Effect of Mulching Manures and Use of *Heterorhabditis Bacteriophora* on Strawberry Fruit Yield *Temnorhynchus baal* and *Meloidogyne javanica* Under Field Conditions

M.M.M. Abd-Elgawad[1*], L.W. Duncan[2], M.M.A. Hammam[1], F. El-Borai[3] and I.E. Shehata[4]
(1) Plant Pathology Department, National Research Centre, El-Behoos St., Dokki, Giza 12622, Egypt; (2) Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, Florida 33850, USA; (3) Gulf Coast Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 14625 CR 672, Wimauma, Florida 33598, USA; (4) Pests and Plant Protection Department, National Research Centre, Dokki, Giza 12622, Egypt.
[*]Email address of the corresponding author: mahfouzian2000@yahoo.com

Abstract

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The importance of safe methods to manage strawberry pests has become more evident with increasing strawberry production and export in Egypt. Root damage by white grubs (*Temnorhynchus baal* larvae) and root-knot nematode (RKN), *Meloidogyne javanica* cause severe losses to strawberry fruit yield. Growers often rely on a few selective chemical pesticides to control these pests and to minimize pesticide residues in order to comply with maximum residue limits allowed for fruit export. Entomopathogenic nematodes (EPNs) that invade and kill grubs and reduce RKN population levels in the soil may be as effective as these insecticides in some cases. We evaluated the profitability of mulching strawberry with commonly used cow and/or chicken manure, with and without application of commercial *Heterorhabditis bacteriophora* in two farms for four consecutive years. All plots received chemical fertilizers. The designed drip irrigation system for EPN delivery in the farms insured efficient and even ($P < 0.05$) application of EPN in all treatments. Timing of EPN application varied due to import regulatory guidelines. All manure mulch treatments increased strawberry yield each year at a farm in Al-Qalyubia governorate, whereas yield remarkably increased in the fourth season at the farm in El-Beheira governorate. Compared to EPN-treated plots, 70% more ($P = 0.06$) insects were recovered in soil beneath dead plants in plots that did not receive EPNs. *Heterorhabditis bacteriophora* boosted fruit yield ($P < 0.05$) in the second and fourth years at El-Beheira and in all years at Al-Qalyubia. It reduced ($P < 0.05$) numbers of *Meloidogyne javanica* galls/egg masses on strawberry roots and juveniles in the soil in both farms. Mulches had no measurable effect on EPN performance, but suppressed pests and boosted plant productivity. The greatest yield enhancement by EPN occurred in the second year at El-Beheira farm when EPNs were applied shortly after planting (October), enabling early season control of the pests, but in the third year at Al-Qalyubia farm where the weather was generally warmer than that at El-Beheira, and therefore more suitable for EPN activity.
Keywords: Strawberry yield, entomopathogenic nematodes, *Meloidogyne*, mulching, Egypt.

Introduction

Strawberry (*Fragaria* × *ananassa* Duchesne) is a broadly cultivated hybrid species of the genus *Fragaria*. It is planted mainly for its fresh fruit but could globally be used in prepared foods and industrial products. Backed by fertile soils, Mediterranean climate, and geographic location**,** its cultivated area in Egypt has been increasing as a specialty crop. The availability of such cheap and favorable resources can enable low production costs, early fruiting and long harvest season, good quality, and closeness to export markets (Abd-Elgawad, 2019a). Globally, Egypt ranks third in strawberry production (FAO, 2023 (UNDATA)). Moreover, recently imported new varieties from Florida, USA are characterized by outstanding flavor, high early yield, low chilling requirement, and excellent shelf life, which have sparked a wide interest in Egyptian strawberry and created additional markets (El-Borai & Whitaker, 2024).

Yet, numerous strawberry varieties are so susceptible to many types of pathogens, parasites, and pests (El-Shemy *et al.*, 2013). Among them, the strawberry root-grub or white grub, *Temnorhynchus baal* Reiche & Saulcy (Coleoptera: Scarabaeidae) can inflict severe damage to the strawberry crop. The serious phytophagous immature stages (larvae of scarab beetles/white grubs) of *T. baal* initially feed on the strawberry rootlets, causing wilting of the plants and yellowing of the foliage. As white grubs continue to feed on the root systems, symptoms intensify with eventual plant mortality. Thus, they cause severe losses to Egyptian strawberry (Atwa & Hassan 2014; Shamseldean & Atwa, 2004).

Growers and stakeholders usually apply a few selective chemical insecticides to control *T. baal* larvae to avoid pesticide residues and comply with maximum residue limits for fruit export. The significance of safe approaches to manage strawberry pests rises as the strawberry production and export grows in Egypt annually. Voluminous literature currently focus on such approaches to manage plant pests and pathogens given the ecological pollution and negative health effects of synthetic chemical pesticides. These methods may use various tactics and strategies, yet, environmentally friendly materials and techniques need to be further optimized (Abd-Elgawad, 2022a; 2022b). For instance, biopesticides such as entomopathogenic nematodes (EPNs) and other biocontrol agents (BCAs) are frequently less effective and/or more expensive in controlling insect

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pests (Askary & Abd-Elgawad, 2021) and plant-parasitic nematodes (PPNs) (Abd-Elgawad & Askary, 2020) than synthetic chemicals. Therefore, production practices that favor the conservation or superior biocontrol of pests should be realized. Moreover, EPNs can be included in integrated pest management (IPM) programs in various ways that make them complementary or superior to these chemicals; they can exert synergistic or additive effects with other agricultural inputs (Koppenhöfer *et al.*, 2020a; 2020b; Sankaranarayanan & Askary, 2017). In this vein, EPN *Heterorhabditis bacteriophora* Poinar is a natural enemy of white grubs that has much potential for biocontrol (Renkema & Parent, 2021). It is typically applied at 2.5–5×10^9 nematode-infective juveniles (IJs) ha^{-1} as an inundative release to rapidly reduce grub populations in turfgrass (Wilson *et al.,* 2003). Unfortunately, critical methyl bromide is sometimes illegally, but effectively, used against pests especially the grub populations in strawberry nurseries/fields in Egypt (Abd-Elgawad, 2019a). Hence, additional devices and tools for insect control are directly required to develop safe and effective management strategies against *T. baal* and PPNs. This is especially important as applications of EPN IJs can result in not only decreasing arthropod pest populations, but could also reduce PPN abundance (Kepenekci *et al.,* 2018). Hammam *et al.* (2019) found that *H. bacteriophora* not only reduced root-knot nematode (RKN), *Meloidogyne incognita* development parameters but also increased strawberry fruit yield.

This study offers knowledge on whether organic matter, very commonly used as mulches in Egyptian strawberry production, impacted mortality rates of *T. baal* and whether *H. bacteriophora* efficacy was influenced by mulching type. Our objective was also to set up a device in the irrigation lines to distribute suspensions of commercial *H. bacteriophora* equally in the irrigation stream of the commonly used strawberry production system in Egypt. The overall goal is to determine the effects of mulching type with/without *H. bacteriophora* on the strawberry fruit yield and pest populations of *T. baal* and PPNs.

Materials and Methods

Establishment of the experiment

Two farms - famous for strawberry cultivation in Egypt - were selected in the governorates of El-Beheira (Badr Centre, All Green Farm) and Al-Qalyubia (El-Dare Village, Tokh Centre, Abou-Nasr Farm) governorates for 4-consecutive-season experimentation (Table 1). Sandy loam (sand 63.3, silt 16.7, clay 20, OM 1.8%, pH 7.4) soil of All Green Farm and loamy sand (sand 82.8, silt 6, clay 11.2, OM 8.7%, pH 7.8) soil of Abou-Nasr 4.5% (t =8.33, P = 0.004) are known to be naturally infested by *Temnorhynchus baal* (Shehata *et al.* 2019; 2020). Different plots in each season were prepared for cultivation, plowing, chemical fertilization, bed setting, and irrigation as well as other cultural practices during the growing season according to El-Shemy *et al.* (2013). To facilitate mulching, three adjacent beds were used for each manure type or untreated check. Bed raising and transplanting were done after mulching the soil with three manure types; chicken (poultry) manure, cow (cattle) manure, or both (chicken + cow) together at a broadcast equivalent rate of 18, 36, or (9 + 18) m^3/Faddan (= 4200 m^2), respectively. An untreated (un-mulched) check was left as a control. Similar four treatments were set up and used for applying commercial *Heterorhabditis bacteriophora*, a total of eight treatments. This nematode species was imported from BASF company (United Kingdom) under product name ‘Nemasys G’ and had disorders in the delivery times due to the Corona pandemic limitations and import quarantine, leading to difference in the number of viable EPN counted before application and the application dates/times. One-meter-wide beds for strawberry cultivation were raised about 45 cm higher than the surrounding soil surface, spaced 0.5 meter apart, and equipped with two longitudinal plastic tubes to irrigate via drippers 4 rows of 0.2-metre-spaced plants. The strawberry transplanting was made 20 days after mulching annually. A device was set up to evenly distribute *H. bacteriophora* via the drip irrigation system. The irrigation tank containing the nematodes was connected to irrigation lines set up at 90-degree angles to help in the even distribution of the nematodes to the two lines (tubes) running in parallel through each EPN-treated bed simultaneously (Figure 1). Meanwhile, non-treated beds were uniformly drip irrigated from another tank with water only. Because all freshly transplanted strawberry was irrigated by both overhead sprinklers and soil drippers to provide plants with optimal water requirements. Transparent polyethylene plastic was used to cover the beds around 35 days after transplanting, with a hole made for each plant to exit from the plastic cover. During this period, aerial irrigation was gradually reduced with increased irrigation via soil drippers. Thereafter, plants mulched by the plastic were irrigated via the drippers only until season-end. Non-treated plots and plots treated with *H. bacteriophora* received similar all other agricultural practices. According to the transplanting date, the grown variety and the environmental conditions, collection of strawberry fruits began at different times in November/December of each season and continued at an average rate of 2-3 times weekly until harvest-end (Table 1). The accumulated yield of each replicate (bed) was recorded throughout the collection period of strawberry fruits.

Sample collection

Just before the end of the fourth season, soil and root samples were collected from each treatment of both farms to assess the presence of plant-parasitic nematodes. Four rhizosphere soil and root subsamples, from four random plants at each bed (replicate), were taken with a hand trowel (ca 8 cm diameter × 20 cm long), mixed and composited into a single composite sample of about 1 kg (about 2 grams of fibrous roots/plant or 8 grams per sample). Consequently, three composite samples, from 12 plants, were taken from each treatment. Each sample was thoroughly mixed, bagged, labeled and taken to the laboratory in ice box for nematode analyses. From each sample, an aliquot of 250 g soil was processed by sieving and decanting methods for nematode extraction (Barker, 1985). The numbers of PPNs in soil were counted using Hawksley slide under light microscope (Abd-Elgawad, 2021a). Fibrous roots from each sample were gently washed free of soil and an aliquot of one g roots per sample was considered to count RKN galls (Hammam *et al.*, 2019). Other one g root samples were stained in 0.015% Phloxine B solution for 20 min before removing the residual Phloxine B to count egg masses (Daykin & Hussey, 1985). Previous identification of RKN species isolated from the same area, via perineal patterns morphology and biochemical esterase phenotype as *Meloidogyne javanica* (Hammam *et al.*, 2022) was adopted.

To maximize the application efficiency of EPN in strawberry fields, we have investigated their delivery through drip irrigation. Five minutes after the onset of EPN application, EPN suspensions (irrigation water) were collected in plastic bags and IJs were counted from three consecutive, but not adjacent, individual drippers for 10 minutes in each EPN treated bed. Each of the 3 drippers (a replicate) in a single line were at a distance of 1.25, 8.25 and 15.25 meter from the nematode tank. As the three drippers have different distances from the EPN tank, our aim was to examine whether there is a trend of increased recovery of EPN with distance from the tank. Three replicates (beds) were examined per each of the four mulching treatments on 19 and 25 December 2018 for All Green (El-Beheira) and Abou-Nasr (Al-Qalyubia) farms, respectively.

Statistical analyses

The paired *t*-test was conducted to test the difference in fruit yield between non-treated plots and plots treated with *H. bacteriophora* including mulching treatments for each farm in each of the four seasons. The absolute yield values were used as they are affected not only by all treatments but also by field conditions. In Abou-Nasr Farm, the large experimental plots set up in 2019/2020 (Table 1) were used to record the monthly (Middle October 2019 - Middle May 2020) relationship between the number of dead plants per bed and the number of the white grubs (*T. baal* larvae) around their roots in each of the eight treatments. To reduce the damage caused by the digging needed to look for the white grubs, we dug carefully only sites of dead plants found in half the length of a bed (replicate), i.e. specific sites in 25-meter-length beds were dug to about 30 cm deep. The number of dead plants was regressed against the number of *T. baal* larvae for data sets in various scenarios. To circumvent the small difference in the number of plants between the different beds as a result of the absence or death of some plants at an early stage, insect numbers were not taken as absolute values. For each strawberry-planted bed, the number of insects was divided by the numbers of the total plants in the bed. The resulting numbers of insects/plant were averaged across the beds (replicates) for each treatment. The resulting averages were used in the paired *t*-test to compare EPN treated with non-treated sections of the mulching treatments (n = 4). Root-knot nematode counts were subjected to analysis of variance and averages of each nematode developmental stage (soil juveniles, galls, and egg masses) were compared using Duncan's new multiple range test.

Results

The strawberry fruit yields were massively different between years in both farms (Tables 2 and 3). Mulching with either or both manures increased strawberry fruit yield at Al-Qalyubia farm whether with or without EPN additions (Table 2). This increase was the highest when both manures were applied together with or without EPN additions. Such inclusive and positive mulch effects were so apparent on the farm of El-Beheira governorate in the fourth season only (Tables 3 and 4). When the strawberry fruit yield was graded into export and local consumption, mulching with either or both manures generally boosted the export yield at El-Beheira farm especially in the fourth season with/without EPNs (Table 4).

Table 1. Details related to two strawberry farms in Egypt where infective juveniles (IJs) of *Heterorhabditis bacteriophora* were applied for four growing seasons.

	Growing season at Al-Qalyubia governorate				**El-Beheira governorate**			
Description	**2018/19**	**2019/20**	**2020/21**	**2021/22**	**2018/19**	**2019/20**	**2020/21**	**2021/22**
Transplant date	30/9/2018	9/9/2019	4/10/2020	7/10/2022	18/10/2018	10/10/2019	18/9/2020	28/9/2022
Harvest-end date	4/6/2019	20/6/2020	2/3/2021	30/6/2022	12/5/2019	13/5/2020	25/5/2021	20/6/2022
Variety	Festival	Festival	Festival	Festival	Red Merlin	Red Row	Red Row	K13, Red Cleo, Sensation
No. of replicates per treatment × length beds (m)	3×25	3×50	3×50	3×50	4×25	3×25	3×25	3×65
Times EPN used	Thrice	Once	Once	Once	Twice	Once	Once	Once
Application dates	25 Dec; 13 Mar & 16 April	22 Oct, 2019	14 Jan, 2021	1st Dec	19 Dec, 13 March	22 Oct	14 Jan	1st Dec
Number IJs m^2 of a bed	250000 each time	148,800	123,000	150,000	333,333 each time	208,335	164,000	150,000
Fruit’s use	Local	Local	Local	Local	Local& Export	Local & Export	Local & Export	Local & Export

Figure 1. Oxygen gas tube pushes the oxygen to transfer nematode suspension from a nearby tank into the main irrigation tubes (upper), and a connection set up at 90-degree angles can help in the even distribution of the nematodes to the lines/tubes with drippers (lower).

The nematode-infective juvenile concentration or rate (Table 1) range was 12.3 to 33.3, with average 20.5 IJs cm^{-2} in the six field trials (2 farms × 3 seasons). Nematode application across all mulching treatments increased strawberry fruit yield compared to no EPN in each of the four years at Al-Qalyubia farm and in the last three years at El-Beheira farm. The highest improvement was in the third year at Al-Qalyubia (Table 2) and in the second year at El-Beheira farm (Tables 3 and 4). The averages of strawberry fruit yield increase across all EPN-treated plots in years 1, 2, 3, and 4 were -2.0, 11.34, 2.1, and 4.45% in El-Beheira farm and 6.72, 9.55, 174.94 and 21.57% in Al-Qalyubia farm, respectively. Paired *t*-test revealed that *Heterorhabditis bacteriophora* increased yield significantly ($P < 0.05$) in each of the four years at Al-Qalyubia farm (Figures 2 and 4) and only in the second and fourth years at El-Beheira (Figures 3 and 4). Yield changes in EPN-treated plots relative to EPN-free treatments were not significant ($P > 0.05$) at El-Beheira in the first and third years (Figure 3). Yet, EPN application across all treatments significantly ($P = 0.001$, $n = 24$) enhanced local and export strawberry fruit yield compared to no EPN application in the first three years at El-Beheira farm. When EPN application rate was equal in both farms (Figure 4), strawberry yield increase scored 21.6% in Al-Qalyubia (t = 2.353, P = 0.032), and 4.5% in El-Beheira (t =8.33 P=0.004) in the fourth growing season.

The percentages of dead plants in EPN treatments with no chicken, cow, or both manures in the second season at Al-Qalyubia farm were 18.4, 16.6, 18.8, and 33.9%. The corresponding percentages of dead plants in EPN-free treatments were 24.5, 19.5, 25.5, and 39.3%. Thus, these percentages of dead plants in EPN treatments were less than that in EPN-free treatments for each mulching treatment. The mean number of white grubs (*T. baal* larvae) per plant was fewer in beds treated with EPNs than that in EPNs-free beds for each kind of the mulching treatments (n = 4); overall mean ± standard error was 0.0871 ± 0.0106 in EPN-treated beds versus 0.1505 ± 0.0306 in EPNs-free beds using paired t-test (t = 2.64, P = 0.077). The expected correlations between numbers of scarab grubs *T. baal* larvae and their corresponding strawberry dead plants under all mulching scenarios whether with or without *H. bacteriophora* application were found to be highly significant (Table 5).

The effect of different mulching treatments with/without applying *H. bacteriophora* on population densities of *Meloidogyne javanica* infecting strawberry roots at the two farms was variable (Table 6). Generally, *H. bacteriophora*-treated plots had fewer ($P < 0.05$) numbers of *M. javanica* second-stage juveniles (J2) in soil as well as galls and egg masses on strawberry roots than those at *H. bacteriophora*-free plots. Such differences were more pronounced in the three *M. javanica* population parameters at All Green farm than those in Al-Qalyubia farm. Based on counting the EPNs delivered by the drippers, dripper-1 released more IJs than dripper-3 in Al-Qalyubia (P= 0.006, F=13.47, df = 2) and El-Beheira (P= 0.026, F=7.134, df = 2) (Table 7). However, there was no significant difference among the four mulching treatments in nematode numbers released from the drippers in Al-Qalyubia (P = 0.688, F= 0.512, df = 3) or El-Beheira (P = 0.506, F = 0.872, df = 3).

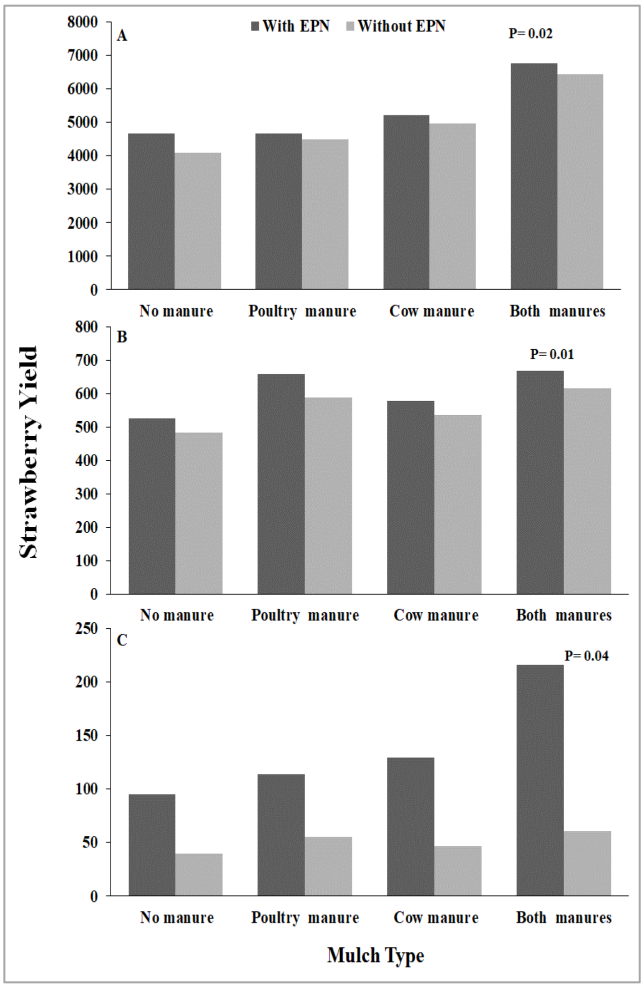

Figure. 2 Probability levels of paired t-test for the difference in fruit yield between non-treated plots and plots treated with *Heterorhabditis bacteriophora* including mulching treatments for Al-Qalyubia farm in A: the first season, B: second season, C: third season.

Table 2. Strawberry fruit yield as influenced by different mulching treatments with/without applying entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* at Abou-Nasr Farm, Al-Qalyubia, Egypt in four seasons.

Treatment	Growing season 2018/2019	% Increase	Growing season 2019/2020	% Increase	Growing season 2020/2021	% Increase	Growing season 2021/2022	% Increase
EPN + cow and chicken manures	6773	44.94	669	27.2	216	127.4	316	15.8
EPN + cow manure	5210	11.5	579	10.1	129	35.8	309	13.2
EPN + chicken manure	4673	0.0	658	25.1	114	20.0	353	29.3
EPN only	4673	-	526	-	95	-	273	-
Cow and chicken manures	6428	56.9	615	27.4	60.5	53.2	305	43.2
Cow manure	4963	21.1	535	10.8	46.5	17.7	262	23
Chicken manures	4498	9.8	587	21.5	55	39.2	249	16.9
Untreated check	4097	-	483	-	39.5	-	213	-

Each value is the total yield of bed numbers defined in Table 1. % Increase = % increase of mulching treatments relative to the non-mulched check (EPN only or untreated check).

Table 3. Strawberry fruit yield as influenced by different mulching treatments with/without applying entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* at All Green Farm, El-Beheira, Egypt in four seasons.

Seasons	With EPN				Without EPN			
	Control	Poultry	Cattle	Both manures	Control	Poultry	Cattle	Both Manures
First	277184	266040	278489	248896	278947	269859	283087	260638
Second	119731	121164	119154	121761	107419	108755	107139	109436
Third	479415	496326	487740	491540	478315	478916	482295	475430
Fourth	677.25	986.3	1172.3	1031.5	635.4	959.1	1122.47	985.5

Each value is the total yield of bed numbers defined in Table 1.

Table 4. Averages of strawberry fruit yield as influenced by different mulching treatments with/without applying entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* at All Green Farm, El-Beheira, Egypt in four growing seasons.

Treatment (manure)	2018/2019				2019/2020				2020/2021				2021/2022			
	Local	% Inc.	Export	% Inc.	Local	% Inc.	Export	% Inc.	Local	% Inc.	Export	% Inc.	Local	% Inc.	Export	% Inc.
EPN + cow and chicken manures	125751	-21.7	123145	5.7	71704	1.82	50057	1.5	392520	2.5	99020	2.8	689.10	22.5	342.40	198.3
EPN + cow manure	132964	-17.2	145525	24.9	70076	-0.49	49078	-0.47	389950	1.8	97790	1.5	942.39	67.6	229.91	100.3
EPN + chicken manure	139450	-13.2	126590	8.6	71214	1.13	49950	1.3	398407	4.0	97919	1.6	670.20	19.2	316.10	175.3
EPN only	160631	-	116553	-	70421	-	49310	-	383055	-	96360	-	562.45	-	114.8	-
Cow and chicken manures	140006	-12.5	120632	1.4	64216	1.9	45220	1.9	378940	-1.4	96490	2.5	662.05	24.9	323.45	207.5
Cow manure	137308	-14.2	145779	22.5	62729	- 0.5	44410	0.07	387705	0.91	94590	0.52	908.86	71.4	213.61	103.1
Chicken manures	134830	-15.7	135029	13.5	63687	1.0	45068	1.6	392361	2.12	86555	-8.02	652.20	23	306.90	191.7
Untreated check	159977	-	118970	-	63040	-	44379	-	384210	-	94105	-	530.2	-	105.2	-

Each value is the total yield of bed numbers defined in Table 1. % Inc.= % increase of mulching treatments relative to the non-mulched check.

Table 5. Correlation equations when numbers of dead plants were regressed against numbers of observed grubs for various scenarios of mulching Egyptian strawberry field with or without applying entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora.*

Serial No.	Equation	Description	N	r^*
1	$Y= 1.51 x + 44.74$	8 treatments (with/without EPN) × 3 reps across eight months	24	0.829
2	$Y= 1.26 x + 58.41$	4 treatments without EPN × 3 reps across eight months	12	0.932
3	$Y= 3.67 x +59.14$	4 treatments with EPN × 3 reps across eight months	12	0.927
4	$Y = 1.13 x + 11.2$	8 months × 3 reps of a treatment (cow and chicken manure)	24	0.928
5	$Y = 2.16 x + 6.12$	8 months × 3 reps of a treatment (cow and chicken + EPN)	24	0.853
6	$Y = 1.69 x +4.92$	8 months × 3 reps of a treatment (chicken manure)	24	0.887
7	$Y = 1.35 x +5.22$	8 months × 3 reps of a treatment (chicken manure + EPN)	24	0.809
8	$Y = 1.39 x + 3.93$	8 months × 3 reps of a treatment (cow manure)	24	0.941
9	$Y = 1.46 x + 4.56$	8 months × 3 reps of a treatment (cow manure + EPN)	24	0.862
10	$Y = 0.95 x +9.17$	8 months × 3 reps of a treatment (non-mulched beds)	24	0.786
11	$Y = 1.61 x +4.5$	8 months × 3 reps of a treatment (non-mulched beds + EPN)	24	0.856

N = number of plotted points, reps = number of replicates, EPN = entomopathogenic nematodes, r = Correlation coefficient for fit to the equation, * = Significant at $P \leq 0.01$.

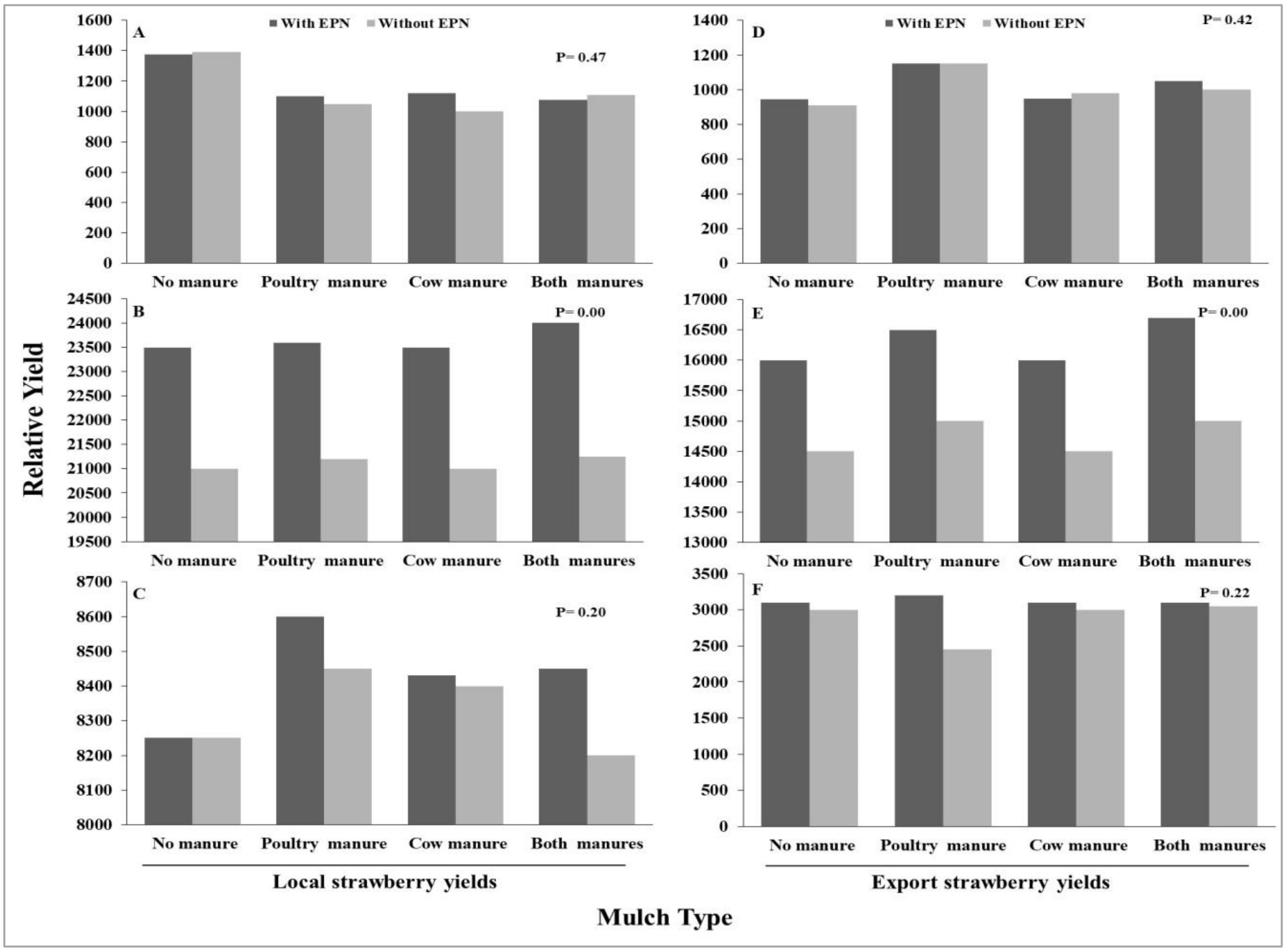

Figure. 3 Probability levels of paired *t*-test for the difference in fruit yield between non-treated plots and plots treated with *Heterorhabditis bacteriophora* including mulching treatments for El-Beheira farm in the first season (A and D), second season (B and E), third season (C and F).

Table 6. Effect of different mulching treatments with/without applying entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* on numbers of second-stage juveniles (J2), galls, and egg masses of *Meloidogyne javanica* infecting strawberry roots at two farms in Egypt.

Treatments	*M. javanica* J2 in 250 g soil	Galls in 1 g fibrous roots	Egg masses in 1 gram root
All Green (El-Beheira) Farm			
Control without EPN	2903.8 a	150.0 a	93.0 a
Control with EPN	2030.0 c	123.0 bc	63.0 bc
Poultry without EPN	2099.0 b	119.0 bc	59.5 bc
Poultry with EPN	1700.0 b	146.8 a	84.0 a
Cattle without EPN	1368.4 d	113.0 c	52.6 bc
Cattle with EPN	1066.0 de	140.0 ab	66.88 b
Both manures without EPN	911.4 e	116.0 c	56.4 bc
Both manures with EPN	820.0 e	102.0 c	48.6 c
Al-Qalyubia Farm			
Control without EPN	852.3 a	54.0 ab	34.6 a
Control with EPN	577.5 b	69.6 a	33.0 a
Poultry without EPN	597.5 b	36.8 c	19.38 b
Poultry with EPN	625.63 b	41.0 c	17.0 b
Cattle without EPN	514.00 b	49.0 bc	26.0 ab
Cattle with EPN	263.00 c	52.5 ab	21.0 b
Both manures without EPN	421.13 bc	44.0 bc	19.0 b
Both manures with EPN	456.38 bc	61.8 ab	32.4 a

*Averages followed by the same letters in the same column are not significantly different at P=0.05 according to Duncan's new multiple range test.

Table 7. Average numbers of *Heterorhabditis bacteriophora*-infective juveniles emerging from three consecutive drippers (Dripper-1, Dripper-2, and Dripper-3) in single lines at a distance of 1.25, 8.25 and 15.25 m from the nematode tank, respectively, for each of four mulch treatments*

Drippers	Location	Dripper-1	Dripper-2	Dripper-3
	Al-Qalyubia	206.5 A	151 B	130.5 B
	El-Beheira	458 A	314.4 AB	250.78 B
Treatments	Cow manure	Poultry manure	Both manures	No manure
Al-Qalyubia	166.4	149.6	165.5	169.2
El-Beheira	291.4	319.2	378.8	374.9

*Means in a row sharing a common letter are not significantly (P < 0.05) different according to Duncan's New Multiple Range Test (means of three replicates). Mulching treatments included average number of released nematodes using the total of the three drippers per each treatment; i.e. no. nematodes/dripper in each treatment (cow and/or chicken manure in addition to untreated check).

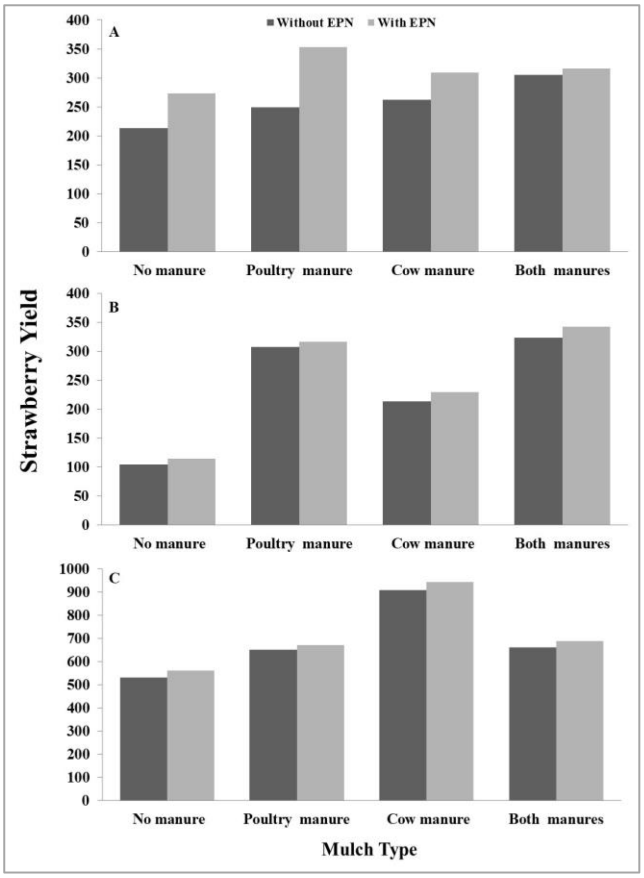

Figure 4. Strawberry yields (kg) for non-treated plots and plots treated with *Heterorhabditis bacteriophora* including mulching treatments in the fourth season. In A: Al-Qalyubia (Abou-Nasr farm), in B: El-Beheira (All Green export farm) and C: El-Beheira (All Green local farm). *H. bacteriophora*–treated plots scored 21.6% (t = 2.353, P = 0.032) and 4.5% (t =8.33, P = 0.004) strawberry yield increase relative to EPN-free plots, respectively.

Discussion

It is clear that each kind of mulching improved yield every year at Abou-Nasr farm, Al-Qalyubia, but did not have the same effect at All Green farm, El-Beheira. This could be due to the assumption that the plants at Abou-Nasr farm did not have enough nutrients from the applied and recommended chemical fertilizers (El-Shemy *et al.,* 2013), and so they received them from the mulch. If so, the routine fertilizers gave plants enough nutrients at All Green farm, and therefore the mulches were not necessary. However, the benefits shown by mulching of well-composted animal manure in both farms are probably due to other benefits provided by the mulch such as water conservation, soil structure improvement, and enhanced nutrient retention. Furthermore, strawberry fruit yield in plots with cow and chicken manures plus *H. bacteriophora* was superior to other mulching treatments in each of the four years at Abou-Nasr farm (Table 2). This superiority of cow and chicken manures plus *H. bacteriophora* compared to other treatments was also apparent at All Green farm in the first and second year out of the four seasons for local and export strawberry fruit yield, respectively (Table 4). Nevertheless, other factors may dictate such differences in mulch effects between the two farms, e.g. the texture and chemical composition of the soil, needs of nutrients for different strawberry varieties, and the interaction of existing soil organisms with manure components. Additionally, the difference in fruit yield among seasons and farms is probably due to many factors such as differences in climate, EPN application dates/times, and variation in severity of other diseases (Anonymous 2021; El-Shemy *et al.*, 2013).

It is apparent that *Heterorhabditis bacteriophora* remarkably increased strawberry fruit yield in each of the four years (seasons) at Abou-Nasr farm (Figures 2 and 4). Yet, such a significant (P < 0.05) increase in yield occurred only in the second and fourth years at All Green farm (Figures 3 and 4). In fact, the yield was so different each year due to biotic/abiotic factors which prevented us from benefiting from summing the yields of the years together for each EPN-mulch type combination. Even when the IJ rate used at both farms was the same, 15 IJs cm^{-2}, in the fourth year, we compared yield increase due to EPN application at both farms (Figure 4) where EPN boosted strawberry yield by 21.6% in Al-Qalyubia (P = 0.032), and only 4.5% in El-Beheira (P = 0.004). Nonetheless, the numbers of the white grubs per plant were consistently fewer in strawberry beds treated with EPNs than that in EPNs-free beds for each of the mulching types with P = 0.077, a probability level which is considered to be not quite statistically significant. In other words, 70% more (P = 0.06) insects were recovered in soil beneath dead plants in plots that did not receive EPN relative to EPN-treated plots. Moreover, the percentages of dead plants in each EPN treatment were less than that in EPN-free treatment for each mulching treatment, which may reflect the significant role of *H. bacteriophora*. It is interesting to note that the most important yield enhancement due to EPNs was in the 2nd year at All Green farm (El-Beheira) when they were applied in October, which is likely due to several factors. Contrary to EPN application in winter (December or January), the rhizosphere soil is warm in autumn (October) and suitable for nematode activity. Moreover, early larval instars, which are more susceptible than late ones and adults may be available at this time (Atwa, 2003). More *H. bacteriophora*-induced mortalities were recorded for the early *T. baal* larval instars than for the late ones (Atwa, 2009). In addition, early EPN application shortly after planting (October) could enable early season control of the newly hatched insect larvae. Therefore, this EPN application time may demonstrate the benefits of *H. bacteriophora*-induced mortality in *T. baal* larvae that kill the plants. On the other hand, the most remarkable yield increase due to EPNs was in the 3rd year at Al-Qalyubia governorate, where the weather was generally warmer than that at El-Beheira governorate and therefore more suitable for EPN biocontrol activity. Such yield increase should be further analyzed in a feasibility study (Anonymous, 2021).

Admittedly, many biotic and abiotic factors can affect EPN persistence and behavior especially in attacking and killing their host insects. Such factors have probably caused these variations in the nematode efficacy. For example, soil moisture and texture (Duncan *et al.*, 2013), salinity (Nielsen *et al.*, 2011), mulching (Hussaini, 2017), and pH (Campos-Herrera *et al.*, 2013) were found to modulate EPN populations directly or indirectly by influencing their host pests or enemies (Campos-Herrera *et al.*, 2019). *Heterorhabditis bacteriophora* capability for *Galleria mellonella* larval infection and reproduction ranged from 72 to 26%, one and five months, respectively, after field inoculation of the infected *G. mellonella* larvae into the strawberry rhizosphere in the field (Shehata *et al.*, 2020). These authors found that the number of IJs, moved to the North of the rhizosphere, was significantly ($P \leq 0.05$) less than that moved to the South or the East as reflected by the numbers of infected insects. Thus, it is possible that the angle of sun rays on the strawberry plant row caused tiny temperature effects. Recently, Renkema & Parent (2021) attributed lower than optimal temperatures for *H. bacteriophora* and *Steinernema scarabaei* infectivity as the likely cause of minimal effects on the Japanese beetle *Popillia japonica* larvae. *Heterorhabditis bacteriophora* was moderately to not-at-all effective against third-instar *P. japonica* when used in autumn at mean soil temperatures less than 15°C (Marianelli *et al.*, 2017; Simões *et al.*, 1993). Therefore, factors that may have a marked influence on EPN foraging behavior, such as persistence, and movement direction, should be further investigated for better harnessing of EPNs as biological pest control agents.

Introducing EPN strategy for managing *T. baal* in strawberry has shown promising results on limited scale (Atwa & Hassan, 2014; Shamseldean & Atwa, 2004; Shehata *et al.*, 2019; 2020). Clearly, favorable shifting of growers from reliance on chemical insecticide applications when *T. baal* larvae can inflict severe losses in strawberry fruit yield to sustainable methods that also target these larvae will require continued large-scale research as herein.

On the other hand, in strawberry fields where root-feeding larvae is a concern, mulching these organic manures pre-transplanting in late autumn followed by an application of *H. bacteriophora* or even other superior EPN species/strains (Adly *et al.*, 2022; Atwa & Hassan, 2014) at the appropriate time and conditions for its activity and when *T. baal* larvae are already in the soil should offer excellent control. The present study may support the assumption that EPN application can offer such appropriate EPN application time as *H. bacteriophora* demonstrated its most favorable effects in October at All Green (El-Beheira) and in January at Abou-Nasr (Al-Qalyubia) farms. In further experimentations, oviposition of *T. baal* adults in mulches/strawberry soil and movement of the hatched larvae to roots will need to be estimated to define the prospects and exact impact of an EPN application time under well-defined biotic/abiotic factors. Once strawberries are cultivated, bedsides and middles with growing grasses are likely to harbor most of the larval *T. baal* population. These sites can be tilled/hand hoeing to kill larvae and successfully decrease *T. baal* populations. Tillage proved to be effective against white grubs (Hussaini, 2017; Szendrei *et al.*, 2005). However, in cases where tillage is not adequate, a strategy for and the economics of applying EPN should be investigated as suggested by Renkema & Parent (2021) against another scarab grub species.

Based on the dripper trials, it can be concluded that the irrigation system for EPN delivery in strawberry farms designed in this study could efficiently and evenly ($P > 0.05$) apply EPN among treatments but not among drippers of single tubes. In contrast, a similar irrigation system of Florida blueberry could efficiently apply EPN via non-significant ($P > 0.05$) differences among drippers of such a single line (Duncan & Abd-Elgawad, 2022). Further studies with more replications are warranted to define the efficacy of different EPN species in the various mulch materials and drippers to determine if EPNs can provide even control of *T. baal* at different drippers' locations.

The longstanding admission that EPNs can offer super arthropod pest management under definite conditions remains mostly unexploited by growers for two main reasons, cost and efficacy (Askary & Abd-Elgawad, 2021). Although the EPN doses herein were ≥ standard ones in two trials, an average of sub-optimal dose was used across the four seasons of both farms to reduce costs. However, we should consider that the cost prices change from year to year, and EPN costs may be lowered with further cost-effective technologies. These latter may address not only less recognized issues of EPNs (Abd-Elgawad, 2019b) but also emerging techniques in EPN production (Shapiro-Ilan *et al.*, 2022), formulation (Askary & Ahmad, 2017; Koppenhöfer *et al.;* 2020a; 2020b), and application (Abd-Elgawad, 2022a). Moreover, applications of EPN IJs are reported to decrease PPN populations in Egypt (Abd-Elgawad, 2017) and elsewhere (Kepenekci *et al.*, 2018).

Hence, the latter authors implied that a relevant feasible study underestimated the value of applying *H. bacteriophora* as it did not include its beneficial effects on RKNs; *M. javanica* herein. Therefore, EPNs should be included among safe techniques to optimize PPN control (Abd-Elgawad, 2021b).

It can be concluded from this study that the three types of manure mulching used could serve not only for greater plant productivity but also to suppress *T. baal* and *M. javanica* populations. Interestingly, manure mulching showed no measurable effect on EPN biocontrol activity, thereby EPN could decrease chemical pesticides utilized against soil pests. Overall, while mulching should help in suppressing *T. baal* populations in strawberry, applying *H. bacteriophora* when biotic and abiotic factors are optimal to the nematode could provide favorable control of both *T. baal* and *M. javanica* populations.

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الملخص

عبد الجواد، محفوظ محمد مصطفى، لاري وايني دانكن، مصطفى محمد عطية محمد همام، فهيم البرعي قورة وابراهيم السيد شحاتة. 2024. تأثير الأسمدة العضوية المغطاة واستخدام *Heterorhabditis bacteriophora* على محصول الفريز/الفراولة وحشرة الجعال (*Temnorhynchus baal*) ونيماتودا تعقد الجذور (*Meloidogyne javanica*) تحت الظروف الحقلية. مجلة وقاية النبات العربية، 42(3): 306–317.
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أصبحت أهمية استخدام الطرائق الآمنة لمكافحة آفات الفريز/الفراولة أكثر وضوحاً مع زيادة إنتاج الفريز/الفراولة وتصديره في مصر. يتسبب تلف الجذور بواسطة يرقات الجعال (*Temnorhynchus baal*) ونيماتودا تعقد الجذور (*Meloidogyne javanica*) في حدوث خسائر فادحة في محصول ثمار الفريز/الفراولة. يعتمد المزارعون في كثير من الأحيان على عدد قليل من المبيدات الكيميائية الانتقائية للسيطرة على هذه الآفات، وتقليل بقايا هذه المبيدات من أجل الامتثال للحدود القصوى لمخلفات التصدير. قد تكون الديدان الخيطية الممرضة للحشرات (EPN) التي تغزو وتقتل يرقات الجعال وتقلل من مستويات تجمعات نيماتودا تعقد الجذور في التربة فعالة مثل المبيدات الحشرية في بعض الحالات. هدف البحث إلى تقييم ربحية تغطية الفراولة باستخدام روث البقر و/أو زَرَق الدجاج شائعي الاستخدام، مع أو بدون استخدام مركب تجاري من نيماتودا الحشرات *Heterorhabditis bacteriophora* في مزرعتين، ولمدة أربع سنوات موسمية متتالية. عوملت جميع قطع الأراضي بهذه الأسمدة. أمكن لنظام الري بالتنقيط المصمم لدينا لتوصيل EPN في المزارع أن يستخدم الديدان الخيطية الممرضة للحشرات بين المعالجات بكفاءة ولكن مع اختلاف توقيت تطبيق نيماتودا الحشرات بسبب إرشادات تنظيم الاستيراد. أظهرت النتائج أن جميع معاملات السماد أدت إلى زيادة إنتاجية الفريز/الفراولة سنوياً في مزرعة بمحافظة القليوبية، بينما زاد الإنتاج بشكل ملحوظ في الموسم الرابع بمزرعة محافظة البحيرة. بالمقارنة مع الخطوط المعالجة بنيماتودا الحشرات. كان عدد يرقات الجعال أعلى بنسبة تزيد عن 70% ($P = 0.06$) تحت النباتات الميتة في تربة الخطوط التي لم تتلق معالجة بنيماتودا الحشرات. أدت المعاملة بنيماتودا الحشرات إلى زيادة إنتاجية الثمار ($P <0.05$) في السنتين الثانية والرابعة في البحيرة وفي جميع السنوات في القليوبية. كما أدت إلى إنخفاض ($P <0.05$) في أعداد العقد الجذرية وكتل البيض على جذور الفريز/الفراولة والأطوار المعدية في التربة للنيماتودا المتطفلة نباتياً *Meloidogyne javanica* في كلا المزرعتين. كان للغطاء البلاستيكي تأثير ملموس في السيطرة على الآفات وتعزيز إنتاجية النبات. سجّلت أكبر زيادة في إنتاجية الفريز/الفراولة في السنة الثانية في مزرعة البحيرة عندما تم تطبيق نيماتودا الحشرات بعد وقت قصير من الزراعة (تشرين الأول/أكتوبر)، مما أتاح السيطرة على الآفات في بداية الموسم، بينما تحققت أكبر زيادة في إنتاجية الفريز/الفراولة في مزرعة القليوبية في السنة الثالثة حيث كان الطقس أكثر ملائمة لنيماتودا الحشرات مقارنة بطقس البحيرة.

كلمات مفتاحية: محصول الفريز/الفراولة، النيماتودا الممرضة للحشرات، *Meloidogyne*، التغطية، مصر.

عناوين الباحثين: محفوظ محمد مصطفى عبد الجواد[1*]، لاري وايني دانكن[2]، مصطفى محمد عطية محمد همام[1]، فهيم البرعي قورة[3] وابراهيم السيد شحاتة[1]. (1) قسم أمراض النبات، معهد البحوث الزراعية والبيولوجية، المركز القومي للبحوث، مصر؛ (2) مركز أبحاث وعلوم الحمضيات، معهد علوم الأغذية والزراعة، جامعة فلوريدا، أمريكا؛ (3) مركز أبحاث وتعليم ساحل الخليج، معهد علوم الأغذية والزراعة، جامعة فلوريدا، أمريكا. *البريد الالكتروني للباحث المراسل: mahfouzian2000@yahoo.com

References

Abd-Elgawad, M.M.M. 2017. Status of entomopathogenic nematodes in integrated pest management strategies in Egypt. Pp. 473-501. In*:* Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. M.M.M. Abd-Elgawad, T.H. Askary and J. Coupland (eds.). CAB International, Wallingford, UK.

Abd-Elgawad, M.M.M. 2019a. Plant-parasitic nematodes of strawberry in Egypt: A review. Bulletin of the National Research Centre, 43:7. https://doi.org/10.1186/s42269-019-0049-2

Abd-Elgawad, M.M.M. 2019b. Towards optimization of entomopathogenic nematodes for more service in the biological control of insect pests. Egyptian Journal of Biological Pest Control, 29:77. https://doi.org/10.1186/s41938-019-0181-1

Abd-Elgawad, M.M.M. 2021a. Optimizing sampling and extraction methods for plant-parasitic and entomopathogenic nematodes. Plants, 10(4):629. https://doi.org/10.3390/plants10040629

Abd-Elgawad, M.M.M. 2021b. Optimizing safe approaches to manage plant-parasitic nematodes. Plants, 10(9):1911. https://doi.org/10.3390/plants10091911

Abd-Elgawad, M.M.M. 2022a. Challenges in field application of biopesticides for successful crop pest management. Pp. 331-366. In*:* Pest management: Methods, applications and challenges. T. Askary (ed.). Nova Science Publishers, Inc., New York, USA. https://doi.org/10.52305/VCWF8745

Abd-Elgawad, M.M.M. 2022b. Understanding molecular plant-nematode interactions to develop alternative approaches for nematode control. Plants, 11(16):2141. https://doi.org/10.3390/plants11162141

Abd-Elgawad, M.M.M. and T.H. Askary 2020. Factors affecting success of biological agents used in controlling plant-parasitic nematodes. Egyptian Journal of Biological Pest Control, 30:17. https://doi.org/10.1186/s41938-020-00215-2

Adly, D, G.M. Nouh and A.E. Eid. 2022. Application of entomopathogenic nematodes to control larvae of *Temnorhynchus baal* (Reiche and Saulcy) under laboratory conditions and in strawberry fields. Egyptian Academic Journal of Biological Sciences (A. Entomology), 15(3):67-77. https://doi.org/10.21608/eajbsa.2022.256736

Anonymous. 2021. Climate change knowledge portal: Egypt. The World Bank Group. Available at: https://climateknowledgeportal.worldbank.org/country/egypt/climate-data-historical (accessed 7 January 2022).

Askary, T.H. and M.J. Ahmad. 2017. Entomopathogenic Nematodes: mass production, formulation and application. Pages 261-286. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. M.M.M. Abd-Elgawad, T.H. Askary and J. Coupland (eds.). CAB International, Wallingford, UK.

Askary, T.H. and M.M.M. Abd-Elgawad. 2021. Opportunities and challenges of entomopathogenic nematodes as biocontrol agents in their tripartite interactions. Egyptian Journal of Biological Pest Control, 31:42. https://doi.org/10.1186/s41938-021-00391-9

Atwa, A.A. 2003. Identification, mass culture, and utilization of entomopathogenic nematodes against insect pests. Ph.D. thesis, Faculty of Agriculture, University of Cairo, Giza, Egypt. 172 pp.

Atwa, A.A. 2009. Comparison between inoculative and inundative release for controlling scarab beetles in strawberry using entomopathogenic nematodes under field conditions. Bulletin of Faculty of Agriculture, University of Cairo, 60(2):197-205.

Atwa, A.A. and S.H. Hassan. 2014. Bioefficacy of two entomopathogenic nematodes against *Spodoptera littoralis* Boisduval (Lepidoptera) and *Temnorhynchus baal* Reiche (Coleoptera) larvae. Journal of Biopesticides, 7(2):104-109. httpa://doi.org/10.57182/jbiopestic.7.2.104-109

Barker, K.R. 1985. Nematode extraction and bioassays. Pp. 19-35. In*:* An Advanced treatise on *Meloidogyne* Vol. II. K.R. Barker, C.C. Carter and J.N. Sasser (eds.). North Carolina State University Graphics, North Carolina, USA.

Campos-Herrera, R., E. Pathak, F.E. El-Borai, A. Schuman, M.M. Abd-Elgawad and L.W. Duncan. 2013. New citriculture system suppresses native and augmented entomopathogenic nematodes. Biological Control, 66(3):183-194. https://doi.org/10.1016/j.biocontrol.2013.05.009

Campos-Herrera, R., R.J. Stuart, E. Pathak, F.E. El-Borai and L.W. Duncan. 2019. Temporal patterns of entomopathogenic nematodes in Florida citrus orchards: Evidence of natural regulation by microorganisms and nematode competitors. Soil Biology and Biochemistry, 128:193-204. https://doi.org/10.1016/j.soilbio.2018.10.012

Daykin, M.E. and R.S. Hussey. 1985. Staining and histopathological techniques in nematology. Pp. 39-48. In: An Advanced treatise on *Meloidogyne* Vol. II. K.R. Barker, C.C. Carter and J.N. Sasser (eds.). North Carolina State University Graphics, North Carolina, USA.

Duncan, L.W., R.J. Stuart, F.E. El-Borai, R. Campos-Herrera, E. Pathak, M. Giurcanu and J.H. Graham. 2013. Modifying orchard planting sites conserves entomopathogenic nematodes, reduces weevil herbivory and increases citrus tree growth, survival and fruit yield. Biological Control, 64(1):26-36. https://doi.org/10.1016/j.biocontrol.2012.09.006

Duncan, L.W. and M.M.M. Abd-Elgawad. 2022. Preparing and evaluating IPM tactics for increasing strawberry and citrus production. Final Report of US-Egypt mutual Research Project cycle 17 (Project No. 172). Egyptian Academy of Scientific Research and technology, STDF, Egypt.

El-Borai, F. and V.M. Whitaker. 2024. UF/IFAS strawberries beyond the USA: A success story from Egypt. UF/IFAS Extension, Horticultural Sciences Department, series No. HS1411.

El-Shemy, A.A., Y.S. Khafagy and A.M.M. Al-Genteery. 2013. Cultivation and production of strawberry. Cairo: Tech issue no. 9/2013, General Directorate of Agricultural Culture. Egyptian Ministry of Agriculture. (In Arabic).

Essa, T.A.A. 2015. Response of some commercial strawberry cultivars to infection by wilt diseases in Egypt and their control with fungicides. Egyptian Journal of Phytopathology, 43(1):113-127. https://doi.org/10.21608/ejp.2015.94601

Hammam, M.M., W.M. El-Nagdi, H. Abd-El-Khair and M.M.M. Abd-Elgawad. 2019. Biological management of the root-knot nematode on strawberry in Egypt. Egyptian Journal of Agronematology, 18(1): 1-17. https://doi.org/10.21608/ejaj.2019.52840

Hammam, M.M., H. Abd-El-Khair, W.M. El-Nagdi and M.M.M. Abd-Elgawad. 2022. Can agricultural practices in strawberry fields induce plant–nematode interaction towards *Meloidogyne*-suppressive soils?. Life, 12(10):1572. https://doi.org/10.3390/life12101572

Hussaini, S.S. 2017. Entomopathogenic nematodes: Ecology, diversity and geographical distribution. Pp. 88–142. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. M.M.M. Abd-Elgawad, T.H. Askary and J. Coupland (eds.). CAB International, Wallingford, UK.

Kepenekci, I., S. Hazir, E. Oksal and E.E. Lewis. 2018. Application methods of *Steinernema feltiae*, *Xenorhabdus bovienii* and Purpureocillium lilacinum to control root-knot nematodes in greenhouse tomato systems. Crop Protection, 108:31-38. https://doi.org/10.1016/j.cropro.2018.02.009

Koppenhöfer, A.M., D.I. Shapiro-Ilan and I. Hiltpold. 2020a. Entomopathogenic nematodes in sustainable food production. Frontiers in Sustainable Food Systems, 4:125. https://doi.org/10.3389/fsufs.2020.00125

Koppenhöfer, A.M., O.S. Kostromytska and W. Shaohui. 2020b. Optimizing the use of entomopathogenic nematodes for the management of *Listronotus maculicollis* (Coleoptera: Curculionidae): split applications and combinations with imidacloprid. Crop Protection, 137:105229. https://doi.org/10.1016/j.cropro.2020.105229

Marianelli, L., F. Paoli, G. Torrini, C. Mazza, F. Benvenuti, G. Binazzi, G. Sabbatini Peverieri, D. Bosio, E. Venanzio and S. Giacometto. 2017. Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (Coleoptera, Scarabaeidae) in Piedmont Region (Italy). Journal of Applied Entomology, 142(3):311-318. https://doi.org/10.1111/jen.12470

Nielsen, A.L., K.O. Spence, J. Nakatani and E.E. Lewis. 2011. Effect of soil salinity on entomopathogenic nematode survival and behaviour. Nematology, 3(7):859-867. https://doi.org/10.1163/138855411X562254

Renkema, J.M. and J.P. Parent. 2021. Mulches used in highbush blueberry and entomopathogenic nematodes affect mortality rates of third-instar *Popillia japonica*. Insects, 12(10):907. https://doi.org/10.3390/insects12100907

Sankaranarayanan, C. and T.H. Askary. 2017. Status of Entomopathogenic Nematodes in Integrated Pest Management Strategies in India. Pp. 362-382. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. M.M.M. Abd-Elgawad, T.H. Askary and J. Coupland (eds.). CAB International, Wallingford, UK.

Shamseldean, M.M. and A.A. Atwa. 2004. Laboratory and field tests of entomopathogenic nematodes against the scarab beetle *Temnorhynchus baal* (Reiche) a novel insect pest of strawberry in Egypt. Egyptian Journal of Biological Pest Control, 14(1):127-133.

Shapiro-Ilan, D., L.L. Garrigos and R. Han. 2022. Production of entomopathogenic nematodes. Pp. 293-316. In: Mass production of beneficial organisms: Invertebrates and entomopathogens, 2nd Edition. J. Mrales-Ramos, G. Rojas and D.I. Shapiro-Ilan (eds.). Academic Press, Amsterdam, Holland. https://doi.org/10.1016/C2018-0-00128-0

Shehata, I.E., M.M. Hammam, F.E. El-Borai, L.W. Duncan and M.M.M. Abd-Elgawad. 2019. Comparison of virulence, reproductive potential, and persistence among local *Heterorhabditis indica* populations for the control of *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae) in Egypt. Egyptian Journal of Biological Pest Control, 29:32. https://doi.org/10.1186/s41938-019-0137-5

Shehata, I.E., M.M. Hammam, F.E. El-Borai, L.W. Duncan and M.M.M. Abd-Elgawad. 2020. Traits of the entomopathogenic nematode, *Heterorhabditis bacteriophora* (Hb-EG strain), for potential biocontrol in strawberry fields. Egyptian Journal of Biological Pest Control, 30:40. https://doi.org/10.1186/s41938-020-00233-0

Simões, N., C. Laumond and E. Bonifassi. 1993. Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. Journal of Nematology 25(2):480-485.

Szendrei, Z., N. Mallampalli and R. Isaacs. 2005. Effect of tillage on abundance of Japanese beetle, *Popillia japonica* Newman (Col., Scarabaeidae), larvae and adults in highbush blueberry fields. Journal of Applied Entomology, 129(5):258-264. https://doi.org/10.1111/j.1439-0418.2005.00961.x

Wilson, M.J., E.E. Lewis, F. Yoder and R. Gaugler. 2003. Application pattern and persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Biological Control, 26(2):180-188. https://doi.org/10.1016/S1049-9644(02)00125-1

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