Saudi Journal of Biological Sciences 28 (2021) 7006–7011

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Management of root-knot nematode, *Meloidogyne incognita* and soil borne fungus, *Fusarium oxysporum* in cucumber using three bioagents under polyhouse conditions

J.A. Patil *, Saroj Yadav, Anil Kumar

[a] Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana 125 004, India

ARTICLE INFO

Article history:
Received 26 May 2021
Revised 23 July 2021
Accepted 27 July 2021
Available online 30 July 2021

Keyword:
Seed treatment
Bio-agents
Meloidogyne incognita
Disease complex
Management
Cucumber
Polyhouse

ABSTRACT

Complex diseases caused by *Meloidogyne incognita* and Fusarium fungus in cucumber is the most destructive disease under polyhouses. The experiment was conducted in the polyhouse of the Department of Horticulture, CCS HAU, Hisar, Haryana, India during summer season (2015–16) to evaluate the potential of bacterial and fungal biocontrol agents against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* in cucumber. Bioagents - *Trichoderma viride* (Tv), *Pseudomonas fluorescence* (Pf), *Purpureocillium lilacinum* (Pl) were taken 10 and 20 g kg^{-1} seed and bioagents liquid formulation, 10- and 15-ml kg^{-1} seed, were mixed with the potted soil. Chemical as well as untreated check were also maintained. All the treatments significantly improved the plant growth parameter, viz., shoot length (SL), root length (RL), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW) and dry root weight (DRW) as compared to untreated check. However, significant reduction in nematode population and maximum improvement in plant growth parameter was recorded with carbofuran followed by higher dose of bioagents liquid formulation. Among the bioagents, bioagents liquid formulation was most effective in suppressing root knot nematode galling (43 / root system) and final population in soil (131 J_2s / 200 cc soil) and fungus wilt incidence (25 %) at 30th day of after germination and significantly improved the plant growth parameters - shoot length (147.3 cm), fresh shoot weight (55.6 g), dry shoot weight (22.51 g) and dry root weight (4.50 g) from other bioagents. Bioagents liquid formulation was effective in suppression of root-knot nematode and fungus complex disease than the powder formulations of bioagents. More studies should be needed in future to evaluate the efficacy of bioagents as seed treatments and soil applications under field conditions.

1. Background

Protected agriculture is considered an important means of increasing the productivity and quality of most vegetable crops (Hanafi, and Papasolomontos,1999). Recently, there has been an increase in interest in protected agriculture (PA) in India. Vegetable crops are grown worldwide as a source of nutrients and fiber in the human diet (Punja, and Utkhede, 2003). Restricted air exchange results in the atmospheric humidity being much higher inside insulated greenhouses than conventional ones which encourage several plant diseases and cause physiological disorders (El-Mougy et al., 2012). *Meloidogyne incognita* and Fusarium disease complex most destructive or notorious pest in horticulture crops and causes severe losses throughout the world (Akhtar et al., 2005; Hadian et al., 2011). Internationally, root knot nematodes have been causes severe yield losses in abundant crops due to their ability to invade several of crop species (Williamson and Hussey, 1996; Kayani et al. 2017). Likewise, it encourages typically root galling, yellowing of leaves, defoliation, stunting, and wilting of infested plants (Sasser et al., 1983). Management of root-knot nematodes and soil borne fungus is a major worldwide challenge for polyhouse growers all over the world. Numerous agricultural practices have been tested against root-knot nematodes (Collange et al., 2011). But they are not much effective against both pathogens. An actual approach toward control of root-knot nematode (RKN) and fungus is given by the use of nematicides

* Corresponding author.
E-mail address: rajhau99@gmail.com (J.A. Patil).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.07.081
1319-562X/

(Giacometti et al., 2010; Nicolopoulou-Stamati et al., 2016). One possible potential and supportive substitute to nematicides is use of bio-control agents (Dutta and Thakur, 2017). Among the biological control agents that have been evaluated are egg-parasitic fungi, antagonist's fungi, antagonist's bacteria, and predatory nematodes has played an important role to suppress growth of plant pathogens and enhance growth of agricultural crops (Rao et al., 1998; Kiewnick and Sikora, 2004; Abdelmoneim, 2006: Al-Shammari et al., 2013).

Thus, the purpose of the present study is to assess the potential antagonistic effect of bio-agents - powder formulations of *Trichoderma viride* (Tv), *Pseudomonas fluorescence* (Pf), *Purpureocillium lilacinum* (Pl) and combined liquid formulation (*T. viride* + *P. fluorescence* + *P. lilacinum*) against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* their effect on growth development of cucumber under polyhouse conditions.

2. Material and methodology

This experiment was performed during summer season in the year 2015–16 in the polyhouse of the Department of Horticulture, CCS HAU, Hisar, Haryana (India) at latitude 29°10′ N, longitude 75°46′ E, altitude 215.2 m a.s.l.

2.1. Nematode and fungus culture

During survey in the protected structures (Patil, 2017), naturally infested cucumber roots showing galls were collected and brought to the laboratory. Nematode and fungus were isolated, identified (Patil et al., 2018) and pure culture of both the organisms were maintained under laboratory. Sand maize meal media was used for mass production of fungus and pure culture of nematode was established on eggplant seedlings in the screen house of department of Nematology. Nematode juveniles (J_2) were collected by sodium hypochlorite method (Ehwaeti et al., 1998).

2.2. Source of bioagents

Powder formulation of *Trichoderma viride*, *Pseudomonas fluorescence*, *Purpureocillium lilacinum* and combined liquid formulation of (*T. viride* + *P. fluorescence* + *P. lilacinum*) were procured from IIHR, Bangalore were used in this study.

Carboxy methyl cellulose (CMC) was used as an adhesive for treating cucumber seeds with fungal spore suspension and bacterial cell suspension ($1x10^8$ cfu ml^{-1}). For preparing 1% (v/w) adhesive solution, 100 mg of adhesive was added to 10 ml of fungal and bacterial suspension. Now required number of seeds was taken in a Petri plate and the fungal as well as bacterial suspension with the adhesive was added drop by drop on the seeds stirring continuously and stopped when all the seeds got smeared with the suspension. After treating, the seeds were dried in shade for 6 h and used for sowing.

2.3. Nematode's extraction

Nematode from soil was extracted by Cobb's method (Cobb, 1918) followed by modified Baermann funnel method (Schindler, 1961).

2.4. Experimental setup

This experiment was conducted in polyhouse (24 ± 2 °C) to evaluate the effect of seed treatment of bio-agents in cucumber for the control of *M. incognita* and soil borne fungus in 15 cm dia. earthen pots (1 kg capacity). Autoclaved soil (sand, sandy loam and peat moss, 2:1:1 respectively) were filled in the earthen pots. Previously *in-vitro* tested bioagents (*T. viride*, *P. fluorescence*, *P. lilacinum*) (un-published data) from two promising doses: 10 and 20 g kg^{-1} seed and bio-agents liquid formulation (*T. viride* + *P. fluorescence* + *P. lilacinum*) 10 and 15-ml kg^{-1} seed were selected for the present study. Bio-agents seed treatment was performed against only nematode, only fungus and nematode + fungus simultaneously inoculated. Treatments were arranged in completely randomized design with four replications. Four seeds of cucumber (cv. Sania) treated with bio-agents sown to each pot. One plant per pot was maintained. Sterilized soil was inoculated with 1000 J_2 kg^{-1} soil of root-knot nematode by pencil hole method and fungus 50 g kg^{-1} soil was mixed in upper layer of soil in pots.

2.5. Maintenance of plants

General care and maintenance of plants were done as recommended by CCS Haryana Agricultural University, Hisar. Hoagland solution was applied at 30 days after sowing. Hoeing, watering other practices were done as per recommendation.

2.6. Observations

After crop maturity at 60 days after sowing, plants were harvested and 200 cc soil was collected for estimation of nematode population. Observations *viz.* plant characters (shoot and root length, fresh and dry plant and root weight), galls per plant, egg masses on plant root, eggs in egg mass, J_2 per 200 cc soil, damping off (pre-emergence,15 and 30 days after sowing) were taken.

2.7. Statistical analysis

Statistical analysis was done by using SPSS software (SPSS for Windows). Means were compared by analysis of variance (ANOVA) and Duncan's test of multiple comparisons ($P < 0.05$). The data compared with means of treatment as significant and non-significant.

3. Results

3.1. Effect of bio-control agents on plant growth performance

Root-knot nematode, *M. incognita* alone, soil borne wilt fungus, *F. oxysporum* alone and *M. incognita* + *F. oxysporum* concomitantly inoculated had suppressive effects on growth of cucumber cv. Sania (Table 1-3), pathogens infestation either inoculated singly or concomitantly caused significant ($P > 0.05$) reduction in shoot length, root length, fresh shoot, root weight, dry shoot and root weight compared to treated plants. Highest plant growth parameter was observed with bio-agents liquid formulations, when nematode and fungus inoculated individually or concomitantly. All the bio-agents treated plants showed greater length, fresh and dry weight of shoot and roots of cucumber plants compared to control. Among the tested bio-agents, bio-agents liquid formulations 15 ml kg^{-1} seed showed significantly ($P > 0.05$) greater shoot length, root length, fresh and dry shoot and root weight followed by *P. lilacinum* 20 g kg^{-1} seed compared to other treatments and control irrespective of whether nematode and fungus inoculated individually and concomitantly (Tables 1-3). Extensive root rotting had been observed in the cucumber plants inoculated with both the pathogen simultaneously as compared to where nematode and fungi inoculated individually. Data augmented that positive impact of all applied bio-agents was noted on the cucumber plant growth.

Table 1
Effect of bio-agents seed treatment on shoot and root length of cucumber plant infested with root-knot nematode and soil borne fungus.

Treatments	Shoot length (cm)				Root length (cm)			
	Nematode alone	Fungi alone	Nematode + fungi	Pooled Mean	Nematode alone	Fungi alone	Nematode + fungi	Pooled Mean
T. viride 10 g kg^{-1} seed	130.1^{b}	131.8^{bc}	129.1^{b}	130.1	35.0^{b}	39.4 ^{cd}	31.2^{b}	35.2
T. viride 20 g kg^{-1} seed	139.8^{de}	142.4^{ef}	137.1 ^{de}	139.8	42.8 ^{cd}	46.3 ^{fg}	37.6 ^{de}	42.2
P. fluorescence 10 g kg^{-1} seed	132.3^{bc}	129.5^{b}	130.2^{b}	132.3	36.6^{b}	36.5 ^{bc}	32.1 ^{bc}	35.1
P. fluorescence 20 g kg^{-1} seed	142.5^{ef}	139.2 ^{de}	139.1 ^{de}	142.5	44.5 ^{de}	43.8 ^{ef}	40.2 ^{e}	42.8
P. lilacinum 10 g kg^{-1} seed	134.9^{bcd}	127.8^{b}	132.2 ^{bc}	134.9	39.3 ^{bc}	35.1^{b}	33.4 ^{bc}	35.9
P. lilacinum 20 g kg^{-1} seed	146.1^{f}	136.5 ^{cd}	140.2 ^{be}	146.1	47.9 ^{e}	42.9 ^{def}	40.0 ^{e}	43.6
LFB 10 ml kg^{-1} seed	136.8^{cde}	134.5 ^{cd}	134.9 ^{cd}	136.8	41.3 ^{cd}	41.5 ^{de}	35.6 ^{cd}	39.5
LFB 15 ml kg^{-1} seed	152.6 ^{g}	146.5^{f}	144.8^{f}	152.6	52.3^{f}	48.4 ^{g}	44.6^{f}	48.4
Carbosulfan 3% v/w	159.6 ^{h}	153.1 ^{g}	149.8 ^{g}	159.6	57.1 ^{g}	54.0 ^{h}	52.6 ^{g}	54.6
Non-treated control (inoculated)	86.5^{a}	86.4 ^{a}	82.9 ^{a}	86.5	23.3 ^{a}	22.9 ^{a}	20.8 ^{a}	22.3
Non-treated control (non-inoculated)	165.8^{i}	162.4 ^{h}	161.2 ^{h}	111.9	59.6 ^{g}	56.3 ^{h}	55.8 ^{g}	57.2
Pooled Mean	138.8	135.5	134.7		43.6	42.5	38.5	

LFB = liquid formulation of bio-agents, Data are mean of four replications.

Table 2
Effect of bio-agents seed treatment on fresh shoot weight and root weight of cucumber plant infested with root-knot nematode and soil borne fungus.

Treatments	Shoot weight (g)				Root weight (g)			
	Nematode alone	Fungi alone	Nematode + fungi	Pooled Mean	Nematode alone	Fungi alone	Nematode + fungi	Pooled Mean
T. viride 10 g kg^{-1} seed	42.0^{b}	34.1 ^{bc}	30.3 ^{bc}	35.5	13.8^{b}	13.7 ^{bc}	11.8^{b}	13.1
T. viride 20 g kg^{-1} seed	49.6 ^{de}	50.3^{f}	40.4 ^{ef}	46.8	18.0 ^{def}	19.4^{f}	16.9 ^{de}	18.1
P. fluorescence 10 g kg^{-1} seed	43.8 ^{bc}	32.7 ^{bc}	31.7 ^{bc}	36.1	14.7 ^{bc}	13.0 ^{be}	12.7 ^{bc}	13.5
P. fluorescence 20 g kg^{-1} seed	52.4 ^{ef}	44.3 ^{e}	42.7 ^{e}	46.5	18.8 ^{efg}	17.9 ^{ef}	17.3 ^{e}	18.0
P. lilacinum 10 g kg^{-1} seed	45.4 ^{bcd}	30.9^{b}	31.3 ^{cd}	35.9	15.8 ^{bcd}	12.2^{b}	13.7 ^{bcd}	13.9
P. lilacinum 20 g kg^{-1} seed	55.9 ^{fg}	40.7 ^{de}	41.5 ^{e}	46.0	19.9 ^{fgh}	18.6 ^{ef}	18.2 ^{e}	18.9
LFB 10 ml kg^{-1} seed	47.8 ^{cde}	36.6 ^{cd}	35.8 ^{cd}	40.1	16.9 ^{cde}	15.0 ^{cd}	15.8 ^{cde}	15.9
LFB 15 ml kg^{-1} seed	59.7 ^{gh}	52.3^{f}	47.4 ^{fg}	53.1	21.2 ^{gh}	16.4 ^{de}	17.2 ^{de}	18.3
Carbosulfan 3% v/w	61.6 ^{h}	54.5^{f}	51.1 ^{g}	55.7	22.5 ^{hi}	22.6 ^{g}	21.7^{f}	22.3
Non-treated control (inoculated)	23.9 ^{a}	23.7 ^{a}	20.8 ^{a}	22.8	6.8 ^{a}	6.5 ^{a}	4.8 ^{a}	6.0
Non-treated control (non-inoculated)	71.7 ^{i}	64.8 ^{g}	67.7 ^{h}	68.1	24.9 ^{i}	23.0 ^{g}	23.5^{f}	23.8
Pooled Mean	50.3	42.3	40.1		17.6	16.2	15.8	

LFB = liquid formulation of bio-agents, Data are mean of four replications.

Table 3
Effect of bio-agents seed treatment on dry shoot weight and root weight of cucumber plant infested with root-knot nematode and soil borne fungus.

Treatments	Dry shoot weight (g)				Dry root weight (g)			
	Nematode alone	Fungi alone	Nematode + fungi	PooledMean	Nematode alone	Fungi alone	Nematode + fungi	PooledMean
T. viride 10 g kg^{-1} seed	13.7^{b}	15.7 ^{bcd}	13.5^{b}	14.3	3.3^{b}	3.5 ^{abc}	3.0 ^{ab}	3.3
T. viride 20 g kg^{-1} seed	17.8 ^{def}	19.1 ^{fg}	16.8 ^{cde}	17.9	4.1 ^{bcd}	5.9 ^{cde}	3.9 ^{bc}	4.6
P. fluorescence 10 g kg^{-1} seed	14.7 ^{bc}	14.7 ^{bc}	14.2 ^{bc}	14.5	3.6 ^{bc}	3.2 ^{abc}	3.1 ^{ab}	3.3
P. fluorescence 20 g kg^{-1} seed	18.7 ^{ef}	18.2 ^{efg}	17.5 ^{de}	18.1	4.7 ^{cd}	5.1 ^{bcde}	4.5 ^{bc}	4.8
P. lilacinum 10 g kg^{-1} seed	15.7 ^{bcd}	13.7^{b}	15 ^{bcd}	14.8	3.7 ^{bc}	2.9 ^{ab}	3.5 ^{bc}	3.4
P. lilacinum 20 g kg^{-1} seed	19.7 ^{gh}	17.3 ^{def}	17.7 ^{de}	18.2	5.2 ^{de}	4.2 ^{abcd}	5 ^{cd}	4.8
LFB 10 ml kg^{-1} seed	16.8 ^{cde}	16.2 ^{cde}	15.8 ^{bcd}	16.3	3.8 ^{bc}	3.8 ^{abc}	3.7 ^{bc}	3.8
LFB 15 ml kg^{-1} seed	21 ^{gh}	20 ^{gh}	19.0 fg	20.0	6.1 ^{e}	6.8 ^{de}	6.3 ^{de}	6.4
Carbosulfan 3% v/w	22.7 ^{hi}	21.9 ^{hi}	20.8 ^{ef}	21.8	7.9^{f}	7.3 ^{ef}	7.1 ^{e}	7.4
Non-treated control (inoculated)	6.3 ^{a}	7 ^{a}	5.3 ^{a}	6.2	2.0 ^{a}	1.8 ^{a}	1.6 ^{a}	1.8
Non-treated control (non-inoculated)	24 ^{i}	22.8 ^{i}	23.0 ^{g}	23.3	9.8 ^{g}	9.5^{f}	9.3^{f}	9.5
Pooled Mean	17.4	17.0	16.2		4.64	4.16	4.77	

LFB = liquid formulation of bio-agents, Data are mean of four replications.

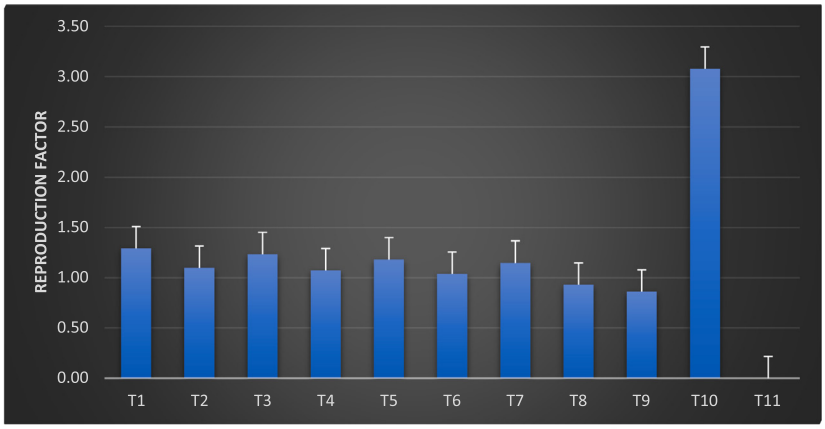
3.2. Effect of bio-agents on nematode reproduction

Root-knot nematode formed big and compound root galls in non-treated inoculated control with nematode alone and nematode and fungus inoculated simultaneously. All applied bio-agents found to suppress significantly ($P > 0.05$), the multiplication of *M. incognita* and *F. oxysporum* compared to control (Table 4, Figs. 1 & 2). Maximum reduction in number of galls plant root^{-1}, number of egg masses plant root^{-1} and soil population 200 cc soil^{-1} was recorded with bio-agents liquid formulation 15 ml kg^{-1} seed irrespective of whether nematode inoculated individually or nematode and fungi both inoculated simultaneously. Among the bio-agents maximum suppression of nematode population was observed with bio-agents liquid formulations followed by *P. lilacinum* as compared to check. It is also clear from the results that the egg masses plant root^{-1} was very few with carbosulfan treated plants but eggs egg mass^{-1} were not affected or non-significant ($P > 0.05$) as compared to non-treated inoculated control. Various

Table 4
Effect of bio-agents seed treatment on nematode reproduction in cucumber crop infested with root-knot nematode and soil borne fungus.

Treatments	Numbers of galls/plant			Numbers of egg masses/plant			Final nematode population / 200 cc soil		
	Nematode alone	Nematode + fungi	Pooled Mean	Nematode alone	Nematode + fungi	Pooled Mean	Nematode alone	Nematode + fungi	Pooled Mean
T. viride 10 g kg^{-1} seed	224.3 ^{i}	218.0 ^{j}	221.2	269.0 ^{j}	255.5 ^{j}	262.3	267.3 ^{j}	255.0 ^{j}	261.2
T. viride 20 g kg^{-1} seed	202.5^{f}	194.3^{f}	198.4	194.0^{f}	187.3^{f}	190.7	227.3^{f}	218.8^{f}	223.1
P. fluorescence 10 g kg^{-1} seed	219.8 ^{h}	212.3^{f}	216.1	226.8 ^{i}	212.8 ^{i}	219.8	255.5 ^{i}	242.0 ^{i}	248.8
P. fluorescence 20 g kg^{-1} seed	193.0 ^{e}	185.8 ^{e}	189.4	186.5 ^{e}	179.5 ^{e}	183.0	222.3 ^{e}	211.5 ^{e}	216.9
P. lilacinum 10 g kg^{-1} seed	216.8 ^{h}	207.5 ^{h}	212.2	217.3 ^{h}	206.5 ^{h}	211.9	244.5 ^{h}	232.5 ^{h}	238.5
P. lilacinum 20 g kg^{-1} seed	182.3 ^{d}	177.5 ^{d}	179.9	172.8 ^{d}	162.5 ^{d}	167.7	215.0 ^{d}	195.8 ^{d}	205.4
LFB 10 ml kg^{-1} seed	210.5 ^{g}	202.8 ^{g}	206.7	207.0 ^{g}	195.0 ^{g}	201.0	237.8 ^{g}	224.5 ^{g}	231.2
LFB 15 ml kg^{-1} seed	172.5^{c}	166.5^{c}	169.5	159.5^{c}	151.3^{c}	155.4	192.5^{c}	184.5^{c}	188.5
Carbosulfan 3% v/w	166.3^{b}	161.3^{b}	163.8	142.8^{b}	138.8^{b}	140.8	178.5^{b}	165.5^{b}	172.0
Non-treated control (inoculated)	323.5 ^{j}	313.8 ^{k}	318.7	442.5 ^{k}	436.3 ^{k}	439.4	636.8 ^{k}	630.5 ^{k}	633.7
Non-treated control (non-inoculated)	0.0 ^{a}	0.0 ^{a}	0.0	0.0 ^{a}	0.0 ^{a}	262.3	0.0 ^{a}	0.0 ^{a}	0.0
Pooled Mean	192.0	185.4		201.7	193.2		243.4	232.8	

LFB = liquid formulation of bio-agents, Data are mean of four replications.



Note: T1: *T. viride* 10 g kg^{-1} seed, T2: *T. viride* 20 g kg^{-1} seed, T3: *P. fluorescence* 10 g kg^{-1} seed, T4: *P. fluorescence* 20 g kg^{-1} seed, T5: *P. lilacinum* 10 g kg^{-1} seed, T6: *P. lilacinum* 20 g kg^{-1} seed, T7: LFB 10 ml kg^{-1} seed, T8: LFB 15 ml kg^{-1} seed, T9: Carbosulfan 3% v/w, T10: Non-treated control (inoculated) T11: Non-treated control (non-inoculated)

Fig. 1. Effect of bio-agents seed treatment on nematode reproduction factor in infested with root-knot nematode alone.

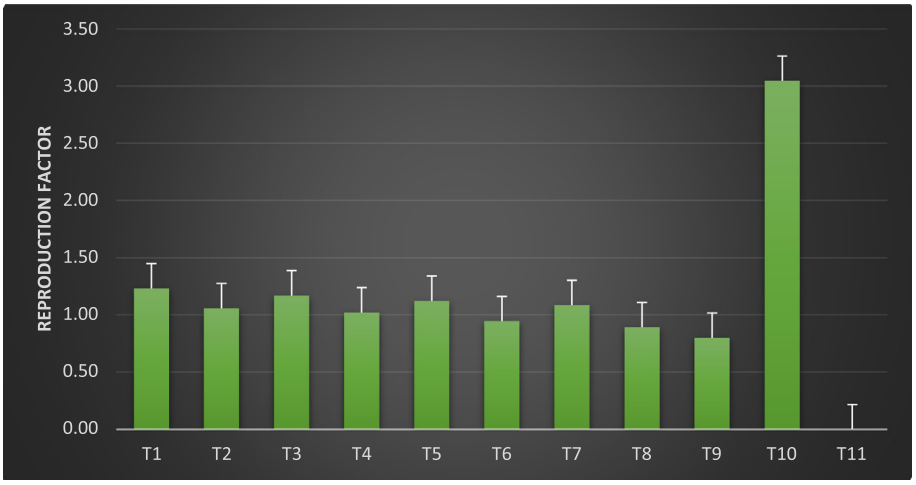


Fig. 2. Effect of bio-agents seed treatment on nematode reproduction factor in infested with root-knot nematode and fungus inoculated simultaneously.

applied bio-agents caused significant reduction in eggs egg mass^{-1} compared to control (non-treated inoculated) and caobosulfan. Lowest reproduction factor of *M. incognita* was calculated with bio-agents liquid formulations 15 ml kg^{-1} seed. Fungal disease suppression has also been observed with the application of bio-agents and carbosulfan as compared to control.

3.3. Per cent disease incidence

It is clear from the data (Table 5) that the all-applied bio-agents and carbosulfan suppress the percent fungal disease irrespective of whether fungi inoculated individually or nematode and fungus both inoculated concomitantly as compared to non-treated inoculated control. Fungus diseases incidence was observed after sowing at 15 and 30 days. Fungal disease incidence after 15 and 30 days of sowing, lowest disease incidence was recorded with bio-agents liquid formulations 15 ml kg-1 seed or *T. viride* 20 g kg^{-1} seed as compared to non-treated inoculated control.

4. Discussion

Bioagents liquid formulation treated seeds was showed significantly higher plant growth followed by *P. lilacinum* irrespective of whether nematode or fungus were inoculated individually or concomitantly. Results are agreements with the studies of Amer Zareen et al. (2001) found that significantly higher plant growth was recorded in *Trichoderma harzianum, T. flavus* and *P. lilacinum* applied as seed/soil drench compared to control and lowest in *P. fluorescence* treated plants. All treatments significantly reduced the nematode populations compared with non-treated inoculated control. Irrespective of nematode inoculated individually or nematode and fungus concomitantly, lowest galls plant root^{-1} and eggs egg mass^{-1} was recorded with bioagents liquid formulation compared to non-treated inoculated control followed by *P. lilacinum*. Dhawan et al. (2004) also observed that seed treatment of okra with *P. lilacinum* (10, 15 and 20 g kg^{-1} seed) improved the okra growth and reduced the *M. incognita* populations. Commercial product of *P. lilacinum* and *Bacillus firmus* applied as soil drenching and seed treatment on soybean and tobacco, improved the plant growth and significantly reduced the root-knot nematode (*Meloidogyne* spp.) populations under field conditions (Lamovsek et al. 2013).

Among all the tested bio-agents, bio-agents liquid formuations (*T. viride* + *P. fluorescence* + *P. lilacinum*) significantly improved the plant growth and reduced disease severity might be due to the combined effects of all bio-agents. Several researchers proved that combined application of bio-agents has been effective in management of *M. incognita* and *F. oxysporum* either inoculated individually or simultaneously in various vegetable crops under *in vivo* or field (protected/open) conditions (Singh and Balodi, 2021; Abo-Elyousr et al., 2014; Singh et al, 2013; Singh, 2013, 2019; Singh and Singh, 2012; Dubey et al., 2007). In present study, combined application of egg parasitic fungus, *P. lilacinum*, antagonistic fungus, *T. viride* and bacterial antagonists *P. fluorescens* was shown increased efficiency in cucumber plants under protected conditions may be the reason that the bio-agents colonisation was more due to favourable conditions (micro climate) maintained in protected structures. Some researchers also found that bio-agents efficacy has been improved for management of complex diseases (*Meloidogyne* spp. and *F. oxysporum*) under protected conditions (Singh and Balodi, 2021; Abo-Elyousr et al., 2014).

Similarly, Rao et al. (1997) found that lowest root-knot index, root-knot nematode (*M. incognita*) population and enhanced fruit yield of okra when seeds are treated with *P. lilacinum*. The nematode population might be reduced due to the parasitic activity of *P. lilacinum* on eggs and all stages of nematodes. *P. lilacinum* spores also attached to the cuticle of second stage juveniles of root-knot nematode (vermiform stages) when they are randomly move in the soil pore spaces. The *P. lilacinum* spores penetrate the cuticle and engulf the root knot nematode juveniles and hyphae of the fungus could entered in the nematode body through natural openings, such as anus and vulva. *P. lilacinum* feed and suck the body contents and ultimately killed the nematode. Moreover, *P. lilacinum* acted as a parasite on the nematode. Greatest reduction in soil nematode populations was found in the presence of both pathogens nematode and fungus followed by nematode alone. All the used bioagents in this study significantly reduced the fungal incidence in cucumber as compared to non-treated inoculated check. Lesser (10 %) fungal disease incidence was observed with the bioagents liquid formulations as compared to nontreated inoculated check followed by *T. viride* (15 %) where both the pathogens inoculated simultaneously. These findings were also agreements with the studies of Druzhinina et al. (2011), Hallmann et al. (2009), Robab et al. (2012) and Vos et al. (2012).

In our study, all bio-agents increased the plant growth and reduced disease severity might be the reason that the bio-agents were rapidly multiplied and colonized in soil rhizosphere. So, crop at the initial/early stage was protected from prior nematode infection or disposer for soil borne fungus. The efficiency of bio-agents (fungal and bacterial antagonists) has been varied, and mainly depends upon their establishment in soil rhizosphere and

Table 5
Effect of bio-agents seed treatment on percent fungal incidence in cucumber crop infested with root-knot nematode and soil borne fungus.

Treatments	Pre emergence damping off					
	Fungi alone			Nematode + Fungi		
	15 days after sowing	30 days after sowing	Pooled Mean	15 days after sowing	30 days after sowing	Pooled Mean
T. viride 10 g kg^{-1} seed	15 ^{a}	25^{b}	20	20 ^{a}	30^{b}	25
T. viride 20 g kg^{-1} seed	10 ^{a}	20 ^{ab}	15	15 ^{a}	15 ^{ab}	15
P. fluorescence 10 g kg^{-1} seed	15 ^{a}	20 ^{ab}	18	20 ^{a}	30^{b}	25
P. fluorescence 20 g kg^{-1} seed	10 ^{a}	15 ^{ab}	13	15 ^{a}	20 ^{ab}	17.5
P. lilacinum 10 g kg^{-1} seed	15 ^{a}	20 ^{ab}	18	20 ^{a}	25 ^{ab}	22.5
P. lilacinum 20 g kg^{-1} seed	5 ^{a}	10 ^{ab}	8	10 ^{a}	15 ^{ab}	12.5
LFB 10 ml kg^{-1} seed	10 ^{a}	20 ^{ab}	15	15 ^{a}	20 ^{ab}	17.5
LFB 15 ml kg^{-1} seed	5 ^{a}	5 ^{ab}	5	5 ^{a}	10 ^{ab}	7.5
Carbosulfan 3% v/w	5 ^{a}	5 ^{ab}	5	5 ^{a}	10 ^{ab}	7.5
Non-treated control (inoculated)	55^{b}	65^{c}	60	60^{b}	60^{c}	60
Non-treated control (non-inoculated)	0 ^{a}	0 ^{a}	0	0 ^{a}	0 ^{a}	0
Pooled Mean	13.2	18.6		16.8	21.4	

LFB = liquid formulation of bio-agents, Data are mean of four replications.

parasitization on target pest (Singh and Mathur, 2010), so seed treatment with bio-agents initially protects the crop from soil borne pathogens.

5. Conclusions

According to the results of this study, bio-agents can be suitable candidates for use in biological control of *M. incognita* and fungus as seed treatment, which, in turns, will be an effective action to reduce the consumption of pesticides and helping to develop safer sustainable agriculture. The results of this study seem to indicate that the liquid formulations of bioagents could be a potential candidate in organic farming/protected cultivation, where a few/less options are available to manage nematode and fungus disease complexes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdelmoneim, T.S., 2006. Using some microorganisms or their products for control of plant parasitic nematodes. In: PhD. Thesis, Faculty of Agriculture. Suez Canal University, Ismailia, Egypt, pp. 1–111.

Abo-Elyousr, K.A.M., Sobhy, A.H., Abdel-Rahim, I.R., 2014. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. J. Phytopathol. 162, 567–574.

Akhtar, H., Anita, S., Kumar, S.P., 2005. Studies on the management of root-knot nematode, *Meloidogyne incognita*-wilt fungus, *Fusarium oxysporum* disease complex of green gram, *Vigna radiata* cv ML-1108. J. Zhejiang Univ. Sci. 6 (8), 736–742.

AL-Shammari, T.A., Bahkali, A.H., Elgorban, A.M., El-Kahky, M.T., Al-Sum, B.A., 2013. The use of *Trichoderma longibrachiatum* and *Mortierella alpina* against root-knot nematode, *Meloidogyne javanica* on tomato. J. Pure Appl. Microbio 7, 199–207.

Cobb, N.A., 1918. Estimating the nema population of the soil. Agricultural Technology Circular-1. Bureau of Plant Industry, U.S.D.A. California, USA, pp. 48.

Amer-Zareen, Khan, N.J., Zaki, M.J., 2001. Biological control of *Meloidogyne javanica* (Treub) Chitwood, root-knot nematode of okra (*Abelmoscus esculentus* L.). Pak. J. Biol. Sci. 4, 990–994.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., Tchamitchian, M., 2011. Root-knot nematode (*Meloidogyne* sp.) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Prot. 30 (10), 1251–1262.

Dhawan, S.C., Narayana, R., Babu, N.P., 2004. Bio-management of root-knot nematode, *Meloidogyne incognita* in okra by *Paecilomyces lilacinus*. Annual Plant Prot. Sci. 12, 356–359.

Druzhinina, I.S., Sedi-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., Kubicek, C.P., 2011. *Trichoderma*: the genomics of opportunistic success. Nat. Rev. Microbiol. 9, 749–759.

Dubey, S.C., Suresh, M., Singh, B., 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. J. Bio. Cont. 40, 118–127.

Dutta, J., Thakur, D., 2017. Evaluation of multifarious plant growth promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling. India. PloS one 12, (8) e0182302.

Ehwaeti, M.E., Phillips, M.S., Trudgill, D.L., 1998. The viability of *Meloidogyne incognita* eggs released from egg masses of different ages using different concentrations of sodium hypochlorite. Nematologica 44 (2), 207–219.

El-Mougy, N., Abdel-Kader, L., Lashin, S.M., 2012. Vegetables root rot disease management by an integrated control measures under greenhouse and plastic houses conditions in Egypt—A review. Int. J. Eng. Innov. Technol 6, 241–248.

Giacometti, R., d'Errico, G., d'Errico, F.P., 2010. *In vitro* nematicidal activity of the experimental formulation tequil against *Meloidogyne incognita* and *Heterodera daverti*. Nematropica, 263–268.

Hadian, S., Rahnama, K., Jamali, S., Eskandari, A., 2011. Comparing neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato. Adv. Environ. Biol. 5 (8), 2052–2057.

Hallmann, J., Davies, K.G., Sikora, R., 2009. Biological Control Using Microbial Pathogens, Endophytes and Antagonists. In: PerryMoensStarr, R NMJ L (Ed.), Root-knot nematodes Wallingford. Oxford shireCAB International, UK, p. 380.

Hanafi, A., Papasolomontos, A., 1999. Integrated production and protection under protected cultivation in the Mediterranean region. Biotechnol. advances 17 (2–3), 183–203.

Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2017. Effects of southern root-knot nematode population densities and plant age on growth and yield parameters of cucumber. Crop Prot. 92, 207–212. https://doi.org/10.1016/j.cropro.2016.09.007.

Kiewnick, S., Sikora, R., 2004. Optimizing the efficacy of *Paecilomyces lilacinum* (strain 251) for the control of root-knot nematodes. Commun. Agric. Appl. Biol. Sci. 69, 373–380.

Lamovsek, J., Urek, G., Trdan, S., 2013. Biological control of root-knot nematodes (*Meloidogyne* spp.): Microbes against the pests. Acta Agric. Slov 101, 263–275.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public Health 4, 148.

Patil, J., 2017. Studies on incidence, interaction and management of root-knot nematode, *Meloidogyne incognita* and soil borne fungus infesting cucumber in polyhouse, Thesis Nematology, CCSHAU, Hisar.

Patil, J., Goel, S.R., Yadav, S., 2018. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber grown under protected cultivation. J. Entomol Zool. Stud. 6 (1), 1004–1007.

Punja, Z.K., Utkhede, R.S., 2003. Using fungi and yeasts to manage vegetable crop diseases. Trend. Biotech. 21 (9), 400–407.

Rao, M.S., Reddy, P.P., Nagesh, M., 1997. Integrated management of *Meloidogyne incognita* on okra (*Abelmoschus esculentus*) by castor cake suspension and *Paecilomyces lilancinus*. Nematologia mediterrerenea 25, 17–19.

Rao, M.S., Reddy, P.P., Nagesh, M., 1998. Evaluation of plant-based formulations of *Trichoderma harzianum* for the management of *Meloidogyne incognita* on eggplant. Nematol. Mediterr. 26, 59–62.

Robab, M.I., Shaikh, H., Azam, T., 2012. Antagonistic effect of *Glomus mosseae* on the pathogenicity of root knot nematode infected *Solanum nigrum*. Crop Prot. 42, 351–355.

Sasser, J.N., Eisenbach, J.N., Carter, C.C., 1983. The international *Meloidogyne* project – its goals and accomplishments. Ann. Rev. Phytopathol. 21, 271–288.

Schindler, A.F., 1961. A simple substitute for a Baermann funnel. Plant Dis. Report 45, 747–748.

Singh, S., 2013. Integrated approach for the management of root-knot nematode, *Meloidogyne incognita* on eggplant under field conditions. Nematol. 15, 747–757.

Singh, S., 2019. Integrated approach for management of root-knot nematode (*Meloidogyne incognita*) in bitter gourd (*Momordica charantia*). Ind. J. Agri. Sci. 89, 71–76.

Singh, S., Balodi, R., Meena, P.N., Singhal, S., 2020. Biocontrol activity of *Trichoderma harzianum Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita Fusarium oxysporum and Rhizoctonia solani*. (Unpublished).

Singh, S., Mathur, N., 2010. Bio-control of root-knot nematode, *Meloidogyne incognita* infesting tomato. Biocont. Sci. Tech. 20, 865–874.

Singh, S., Pandey, R.K., Goswami, B.K., 2013. Biological control activity of *Purpureocillium lilacinum* strains in managing root-knot disease of tomato caused by *Meloidogyne incognita*. Biocont. Sci. Tech. 23, 1469–1489.

Singh, S., Singh, R.K., 2012. Development of an integrated approach for managing root-knot disease of chilli (*Capsicum annum* L.) under field condition. Rus. J. Nematol. 20, 65–73.

Vos, C.M., Tesfahun, A.N., Panis, B., De, W.D., Elsen, A., 2012. Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode (*Meloidogyne incognita*) and the migratory nematode (*Pratylenchus penetrans*). Appl. Soil Ecol.. 61, 1–6.

Williamson, V.M., Hussey, R.S., 1996. Nematode pathogenesis and resistance in plants. Plant Cell 8, 1735–1745.