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Suppression of *Meloidogyne incognita* race 2 with some entomopathogenic agents

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Abstract: Root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is one of the most important species causing yield and quality reduction in crops worldwide. Because they are of soil origin, the control of this nematode is very difficult and uneconomical. Due to environmental problems caused by intensive pesticides used to control plant diseases and pests, research on alternative control methods has increased. One of the most valuable alternative control methods is biological control. This work was conducted to determine the larvicidal effects of isolates of fungi and bacterial species, whose bio-insecticidal effects are known and widely studied, against second-stage juveniles (J_2) of root-knot nematode *M. incognita* race 2. In the experiments, fungal isolates of *Beauveria bassiana* (ET 10, ET 101) and *Aspergillus flavus* (ET 95, ET 102) and bacterial strains of *Brevibacillus brevis* (FD-1), *Bacillus thuringiensis* subsp. *kenyae* (FDP-8, FDP-42), *Bacillus atrophaeus* (FD-17) and *Bacillus cereus* (FD-63) species were used. Fungal isolates were applied in stock suspension (1X), diluted with sterile distilled water to 5X and 10X doses, and bacterial strains were applied at 1×10^6, $1 \times 10^{7,}$ and 1×10^8 cfu mL^{-1} concentrations. In vitro experiments were set up with 3 replications, sterile distilled water was used in the negative control and a commercial nematicide (Velum Prime ® SC 400, Bayer) was used in the positive control. At the end of the 1st, 3rd, and 7th days, the results of the applications were counted and evaluated according to the percent mortality rates. When the percent mortality rates obtained at the end of the 7th day were compared with the negative control, the most effective results were obtained from the 5X dose of ET 102 in fungal isolates (39.88%) and the 1×10^8 cfu mL^{-1} concentration of FD-63 in bacterial strains (65.07%). It is thought that the ET 102 fungal isolate and FD-63 bacterial strain used in this study have the potential to be biological control agents against *M. incognita* race 2 juvenile. It was concluded that these isolates can be included in integrated pest management strategies against root-knot nematodes by making field trials.

Key words: *Meloidogyne incognita*, *Aspergillus flavus, Bacillus cereus, Beauveria bassiana,* suppression, entomopathogenic agents

1. Introduction

Plant parasitic nematodes are a group of pests that cause serious economic losses in agricultural areas. It was reported that annual crop loss from plant parasitic nematodes in the world reaches 173 billion dollars (Elling, 2013). Root-knot nematodes are the one of the most important groups in plant parasitic nematodes and polyphagous species that can cause damage to almost all plant crops widely produced in the world (Gill and McSorley, 2011).

Physical, cultural, and chemical methods are used to control root-knot nematodes but these methods are not always effective enough (Elekçioğlu and Uygun, 1990). In addition to these, chemical nematicides can be toxic to nontarget microorganisms in agricultural systems because, they were developed to control of plant parasitic nematodes which are soil organisms (Abawi and Widmer, 2000). Negative effects on human and environmental health have also been revealed in studies (Guginoet al., 2006).

These negative effects encourage studies to develop alternative control methods against nematodes (Kiewnick and Sikora, 2006; Uysal et al., 2021). Biological control methods are one of the most important approaches to ensure sustainability in agriculture (Mokhtariet al., 2009). In this context, studies on the use of entomopathogenic fungi and bacteria in nematode control have been carried out in recent years (Ceitintas and Abed Salih, 2016; Devi and Bora, 2018; Kepenekciet al., 2017; Yerukalaet al., 2021).

Aspergillus flavus and *Beauveria* spp. are fungi species with proven entomopathogenic effects and great potential, and their activities against plant parasitic nematodes have been demonstrated by various studies (Aminuzzamanet al., 2018; Khanet al., 2000; Liu et al., 2008; Udayet al., 2019;

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Youssefet al., 2020). In addition, beauvericin produced by *B. bassiana* has been proven to have nematicidal activity against *M. incognita* (Anke et al., 1995; Hamillet al., 1969; Suzukiet al., 1977).

In addition to fungi, entomopathogenic bacteria offer an effective alternative option for the control of nematodes with their competitiveness and antagonistic effects (Meyer, 2003). It is thought that Bt crystal proteins can provide a safe and economical control method against nematodes. It was determined that 7 Cry proteins are toxic to plant parasitic nematode juvenile (Frankenhuyzen, 2009). It has been reported that Cry proteins cause damage in the intestines of nematodes (Salehi Jouzaniet al., 2008).

This study was carried out to determine the larvicide effects of isolates of fungi and bacterial species whose bio-insecticidal effects are known and widely studied against root-knot nematode *M. incognita* race 2 juvenile.

2. Materials and methods

2.1. Entomopathogenic fungal and bacterial isolates

The entomopathogenic effects of 4 fungal isolates (ET 10, ET 95, ET 101, ET 102) and 5 bacterial strains (FD-1, FDP-8, FD-17, FDP-42, FD-63) were tested against to *M. incognita* race 2 2nd stage juvenile (J_2) in vitro. Fungal isolates were obtained from the collection kept in tubes containing Potato Dextrose Agar (PDA; Difco) at 4 °C in Atatürk University, Faculty of Agriculture, Department of Plant Protection, Mycology Laboratory. Bacterial strains were obtained from Atatürk University, Faculty of Agriculture, Department of Plant Protection, Plant Clinical Laboratory culture collection. Bacterial strains were stored at –86 °C in stock growth medium containing 30% glycerol and Loria Broth (LB) until use.

2.2. Identification of fungal isolates and bacterial strains

In previous studies, the insecticidal effect of ET 10, one of the fungal isolates, was determined against various pests and it was diagnosed using molecular methods as *Beauveria bassiana* (GB |KY806126|; 99%) (Tozlu et al., 2017). The other three fungal isolates were diagnosed using the MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry, Bruker Daltonics GmbH, Bremen, Germany) technique. These isolates were identified using the MALDI-TOF MS Microorganism Identification device in Hatay Mustafa Kemal University Plant Health Clinic Application and Research Center. Protein isolation from pure fungal cultures was performed using the ethanol-formic acid extraction method. The spectra obtained with the flex control software program (Biotyper 3.0; Microflex LT; BrukerDaltonics GmbH, Bremen, Germany) of the device were compared with the Maldi Biotyper Real-Time Classification (RTC) software, and the diagnosis process was completed. As a result, the data was determined between 2000–3000 and in green color accepted as having high reliability (Chalupováet al., 2014; De Caroliset al., 2012; Soyluet al., 2021).

Extraction and analysis of fatty acid methyl esters of bacterial isolates used in the study were performed with the standard protocol of the Microbial Identification System (MIDI, Inc., Newark, DE) (Paisley, 1995), and the bacterial strains prepared from fresh bacterial cultures was diagnosed with the MIS device.

2.3. Hypersensitivity test

Hypersensitivity test was performed to determine whether bacterial agents are plant pathogens. The tests were carried out using fresh leaves of the *Nicotiana tabacum* L. var. *Samsun* variety grown in pots under controlled conditions. Suspensions (10^8 cells/mL) prepared using bacterial cultures kept in Nutrient agar medium for 24–48 h were injected between 2 leaf veins and symptoms on the leaves were examined. Asymptomatic tobacco leaves were considered negative and symptomatic as positive (Klement, 1964).

2.4. Nematode population

The nematode populations used in the study were obtained from the vegetable fields in Adana province. After the *M. incognita* population was confirmed with scar primers (Tesařováet al., 2003) and esterase enzyme profile (Esbenshade and Triantaphyllou, 1985), it was determined to be race-2 by the North Carolina Host Test and was cultured in Biological Control Central Research Institute and produced as a pure culture. In experiments, *M. incognita* race 2 second stage juvenile (J_2) obtained from the pure culture was used.

2.5. Preparation of fungal and bacterial suspensions

Fungal suspensions were prepared using the method of Liu et al. (2008) in some modification. The Fungal isolates were allowed to grow for 3 weeks in 5 petri dishes containing PDA at 16/8 (L / D) photoperiod, 80% ± 10% relative humidity and 25 ± 1 °C temperature conditions. The initial dose was prepared by scraping the conidia from these cultures into a solution containing 0.05% Tween 80. The mixture was purified from hyphae by filtration through a fine-mesh sieve. Three application doses prepared by diluting the stock suspension (1X) to 5X and 10X doses with sterile distilled water were used in mortality tests after vortex for 3 min.

Bacterial strains drawn on Tryptic Soy Agar in 3 phases were cultured at 27 °C for 24 h and fresh cultures were obtained. After the incubation period, a single colony was transferred to vials containing Tryptic Soy Broth and the vials were allowed to grow aerobically for 24 h at 27 °C on a rotating shaker (150 rpm). By adding sterile TSB to the bacterial suspension, the initial dose was prepared at a

concentration of 1×10^8 cfu mL^{-1} with a turbidimeter, and 1×10^7 and 1×10^6 doses were obtained by diluting.

2.6. Mortality of second stage juveniles (J_2) and statical analysis

To determine the larvicide effects of entomopathogenic fungi and bacteria on *M. incognita* juvenile race 2 (J_2), 500 µL of suspension containing 500 J_2 mL^{-1} was added to sterile eppendorf tubes of 2 mL volume. Then, the prepared bacterial and fungal suspensions were added in 500 µL volume and the final volume was completed to 1 mL. For dead and alive counts, the suspension was mixed with a micropipette, and nematodes in 50 µL volume were counted using light microscope (Zeiss Microscope Axio Imager A2) at the end of the 1st, 3rd, and 7th days. Each experiment was set up with 3 replications. Sterile distilled water was used in the negative control, and a commercial nematicide (Velum Prime ® SC 400, Bayer) with the active ingredient Fluopyram in the IRAC[1] nematicide MOA Classification Group N-3 was used in the positive control ("Mode of Action | Insecticide Resistance Action Committee (IRAC)", 2022). Mortality rates of *M. incognita* were analyzed with the JMP, 5.0 program. Differences between the means were made with the LSMeans Student's test at the probability level of $p < 0.01$.

3. Results

3.1. Identification results

Identification results of fungal isolates and bacterial strains are given in Table 1, Table 2, and Figure 1.

3.2. Mortality tests

Mortality rates at 1X, 5X, and 10X doses at the end of the 1st, 3rd, and 7th days of entomopathogenic fungi applications against *M. incognita* J_2 are indicated in Figure 2, Table 3, and Table 4. In the time-dependent analysis, the highest mortality rate was obtained at the end of the 7th day (100%) in the positive control. This was followed by the ET 102 (5X) (39.88%), ET 10 (1X) (33.36%), ET 95 (1X) (33.28%), ET 102 (10X) (32.42%), ET 102 (1X) (31.80%), and ET 101 (1X) (29.75%) in the counts at the end of the 7th day. The lowest mortality rate was found in the negative control. In the analyzes made according to the doses, the highest mortality rate was obtained from the positive control at all counting times and was statistically in a different group from the other doses. This was followed by ET 10 (7d) (39.88%), ET 95 (7d) (33.36%), ET 102 (7d) (33.28%), ET 10 (3d) (32.24%), and ET 101 (7d) (31.80%) at 1X dose, with percent mortality of isolates at the times indicated. The lowest mortality rate was found in the negative control application compared to all doses.

1 Mode of Action | Insecticide Resistance Action Committee (IRAC) (2022). (accessed on 2022) https://irac-online.org/mode-of-action/

Table 1. Identification results of entomopathogenic fungus isolates.

Fungal Isolates	Isolated from	Identification results	Reference
ET 10	*Sphenoptera antiqua* Illiger (Col.: Buprestidae)	*Beauveria bassiana*	(Tozlu et al., 2011)
ET 95	Soil	*Aspergillus flavus*	In this study
ET 101	Soil	*Beauveria bassiana*	In this study
ET 102	Soil	*Aspergillus flavus*	In this study

Table 2. Diagnostic results of entomopathogenic bacterial strains.

Bacterial Strains	Isolated from	Microbial identification results of bacteria	SIM	HR	Reference
FD-1	Malacosoma neustria (Lep.: Lasiocampidae)	Brevibacillus brevis	0.65	-	Tozlu et al., 2011
FDP-8	Hypera postica (Col.: Curculionidae)	Bacillus thuringiensis subsp. kenyae	0.45	-	Tozlu et al., 2011
FD-17	Yponomeuta evonymella	Bacillus atrophaeus	0.46	-	Tozlu et al., 2011
FDP-42	Apion spp.(Col.: Brentidae)	Bacillus thuringiensis subsp. kenyae	0.47	-	Tozlu et al., 2011
FD-63	Yponomeuta evonymella	Bacillus cereus	0.24	-	Tozlu et al., 2011

SIM: Similarity index, - : Negative reaction, HR: Hypersensitivity test

Analyte Name: H1
Analyte Description:
Analyte ID: ET102
Analyte Creation Date/Tim: 2021-11-05T04:02:09.112
Applied MSP Library(ies): Filamentous Fungi
Applied Taxonomy Tree:

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+)	Aspergillus flavus_oryzae_group (2008_146044 MUZ A. flavus)	1.935	5052
2 (+)	Aspergillus flavus_oryzae_group (1327 MPA A. flavus)	1.845	5052
3 (+)	Aspergillus flavus_oryzae_group (12106124 MVD A. oryzae)	1.838	5052
4 (+)	Aspergillus flavus_oryzae_group (M08 RLH A. flavus)	1.823	5052
5 (+)	Aspergillus flavus_oryzae_group (M09 RLH A. flavus)	1.821	5052

Analyte Name: H2
Analyte Description:
Analyte ID: ET95
Analyte Creation Date/Time: 2021-11-05T04:02:09.119
Applied MSP Library(ies): Filamentous Fungi
Applied Taxonomy Tree:

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (-)	Aspergillus flavus_oryzae_group (1327 MPA A. flavus)	1.523	5052
2 (-)	Aspergillus flavus_oryzae_group (M09 RLH A. flavus)	1.48	5052
3 (-)	Aspergillus flavus_oryzae_group (2008_146044 MUZ A. flavus)	1.479	5052
4 (-)	Aspergillus flavus_oryzae_group (M08 RLH A. flavus)	1.409	5052
5 (-)	Aspergillus flavus_oryzae_group (M07 RLH A. flavus)	1.4	5052

Analyte Name: H3
Analyte Description:
Analyte ID: ET101
Analyte Creation Date/Time: 2021-11-05T04:02:09.115
Applied MSP Library(ies): Filamentous Fungi
Applied Taxonomy Tree:

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+)	Beauveria bassiana 143_32166_838 JMI	1.775	475271
2 (-)	Beauveria bassiana DSM 1344 DSM	1.678	475271
3 (-)	Beauveria bassiana DSM 875 DSM	1.677	475271
4 (-)	Beauveria bassiana 122_42643_1590 JMI	1.661	475271
5 (-)	Beauveria bassiana 143_32166_838 JMI	1.59	475271

Figure 1. MALDI-TOF diagnostic result of ET 95, ET 101, and ET 102 isolates.

The in vitro activities of entomopathogenic bacteria against to *M. incognita* juvenile (J_2) are indicated in Figure 3, Table 5, and Table 6. In the analysis made according to time, the highest mortality rate was found in the positive control, and the lowest mortality rate was found in the negative control. The highest mortality rate was determined at the 1×10^8 concentration of FD-63 (65.07%) at the end of the 7th day. It was followed by the percent mortality rates (50.61%, 44.18%, 43.34%) obtained from the 7th-day counts of FD-1's 1×10^8, FDP-8's 1×10^8 and FD-63's 1×10^7 concentrations, respectively. In the analyzes made according to the concentrations, the highest mortality rate

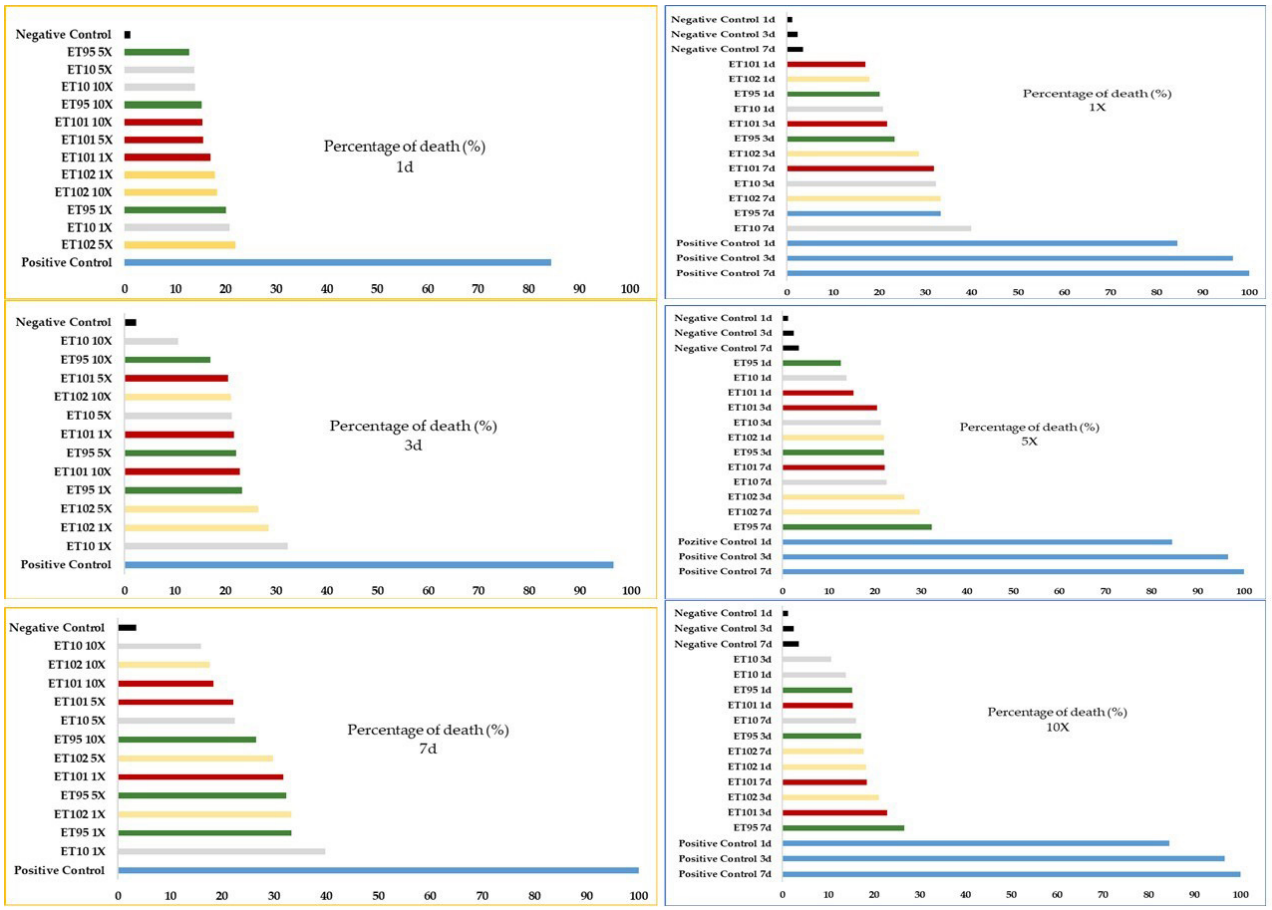


Figure 2. The mortality rates (%) from the bioagent fungus tested against *Meloidgyne incognita* J_2 under controlled conditions (X axis: percentage of death; Y axis: the isolate used).

was found in the positive control, and the lowest mortality rate was determined in the negative control applications. When the percent mortality rate of bacterial strains was examined, the highest mortality rate was determined at the 10^8 concentration of FD-63 (65.07%) at the end of the 7th day. It was followed by the percent mortality rates obtained from 1×10^8 concentrations of bacterial strains FD-1 (7d), FD-63 (3d), and FDP-8 (7d), respectively (50.61%, 48.89%, 44.18%).

4. Discussion

According to the bioassay test results, the most effective fungal isolate was ET 102 (*A. flavus*) and the most effective bacterial isolate was FD-63 (*Bacillus cereus*) in the counts made at the end of the 7th day. It was noted that the tested bioagents were effective against to *M. incognita* second stage juvenile (J_2) to varying degrees. It was found that the average plant weight, plant height and root-gal index decreased in the combined application of *B. bassiana* with *M. incognita* on tomato plants compared to the control group (Ekanayake and Jayasundara, 1994). It is reported that beauvericin produced by *B. bassiana* has a weak nematicidal effect against *M. incognita* (Mayer, 1995). In the study of Liu et al. (2008) in which they evaluated the nematicidal effect of *B. bassiana* culture filtrates at different concentrations against *M. hapla*, they determined that the culture filtrate concentration proportionally changed the hatching percentage and mortality rate. In another study, it was found that the hatching percentages of *M. incognita* and the survival rates of the second stage juvenile were significantly affected in the applications of culture filtrate and spore concentrations of *B. bassiana* in proportion to the dose (Youssefet al., 2020). Aita et al. (2020) investigated the potential of cell wall degrading enzymes (chitinase, β-1,3 glucanase, endo-cellulase and exocellulase) produced using *Trichoderma harzianum, B. bassiana* and *Metarhizium anisopliae* against *M. javanica*; reported that at the end of the third day all bioproducts highly affected the hatching rate (66.7%–87.0%) and the mortality rate (40.0%–90.0%) in the second stage juvenile

Table 3. At the end of the 1st, 3rd, and 7th days, the effects of entomopathogenic fungi on *Meloidgyne incognita* according to their doses.

Mortality ratio (%)*						
Treatment	1 day		3 days		7 days	
Positive Control	84.52	A	96.59	A	100.00	a
ET 102 5X	21.98	B	26.44	BCD	39.88	b
ET 10 1X	20.77	B	32.24	B	33.36	bc
ET 95 1X	20.11	B	23.27	BCD	33.28	bc
ET 102 10X	18.32	B	21.10	BCDE	32.42	bc
ET 102 1X	17.91	B	28.56	BC	31.80	bc
ET 101 1X	16.99	B	21.75	BCDE	29.75	bcd
ET 101 5X	15.46	B	20.57	CDE	26.57	cde
ET 101 10X	15.42	B	22.83	BCD	22.53	cde
ET 95 10X	15.24	B	17.11	DE	22.14	cde
ET 10 10X	13.89	B	10.71	EF	18.38	de
ET 10 5X	13.83	B	21.27	BCDE	17.68	de
ET 95 5X	12.70	B	22.08	BCD	15.99	ef
Negative Control	1.15	C	2.41	F	3.58	f
CV	33.08		25.65		25.55	
LSD	11.43		11.28		13.09	

*In the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p < 0.01$).

Table 4. The effectiveness of entomopathogenic fungi on *M. incognita* at the end of the 1st, 3rd, and 7th days according to the doses.

Mortality ratio (%*)						
Treatment	1X		5X		10X	
Positive Control 7 days	100	A	100.00	A	100.00	a
Positive Control 3 days	96.59	A	96.59	A	96.59	a
Positive Control 1 day	84.52	B	84.52	B	84.52	b
ET 10 7 days	39.88	C	22.53	CDE	15.99	cde
ET 95 7 days	33.36	CD	32.42	C	26.57	c
ET 102 7 days	33.28	CD	29.75	CD	17.68	cde
ET 10 3 days	32.24	CDE	21.27	DE	10.71	efg
ET 101 7 days	31.80	CDE	22.14	DE	18.38	cde
ET 102 3 days	28.56	DEF	26.44	CD	21.10	cde
ET 95 3 days	23.27	DEFG	22.08	DE	17.11	cde
ET 101 3 days	21.75	EFG	20.57	DE	22.83	cd
ET 10 1 day	20.77	FG	13.83	E	13.89	def
ET 95 1 day	20.11	FG	12.70	EF	15.24	de
ET 102 1 day	17.91	FG	21.98	DE	18.32	cde
ET 101 1 day	16.99	G	22.14	DE	15.42	cde
Negative Control 7 days	3.58	H	3.58	FG	3.58	fg
Negative Control 3 days	2.41	H	2.41	G	2.41	g
Negative Control 1 day	1.15	H	1.15	G	1.15	g
CV	19.61		19.92		24.47	
LSD	10.99		9.99		11.31	

*In the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p < 0.01$).

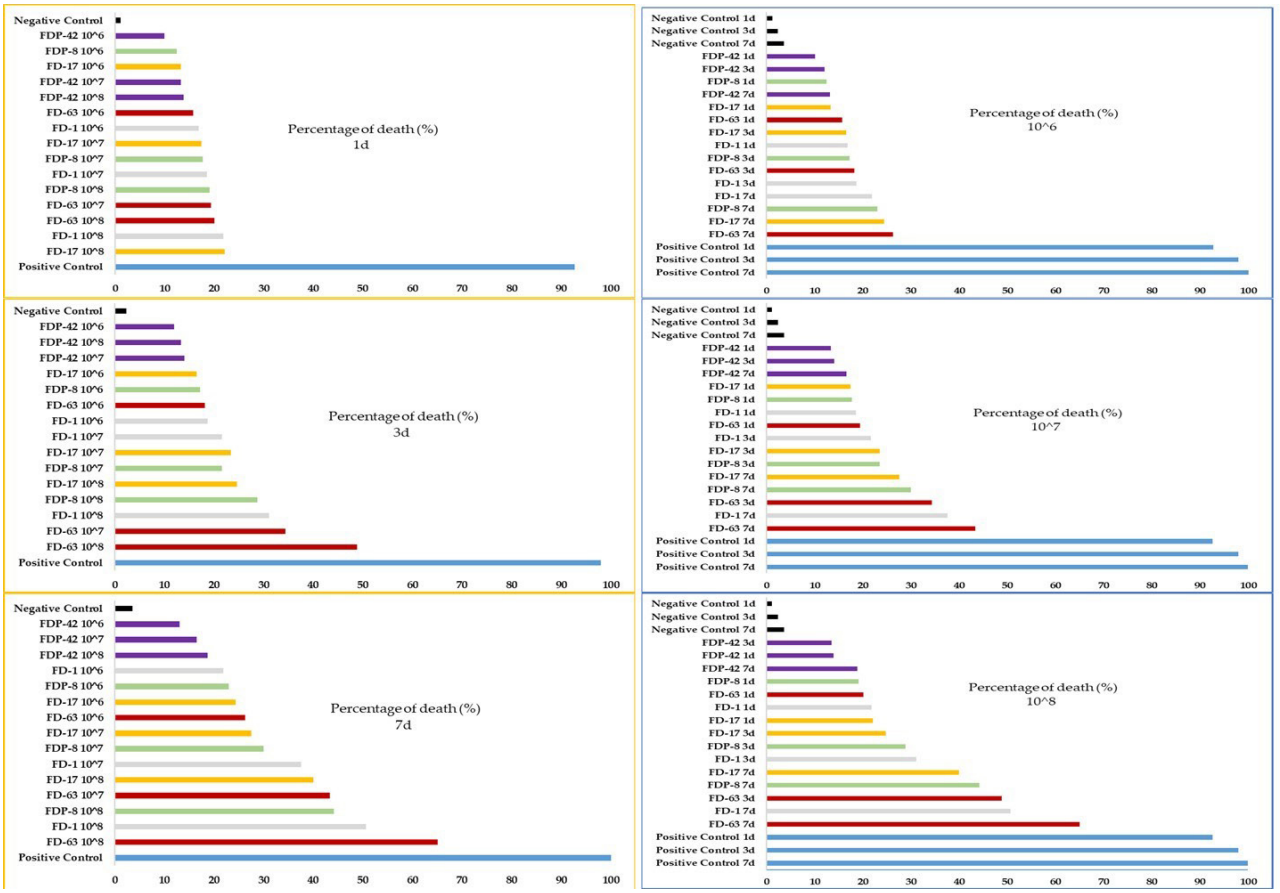


Figure 3. The mortality rates (%) from the bioagent bacteria tested against *Meloidogyne incognita* J_2 under controlled conditions (X axis: percentage of death; Y axis: the isolate used) .

Table 5. At the end of the 1st, 3rd, and 7th days, the efficacy of entomopathogenic bacteria on *Meloidogyne incognita* according to the doses.

Mortality ratio (%)*						
Treatment	1 day		3 days		7 days	
Positive Control	92.70	A	97.98	A	100.00	a
FD-17 10^8	22.08	B	24.72	DEF	40.02	d
FD-1 10^8	21.80	B	31.09	CD	50.61	c
FD-63 10^8	20.13	BC	48.89	B	65.07	b
FD-63 10^7	19.37	BCD	34.39	C	43.34	cd
FDP-8 10^8	19.13	BCD	28.77	CDE	44.18	cd
FD-1 10^7	18.61	BCDE	21.65	EFGH	37.57	de
FDP-8 10^7	17.69	BCDE	21.65	EFGH	30.01	ef
FD-17 10^7	17.47	BCDE	23.41	DEFG	27.59	f
FD-1 10^6	16.84	BCDE	18.69	FGHI	21.86	fgh
FD-63 10^6	15.74	BCDEF	18.22	FGHI	26.28	fg
FDP-42 10^8	13.84	CDEF	13.42	I	18.77	ghi
FDP-42 10^7	13.33	DEF	14.10	HI	16.61	hi

Table 5. (Continued).

FD-17 10^6	13.32	DEF	16.52	GHI	24.40	fgh
FDP-8 10^6	12.42	EF	17.18	FGHI	23.01	fgh
FDP-42 10^6	10.05	F	12.08	I	13.10	i
Negative Control	1.15	G	2.41	J	3.58	j
CV	19.56		18.09		15.05	
LSD	6.61		7.91		8.63	

*In the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p < 0.01$).

Table 6. The effectiveness of entomopathogenic fungi on *Meloiodogyne incognita* at the end of the 1st, 3rd, and 7th days according to the doses.

	Mortality ratio (%)*					
Treatment	10^6		10^7		10^8	
Positive Control 7 days	100.00	A	100	A	100.00	a
Positive Control 3 days	97.98	AB	97.98	AB	97.98	a
Positive Control 1 day	92.70	B	92.70	B	92.70	a
FD-63 7 days	26.28	C	43.34	C	65.07	b
FD-17 7 days	24.40	CD	27.59	EFG	40.02	d
FDP-8 7 days	23.01	CDE	30.01	EF	44.18	cd
FD-1 7 days	21.86	CDEF	37.57	CD	50.61	c
FD-1 3 days	18.69	DEFG	21.65	GH	31.09	e
FD-63 3 days	18.22	EFGH	34.39	DE	48.89	c
FDP-8 3 days	17.18	EFGHI	23.54	FGH	28.77	ef
FD-1 1 day	16.84	FGHI	18.61	HI	21.80	fgh
FD-17 3 days	16.52	FGHI	23.41	FGH	24.72	efg
FD-63 1 day	15.74	GHIJ	19.37	HI	20.13	fgh
FD-17 1 day	13.32	GHIJ	17.47	HI	22.08	fgh
FDP-42 7 days	13.10	GHIJ	16.61	HI	18.77	gh
FDP-8 1 day	12.42	HIJ	17.69	HI	19.13	gh
FDP-42 3 days	12.08	IJ	14.10	I	13.42	h
FDP-42 1 day	10.05	J	13.33	I	13.84	h
Negative Control 7 days	3.58	K	3.58	J	3.58	i
Negative Control 3 days	2.41	K	2.41	J	2.41	i
Negative Control 1 day	1.15	K	1.15	J	1.15	i
CV	13.96		14.10		14.68	
LSD	6.12		7.28		8.77	

*In the same column by the same letter are not significantly different to the test of LS Means Differences Student's ($p < 0.01$).

on the first day. It has been reported that the culture filtrate of 9 different *B. bassiana* isolates caused death to *M. incognita* second stage juvenile at rates ranging from 15.39% to 99.79% after 48 h (Zhaoet al., 2013). In studies on plant growth and yield parameters; it was found that *B. bassiana* ARSEF 6646 isolate against *M. incognita* significantly affected root-gal index, plant height and plant fresh weight on tomato and eggplant plants (Cetintas and Abed Salih, 2016) and Kepenekci et al. (2017), stated that 1×10^8 cfu ml^{-1} concentration of two *B. bassiana* isolates [F-56 (4.59 ± 0.54) and F-63 (2.77 ± 0.59)] in tomato significantly suppressed *M. incognita* and *M. javanica* populations and increased tomato yield.

Bacterial preparations used in plant parasitic nematode control are generally recommended as seed application (Uysal and Özdemir, 2020). Seed applications are an environmentally friendly strategy that promotes productivity and sustainability, and is beneficial to agricultural systems with a knock-on effect (Bayırlı, 2020). It is seen that there are many studies carried out using *B. cereus* on this subject. Oka et al. (1993) found that *B. cereus* has nematicidal activity against *M. javanica* eggs and juvenile; Xiao et al. (2013) stated that *B. cereus* X5 has a biofumigation effect that causes an increase in plant biomass in the roots and rhizosphere and a decrease in the number of *Meloidogyne* sp. and Sela et al. (1998) reported that a collagenolytic/proteolytic enzyme was isolated from *B. cereus*, which is capable of damaging the nematode cuticle and is stable at temperatures between 4–40 °C, which can show activity in a wide pH range (5.4–8.2). Siahpoush et al. (2011) in a study of cucumber roots, they investigated the effects of antagonist bacteria *B. cereus* (1×10^9 cfu/mL) and salicylic acid alone or in combination against *M. javanica*, and determined that its chemical combination with entomopathogen reduced rot in cucumber roots, egg packs and egg numbers. Gao et al. (2016) determined that *B. cereus* strain S2 caused 90.96%, 81.36%, and 67.42% mortality against *M. incognita* in vitro, pot and field trials, respectively; compounds that cause nematicidal effects are defined as C16 sphingosine and phytosphingosine by LC-MS. In the same study, it was also determined that after the application of sphingosine, ROS was stimulated in the intestinal region and disappeared in the genital region in nematodes. Wang et al. (2018) reported a significant decrease in *M. incognita* population and gal index, and an increase of 47.4% in tomato yield when prevaccinated with *B. cereus* BCM2 in tomato compared to the control group. Dawar et al. (2014) found that in their study with *Bacillus* spp., *B. subtilis*, *B. Thuringiensis*, and *B. cereus* species, *M. javanica* juvenile hatches were significantly reduced and gall formation was prevented.

Entomopathogenic fungi and bacteria are successfully used in biological control studies, which are important alternatives in pest control. Although many studies have been conducted on the effects of these bioagents on insects and their effects have been revealed, information on their effects against nematodes needs to be developed with more studies (Liuet al., 2008; Özdemir and Arici, 2021; Yerukalaet al., 2021).

5. Conclusions

Chemical nematicides used for the control of nematodes are not economical when used in large areas as well as the difficulties of application. However, it is of great importance to increase the ratio of alternative methods in pest management in order to reduce the negative effects of the intensive use of chemical pesticides on human and environmental health and natural balance. Biological control is considered a difficult method due to the low efficiency compared to synthetic chemicals and the need for special conditions for their application. For this reason, these difficulties should be eliminated by formulating effective bioagents and their use should be expanded. In addition, the inclusion of biological control agents in integrated pest management (IPM) strategies instead of cultural, physical and chemical control against plant parasitic nematodes is very important for sustainability in agriculture.

It is thought that the isolates used alone cannot be effective as a controlling factor, it is necessary to determine the mixture effects in future studies and to evaluate the results by making field applications.

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