + *T. viride* at 50 g/m^2 along with untreated control were also maintained for comparison purposes. Talc-based formulations of bio-agents *i.e., P. lilacinum* (2 X 10^6cfu/g) and *P. fluorescens* (2X10^8 cfu/g) were procured from the Indian Institute of Horticulture Research (IIHR), Bengaluru. Whereas, *T. viride* (2 X 10^6 cfu/g) was procured from T-Stanes and Company Limited, Coimbatore. Fifteen days prior to seed sowing, the organic amendments and bio-agents were mixed thoroughly in desired amounts in poly bags and moisture was maintained by sprinkling water each week. It was kept as such for two weeks for population build-up of bio-agents. Its application was done at the time of sowing. Three replications were maintained for each treatment. Observations shoot length (cm), shoot weight (g), root length (cm), root weight (g) and yield kg per plant and number of galls per plant, number of egg masses per plant, number of eggs and J2 per egg mass and final nematode population per 200 cc soil along with isolation of bio-agent from egg mass were recorded.

Experimental results

Plant growth parameters

Vine length (cm)

Maximum vine length (186.00, 183.00 and 189.58 cm) was found in T15 *i.e.*, neem cake at 250 g + *Trichoderma viride* at 50 g/m^2 followed by T_{16} *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (184.60, 181.50 and 187.75 cm) in 2020, 2021 and pooled average of both years, respectively. Among various combinations, except check, maximum vine length (185.37, 181.00 and 188.56 cm) was recorded in T_4 *i.e.*, neemcake at 500 g + *Purpureocillium lilacinum* at 50 g/m^2.

Root length (cm)

Highest root length (40.10, 40.00 and 40.05 cm) was found in T_{15} *i.e.*, neemcake at 250 g + *T. viride* at 50 g/m^2 followed by T_{16} *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (39.77 cm, 37.00 cm and 38.38 cm) in 2020, 2021.

Vine weight (g)

The increase in vine weight was significant in all the treatments except the untreated control. Maximum vine weight (196.17, 191.50 and 193.83 g) was found in T_{15} *i.e.*, neemcake at 250 g + *T. viride* at 50 g/m^2 followed by T_{16} *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (194.60, 189.50 and 192.05 g) in 2020, 2021.

Root weight (g)

Highest root weight (27.98, 28.80 and 28.89 g) was found in T15 *i.e.*, neemcake at 250 g + *T. viride* at 50 g/m^2 followed by T16 *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (26.75, 24.00 and 25.37 g) in 2020, 2021.

Table 1: Efficacy of organic amendments fortified with bio-agents for environment-friendly management of root-knot nematode (2020)

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	Nema to Depopulation per 200 cc soil	Yield (kg per plant)
Poultry manure 250 g + *Purpureocillium Lilacinum* 50 g/m^2 (T_1)	181.11	36.00	176.15	21.35	84.00	62.17	178.03	904.00	2.41
Poultry manure 500 g + *Purpureocillium Lilacinum 50* g/m^2 (T_2)	184.66	39.10	190.00	24.00	78.00	55.10	174.16	842.00	2.60
Neem cake 250 g + *Purpureocillium Lilacinum 50* g/m^2 (T_3)	182.00	37.13	180.25	22.08	83.00	56.34	177.08	901.00	2.45
Neem cake 500 g + *Purpureocillium Lilacinum 50* g/m^2 (T_4)	185.37	40.00	196.00	25.06	77.00	52.17	172.12	813.33	2.72
Vermi compost 250 g + *Purpureocillium Lilacinum 50* g/m^2 (T_5)	181.00	36.10	170.16	18.00	91.00	63.00	178.13	903.00	2.42
Vermi compost 500 g + *Purpureocillium Lilacinum 50* g/m^2 (T_6)	183.60	39.00	185.33	22.98	80.00	58.00	175.34	890.00	2.46
Poultry manure 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_7)	177.13	30.13	169.99	17.75	94.00	65.11	182.04	951.66	2.00
Poultry manure 500 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_8)	179.00	34.22	171.00	19.98	92.00	61.03	180.00	964.66	1.89
Neem cake 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_9)	177.38	30.00	166.25	17.70	94.00	67.71	182.11	950.00	1.78
Neem cake 500 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_{10})	180.31	35.90	175.00	20.70	89.00	63.04	179.33	996.00	2.20
Vermi compost 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_{11})	176.20	28.08	164.72	17.00	95.00	69.00	184.07	1,000.00	1.68
Vermi compost at 500 g +	178.00	34.01	173.15	20.15	90.02	65.10	180.69	913.00	1.87

Pseudomonas Fluorescens at 50 g/m² (T12)									
Poultry manure 250 g + *Trichoderma viride* 50 g/m² (T13)	184.05	35.65	177.45	21.00	87.00	61.82	174.03	900.00	2.30
Poultry manure 500 g + *Trichoderma viride* 50 g/m² (T14)	182.33	38.00	182.00	23.10	88.00	58.50	176.66	887.00	2.36
Neem cake 250 g + *Trichoderma viride* 50 g/m² (T15)	186.00	40.10	196.17	27.98	75.10	47.00	167.00	790.00	2.75
Vermi compost 250 g + *Trichoderma viride* 50 g/m² (T16)	184.60	39.77	194.60	26.75	78.08	50.00	172.00	810.00	2.60
Untreated Control (T17)	134.69	19.98	137.67	13.40	124.35	100.11	244.87	1,120.00	0.95
S.Em±	9.144	2.343	4.482	1.31	2.553	3.949	5.747	23.692	0.157
C.D.(P=0.05)	12.93	6.76	26.39	3.80	7.36	11.39	16.58	68.38	0.45

Initial nematode Population: 400 J2 per 200 cc soil Replications: 3 Date of sowing: 25-03-2020 Date of termination: 2-06-2020

Table 2: Effect of organic amendments fortified with bio-agents for eco-friendly management of root-knot nematode (2021)

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)
Poultry manure 250 g + *Purpureocillium Lilacinum 50 g*/m² (T1)	174.25	31.50	180.10	19.50	103.00	75.33	203.10	1033.50	2.16
Poultry manure 500 g + *Purpureocillium Lilacinum 50 g*/m² (T2)	179.98	35.99	186.25	22.00	99.00	68.25	191.20	953.20	2.82
Neem cake 250 g + *Purpureocillium Lilacinum 50 g*/m² (T3)	178.00	32.57	174.00	20.00	101.80	70.00	201.25	1010.00	2.30
Neem cake 500 g + *Purpureocillium Lilacinum 50 g*/m² (T4)	181.00	37.00	188.00	23.20	94.00	68.00	186.25	922.08	2.90
Vermi compost 250 g + *Purpureocillium Lilacinum 50 g*/m² (T5)	168.10	27.00	172.98	16.99	107.00	75.50	211.25	1087.25	1.88
Vermi compost 500 g + *Purpureocillium Lilacinum* 50 g/m² (T6)	179.25	35.00	180.70	22.00	100.00	66.66	194.20	907.00	2.50
Poultry manure 250 g + *Pseudomonas Fluorescens* 50 g/m² (T7)	132.94	26.85	172.00	16.90	108.50	69.33	212.00	1100.98	1.66
Poultry manure 500 g + *Pseudomonas Fluorescens* 50 g/m² (T8)	169.90	27.90	173.98	17.00	106.99	74.80	209.50	1078.00	2.00
Neem cake 250 g + *Pseudomonas Fluorescens* 50 g/m² (T9)	165.00	26.20	170.10	16.58	109.00	77.02	214.60	1108.50	1.45
Neem cake 500 g + *Pseudomonas Fluorescens* 50 g/m² (T10)	172.02	30.00	179.00	18.00	105.20	70.36	206.00	1049.00	2.08
Vermi compost 250 g + *Pseudomonas Fluorescens* 50 g/m² (T11)	164.00	25.00	160.25	16.00	110.50	78.50	216.25	1120.25	1.33
Vermi compost at 500 g + *Pseudomonas Fluorescens* at 50 g/m² (T12)	170.08	28.35	175.00	18.75	106.80	73.00	208.40	1066.00	1.97
Poultry manure 250 g + *Trichoderma viride* 50 g/m² (T13)	176.00	32.00	176.00	19.00	102.25	71.26	201.00	1021.50	2.25
Poultry manure 500 g + *Trichoderma viride* 50 g/m² (T14)	178.50	34.00	179.00	21.00	100.98	69.81	197.58	1002.97	2.33
Neem cake 250 g + *Trichoderma viride* 50 g/m² (T15)	183.00	40.00	191.50	28.80	90.12	63.37	180.00	885.20	3.00
Vermi compost 250 g + *Trichoderma viride* 50 g/m² (T16)	181.50	37.00	189.50	24.00	93.00	69.66	185.20	901.00	2.95
Untreated Control (T17)	130.34	18.00	133.00	11.00	128.20	109.00	239.70	1193.50	1.06
S.Em±	9.396	1.077	7.87	1.12	5.15	3.94	7.460	25.57	0.065
C.D. (P=0.05)	27.12	3.10	22.73	3.25	14.88	11.39	21.53	73.81	0.189

Initial nematode population: 520 J2 per 200 cc soil Replications: 3 Date of sowing: 10-04-2021 Date of termination: 16-07-2021

Table 3: Pooled effect of organic amendments fortified with bio-agents for environment-friendly management of root-knot nematode in a playhouse in cucumber crop (2020 & 2021)

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)
Poultry manure 250 g + *Purpureocillium Lilacinum 50 g*/m² (T1)	175.20	33.75	180.60	20.42	93.50	68.75	190.56	968.75	2.28
Poultry manure 500 g + *Purpureocillium Lilacinum* 50/m² (T2)	182.32	37.54	188.12	23.00	88.50	61.67	182.68	897.60	2.59
Neem cake 250 g + *Purpureocillium Lilacinum 50 g*/m² (T3)	179.12	34.85	178.00	21.04	92.40	63.17	189.16	955.50	2.37
Neem cake 500 g + *Purpureocillium Lilacinum 50 g*/m² (T4)	188.56	38.50	192.00	24.13	85.50	58.08	179.18	867.70	2.75
Vermi compost 250 g + *Purpureocillium Lilacinum 50 g*/m² (T5)	169.13	31.55	176.99	17.49	99.00	69.25	194.69	995.12	2.15
Vermi compost 500 g + *Purpureocillium Lilacinum 50 g*/m² (T6)	181.42	37.00	183.01	22.49	90.00	62.33	184.77	898.50	2.48

Poultry manure 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_7)	151.46	28.49	174.56	17.32	101.25	67.22	197.02	1026.32	1.83
Poultry manure 500 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_8)	170.45	31.06	176.49	18.49	99.49	67.91	194.75	1021.33	1.94
Neem cake 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T9)	165.62	28.10	173.74	17.14	101.50	72.36	198.35	1029.25	1.61
Neem cake 500 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_{10})	173.51	32.95	179.65	19.35	97.10	66.70	192.66	1022.50	2.14
Vermi compost 250 g + *Pseudomonas Fluorescens* 50 g/m^2 (T_{11})	164.36	26.54	168.22	16.50	102.75	73.75	200.16	1060.12	1.50
Vermi compost at 500 g + *Pseudomonas Fluorescens* at 50 g/m^2 (T_{12})	171.61	31.18	176.50	19.45	98.41	69.05	194.54	989.50	1.92
Poultry manure 250 g + *Trichoderma viride* 50 g/m^2 (T_{13})	176.72	33.82	176.72	20.00	94.62	66.54	187.51	960.75	2.27
Poultry manure 500 g + *Trichoderma viride* 50 g/m^2 (T_{14})	180.25	36.00	180.50	22.05	94.49	64.15	187.12	944.98	2.34
Neem cake 250 g + *Trichoderma viride* 50 g/m^2 (T_{15})	189.58	40.05	193.83	28.89	82.61	55.68	173.50	837.60	2.87
Vermi compost 250 g + *Trichoderma viride* 50 g/m^2 (T_{16})	187.75	38.38	192.05	25.37	85.54	59.84	178.60	855.50	2.77
Untreated Control (T_{17})	132.51	18.99	135.84	12.20	126.27	104.55	242.28	1156.75	1.00
S.Em±	6.38	1.54	5.48	0.88	4.43	3.64	6.87	30.75	0.10
C.D.(P=0.05)	17.98	4.33	15.45	2.48	12.48	10.26	19.35	86.63	0.28

Yield (kg per plant)

As per the data of years 2020 and 2021 and pooled average of both years each combined application of organic amendments with bio-agents significantly enhanced the yield (kg/plant) when compared to untreated control. Maximum yield (2.75, 3.00 and 2.87 kg) was found in T_{15} *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2.

Nematode Reproduction Parameters

Number of galls per plant: As per the data of years 2020 and 2021 and pooled average of both years each treatment suppressed the galls formation in plants over untreated control. Minimum galls (75.10, 90.12 and 82.16 per plant) were found in T15 *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2.

Number of egg masses per plant: Minimum egg masses in cucumber crop (47.00, 63.37 and 55.68) was found in T_{15} *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2 followed by T_{16} *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (50.00, 69.66 and 59.84) over untreated control (100.11, 109.00 and 104.55) in 2020, 2021.

Number of eggs and J2 per egg mass: Every treatment reduced the number of eggs and J2 per egg mass significantly when a comparison was made with the control. A minimum number of eggs and J2 per egg mass (167.00, 180.00 and 173.50) was found in T_{15} *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2 followed by T_{16} *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (172.00,185.20 and 178.60) over untreated control (244.87, 239.70 and 242.28) in 2020, 2021.

Nema to depopulation per 200cc soil: All treatments reduced the nematode population per 200 cc soil as compared to control. Minimum J2s of root-knot nematode (790.00, 885.20 and 837.60) were found in T^{15} *i.e.*, neem cake at 250 g + *T. viride* at 50 g/m^2 followed by T16 *i.e.*, vermicompost at 250 g + *T. viride* at 50 g/m^2 (810.00, 901.00 and 855.50) in 2020, 2021.

Discussion

Environment-friendly approaches are the need of the hour as these are safe for human beings and the environment. Many of the chemicals used for nematode management are phased out already. Therefore, organic amendments fortified with bio-agents were used for root-knot nematode management. Relatively rapid decline in nematode population level occurred due to the decomposition of organic amendments and release of toxic compounds and an increase in nematode antagonists. Combined application of (checks) neem cake at 250 g + *Trichoderma viride* at 50 g/m^2 and vermicompost at 250 g + *T. viride* at 50 g/m^2 was best and equally efficacious in containment of root-knot nematode problem and production of high yield of Cucumber. These results are coinciding with the findings of Rao *et al.* (1997) [17] who reported that neem cake and *T. harzianum* significantly increased plant growth and reduced root galling and the final population of root-knot nematode in tomato crops. Antibiotics like trichodermin, dermadin, trichoviridin and sesquiterpene heptalic acid are produced by *T. viride* which are involved in the suppression of nematodes (Askary and Martinelli, 2015) [4]. Role of *Trichoderma* in improving plant health has been demonstrated in many crops including Tobacco, Tomato and Radish (Windham *et al.*, 1986) [21], Tomato (Morsy *et al.*, 2009; Kumari *et al.*, 2020) [15, 14], Bean (Erper *et al.*, 2013) [8]. However, among various treatments except checks, combined application of neem cake at 500 g + *Pupureocillium lilacinum* at 50 g/m^2 was the most efficacious in the management of root-knot nematode in Cucumber followed by poultry manure at 500 g + *P. lilacinum* at 50 g/m^2 and vermicompost at 500 g + *P. lilacinum*at 50 g/m^2. These findings are in contrast to the studies done by other workers that combined application of *P. lilacinum* + neem cake on banana lead to the maximum increase in the plant health and minimum gall index and population of root-knot nematode (Sundraraju and Kiruthika, 2009) [19]. Khan and Saxena (1997) [11] reported that *P. lilacinum* at 1.0 g/kg gave better control against *M. incognita* and performed the highest decrease in nematode population in soil, number of developmental stages, females and number of egg masses on Cucumber plants comparing with the other treatments. Nagesh *et al.*, 2003 [16] carried out an experiment in chrysanthemum against root-knot nematode by incorporating *P. liacinum* + neem cake. Reduction in root-knot nematode was reported along with enhancement of yield in chrysanthemum through this combined application. *P. lilacinum* was mainly an egg parasite (Chen and Chen, 2003) [6]. For the management of *M. graminicola* in rice different combinations of organic amendments and bio-control agents were used. The most propitious combination was neem cake + vermicompost + *Trichoderma* spp. in reducing the nematode in roots and soil (Kumar *et al.*, 2016) [12]. Application of *P. lilacinum* ($2x10^6$ cfu/g) at 50 g/m^2 in nursery bed + *P. lilacinum* at 5 kg/ha along with 2.5 tons of FYM/ha reduced galls and root-knot nematode population both in roots and soil (Wagh and Pramanik, 2014) [20]. Our present investigations focus on the use of bio-agents and organic amendments in sustainable agriculture and open surprise as for the use of bio-nematicides, which are promising as well as ecologically sound and safe. Consumers have been demanding higher food security and environmental quality and this situation will not be different in the future. In this context, efforts in discovering new non-chemical or eco-friendly strategies for nematode management should be continued.

Conclusion

Environment-friendly options for the management of root-knot nematode in protected conditions are of prime need in this era as everyone is concerned about the health risk of having chemical-rich vegetables and fruits. Cultivation of vegetables and fruits with least use of chemicals is the need of the hour. In this present study it was concluded that the application of neem cake at 250 g + *T. viride* at 50 g/m^2 was the most promising combination for keeping the level of root-knot nematode below the economic threshold level in poly house.

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