Egypt. J. Agronematol., Vol. 23, No.2, PP.54- 66 (2024)
DOI: 10.21608/EJAJ.2024.382411

Influence of Chitosan Alone or in Combination with Chicken Manure, and Humic acid on Root-Knot Nematode, *Meloidogyne incognita* Infecting Faba Bean

Sahar H. Abdel-Baset[1*]; El-Blasy S.A.S.[2] and Ahmed M.I.M. [3]

[1]Department of Nematode Diseases Research, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

[2]Legume and Forage Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

[3]Seed Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

1* Corresponding author email: drsaharhassan14@gmail.com

***Rec**eived :15 August 2024* *Revised: 26 September2024* *Accepted: 28 September 2024*

ABSTRACT

A greenhouse experiment evaluated the host susceptibility of three Egyptian faba bean (*Vicia faba* L.) cultivars (Sakha 4, Giza 716, and Giza 843) to root-knot nematode, *Meloidogyne incognita*. Results showed that Giza 843, and Sakha4 were the most susceptible hosts. However, Giza716 considered moderately resistant host. With increasing *M. incognita* inoculum levels, the final nematode population in soil and egg masses/root system on faba bean plants increased. Plant growth decreased only slightly at lower initial inoculum levels; however with increasing levels of inoculum and plant growth characteristics were significantly reduced compared to control plants. Some organic amendments (chitosan, chicken manure and humic acid) were evaluated alone or in combination, and nematicide (Fosthiazate 10 G) against *M. incognita*. All treatments significantly reduced number of galls, egg masses on root, and final juveniles' population (J_2s) in soil. The nematicide, Fosthiazate achieved the highest decrease in the percentage of galls number (81%), number of egg masses (83.7%), and final nematode population (89.5%). Combination of chitosan + chicken manure induced a reduction in galls number (65%), egg masses number (75.6%), and final nematode population (84%). Meanwhile, Chitosan + humic acid reduced galls number (48.8%), egg masses number (56.7%), and final nematode population (74.7%). Results showed that all treatments considerably increased shoot and root fresh weights, root weight, shoot length, pod weight, and number of nodules per plant compared to control (nematode only). In addition, all treatments increased the chemical constituent's contents of elements N, P, K, protein, and carbohydrates in faba bean seeds.

Keywords: Root-knot nematode, *Meloidogyne incognita*, faba bean cultivars, chitosan, chicken manure and humic acid.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the earliest crop of legumes, cultivated primarily for its significant protein content used as animal feed and human sustenance. In association with the soil bacterium, *Rhizobium leguminosarum*, faba beans, like other grain legumes, fix atmospheric nitrogen, which supports sustainable agriculture. Because of this special ability, farmers are less dependent on applying large amounts of artificial fertilizers to maintain the quality of soil and water. Nonetheless, the global acreage planted for faba beans is decreasing.

(Jensen et al., 2010). Serious diseases in faba bean crops can be caused by a variety of airborne fungi, as well as soil-borne pathogens linked to complexes of root and foot rot, and nematodes (Youssef and El-Nagdi, 2004 and Stoddard et al. 2010).

Root-knot nematodes, *Meloidogyne* spp., are the main plant-parasitic nematodes that attack numerous plants and greatly reduce the output of leguminous crops cultivated in infested soil (Montasser et al., 2017). Among the most economically important species of root-knot nematodes, *M. incognita,* harms plant growth and yield and is thought to causes a loss of $100 billion annually (Mukhtar et al., 2014). Researchers, farmers, and scientists have been urged to develop alternate methods of controlling root-knot nematodes that do not harm the environment (Mashela et al., 2008). Crop protectionists have reported using numerous alternative tactics, including applying soil organic amendments made of crop wastes and animal manures (Abubakar and Majeed, 2000). The application of organic soil amendments is not only beneficial to nematode management, but also enhances plant growth and yield (Pakeerathan et al., 2009). Applying organic substrates, on the other hand, promotes the growth of specialized microflora around the rhizosphere, which will aid in the decrease of nematodes that parasitize plants in the soil.

Numerous researchers have examined the utilization of plant components and organic matter to control nematodes in agronomic crops, and they found that, nematode populations are positively or negatively correlated with the amount of organic materials (Asif et al., 2017). The deacetylate chitosan is polymer derived from chitin. It comprises crustaceans' outer shells and certain fungi's cell walls. Chitosan has been found to exhibit elicitor activity by inducing systemic and local resistance mechanisms in tomato plants to root-knot nematode, *M. incognita* (Radwan et al., 2012).

Therefore, the objectives of this study were to 1- Screening different local Egyptian faba bean cultivars against root-knot nematode, *M. incognita***,** 2- Determine the impact of increasing population densities (Pi) of *M. incognita* on the more susceptible cultivar's growth and 3- Investigate the effect of chitosan, humic acid, and chicken manure, either individually or in combination on *M. incognita* reproduction, as well as some faba bean biochemical parameters.

MATERIALS AND METHODS

1-Screening test

Experiments in greenhouses were carried out at Ismailia Agricultural Research Station, Egypt, to assess three faba bean cultivars' growth response to root-knot nematode, *Meloidogyne incognita* during winter season 2021-2022.

Susceptibility of testing cultivars for root-knot nematode, *M. incognita* infection

The reaction of three faba bean cultivars, Sakha 4, Giza 716, and Giza 843 was evaluated under greenhouse conditions to *M. incognita*. The cultivars of faba bean were obtained from Field Crop Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were surface-sterilized before being sown in 25cm clay pots filled with steam-sterilized soil in a 1:4 ratio of sandy clay. Thinning was carried out a week after seeding, with a seedling of each pot. Each seedling (two-week-old) was inoculated with approximately 3000 newly hatched second-stage juveniles (J_2s) of *M. incognita* obtained from pure cultures maintained and propagated on tomato cultivar 888. Each inoculated cultivar was replicated three times. Treatment was carried out with nematode only (control treatment). A randomized complete block design (RCBD) was used to order the treatments. Ninety days after the nematode inoculation, the experiment was terminated; the plant growth and nematode assessment data were recorded.

Data Collection

Nematode Assessment

After uprooting the plants of each cultivar, the roots were cleaned with mild trickle of water. Then, the nematode galls were rated at 1-9 scale of the gall index (GI), gall size (GS) and percentage of galls area (GA) according to Sharma et al. (1994). For each replicate, the sum of GI, GS, and GA is divided by three to determine the damage index (DI). The following scheme was used to determine each plant variety's host susceptibility (designation of resistance) based on DI: plants have DI = 1 indicates a plant's high resistance; DI = 2 to 3 indicates resistance; DI = 4 to 5 indicates moderate resistance; DI = 6 to 7 indicates susceptibility; and DI = 8 to 9 indicates high susceptibility (Sharma et al., 1994). The number of egg masses/root systems, and second-stage juveniles (J_2s) for each pot were counted (Goodey, 1957).

Plant growth assessment

Carefully, uprooted plants and the root and shoot systems were separated at the first basal node. Root systems were cleaned carefully and thoroughly before being measured for fresh weight (g) and length (cm). Fresh shoot weights (g), shoot length (cm). Number of pods and number of nodules per root system were recorded. The percentage of plant reduction (R %) was recorded using the formula.: R%= Control plants – Infected plants / Control plants ×100

2-Effect of initial *M. incognita* population density, on its reproduction on faba bean

Two-week-old faba bean seedlings cv. Giza 843 were inoculated as performed by pipetting the desired nematode inoculum density on and around each seedling. The range of Pi used for each cultivar was approximately from 1000, 2000, to 3000 *M. incognita* newly hatched second stage juveniles (J_2s) per seedling. The untreated control treatment was not inoculated (Pi = 0). Each treatment was replicated three times and arranged in a randomized complete block design (RCBD). The experiment was terminated ninety days after nematode inoculation, and plant growth and nematode assessment data were recorded.

3-Nematicidal activity of some organic amendments on *M. incognita* on faba bean

Humic acid and chicken manure (organic matter = 84.12%, pH = 7.0, C/N ratio = 18:1) were obtained from Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center, Giza, Egypt. Chitosan (molecular weight= 3000Da, deacetylation 95%, water solubility=100%, pH =7, moisture content =10%) was obtained from Sigma-Aldrich Chemical Co..The nematicide, Fosthiazate 10G (Nemathorin®) was used for comparison. Nematode only was used (control treatment).

Application of treatments

1- Chitosan (150 kg Fed^{-1})
2- Chicken manure (2 tons Fed^{-1})
3- Humic acid (15 kg Fed^{-1})
4- Chitosan + chicken manure
5- Chitosan + humic acid
6- Chicken manure+ humic acid
7- Fosthiazate 10 G (12.5 kg Fed^{-1})
8- Control (left without any amendment or chemical)

All the tested materials were incorporated uniformly, 7 days before transplanting in the top of soil pot. Seeds of faba bean cv. Giza 843 were sown, at three seeds per pot, and thinned to one at one week after planting. There were eight treatments in three replicates arranged in a randomized complete block design (RCBD). Each seedling (two-week-old) was inoculated with approximately 3000 newly hatched juveniles (J_2s) of *M. incognita*, the experiment after 90 days from inoculation was terminated, and the previously indicated data on plant growth parameters as well as nematode assessment were recorded.

Data Collection

Nematode Assessment

Number of galls, egg masses/ root system, and their indices as well, the number of second stage juveniles (j_2s) in each pot were recorded.

Plant Growth Assessment

Fresh shoot and root weights (g), and their length (cm), pod weights (g), and number of nodules per root system were measured as mentioned before.

Effect of organic soil amendments on chemical analysis:

For the analysis of chemicals, ten pods from each treatment were randomly selected to determine N, P, and K in and K in dry seeds using Microkjeldahl method of ADAS (1981), and Chapman and Pratt (1978) and the flame photometer for K. The protein percent was calculated according to Pregl (1945). While the percentage of total carbohydrates was determined based on Dubois et al. (1951).

Statistical analysis

All experiments were performed twice in a completely randomized design with 3 replicates in each treatment. Data were subjected to analysis of variance (ANOVA) using MSTAT-C program version 2.10 (Anonymous, 1991). Means were compared by Duncan’s multiple range test at $P \leq 0.05$ probabilities (Duncan, 1955). Relationships between initial nematode densities (Pi) and faba bean yield were depicted as regression lines for derivation linear and quadratic equations and determination correlation coefficient (r) between nematode infection and faba bean yield.

RESULTS

Susceptibility of testing faba bean cultivars infected with *M. incognita*

According to the rating scale, based on damage index (DI), three examined cultivars in Table (1) differed in their susceptibility to *M. incognita*. Giza 843, and Sakha 4 cultivars are the most susceptible (DI=7), and (DI=6), respectively. However, Giza 716 (DI=5) could be considered moderately resistant.

Root-knot nematode, *M. incognita*, had a considerable negative effect on the growth and yield indices of faba bean cultivars Table (2). The fresh shoot weights of roots and, their lengths, in addition pods weight and number of nodules/ plant of all the cultivars were reduced significantly because of nematode infection.

Table 1: Susceptibility of three faba bean cultivars infected with *Meloidogyne incognita*.

Cultivars	No. of galls/ root system	G.I	Gall size (GS)	Gall area (GA)	Damage index (DI)	Host susceptibility / Resistance	No. of J_2s/250 g soil	No. of egg masses/root system	E.I
Sakha 4	62.6^{b}	7.0^{b}	5.0^{b}	5.0^{b}	6.0^{b}	Susceptible	220^{b}	28.0^{a}	5.0^{ab}
Giza 716	49.0^{c}	6.0^{c}	5.0^{b}	5.0^{b}	5.0^{n}	Moderately resistant	175 ^{c}	19.0^{b}	4.3^{b}
Giza 843	76.0^{a}	8.0^{a}	7.0^{a}	7.0^{a}	7.0^{a}	Susceptible	253^{a}	30.0^{a}	5.3^{a}
LSD 0.05	5.1	0.19	1.3	1.3	0.92		29.7	5.5	0.94

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). * Gall index (GI) or egg-masses index (EI) was assessed based on Sharma et al., (1994).

Table 2: Plant growth response of three faba bean cultivars to *Meloidogyne incognita* infection.

Cultivars	Shoot						No. of pods / plant		
	Fresh weights (g)			Length (cm)					
	Control plants	Infected plants	R%	Control plants	Infected plants	R%	Control plants	Infected plants	R%
Sakha 4	41.0^{b}	28.6^{b}	30.0	61.0 ^{a}	45.0^{b}	26.0	9.0^{b}	6.6^{a}	26.6
Giza 716	32.0^{c}	23.0^{c}	28.0	58.0^{a}	48.0^{a}	17.0	9.6^{ab}	8.0^{a}	16.6
Giza 843	50.0^{a}	33.6^{a}	32.8	59.0^{a}	39.0^{c}	33.8	11.0^{a}	6.6^{a}	40,0
LSD 0.05	3.8	2.97		3.05	1.99		1.7	1.88	
	Root								
Cultivars	Fresh weights (g)			Length (cm)			No. of nodules / plant		
	Control plants	Infected plants	R%	Control plants	Infected plants	R%	Control plant	Infected plants	R%
Sakha 4	10.3^{b}	8,0^{a}	22.0	59^{a}	43^{a}	27	77^{a}	44^{a}	42.8
Giza 716	14.3^{a}	10,0^{a}	30.0	54^{b}	43^{a}	20	62^{b}	36^{b}	41.0
Giza 843	11.0^{b}	7.6^{b}	31.0	62^{a}	40^{a}	33	85^{a}	48^{a}	43.0
LSD 0.05	2.2	1.76		4.1	3.46		9.2	6.6	

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). R%= percentage of reduction =cp-ip/cpX100

Reproduction of *M. incognita* as influenced by initial population density

Data in Table (3) displayed the root galling severity and damage index (DI) on faba bean cv. 843 increased as *M. incognita* inoculum level increased. Gall, and egg mass indices were increased severity on faba bean plants as the maximum indices rate was with at Pi (3000 j_2s) was7.3, and 5.0, respectively. Furthermore, data demonstrated that the damage index (DI) increased as inoculum levels increased.

Table 3: Reproduction of *Meloidogyne incognita* on faba bean cv. Giza 843 at different initial population densities (Pi) under greenhouse experiment.

Pi (*M. incognita*)	No. of galls/ root system	G.I	Damage index (DI)	No. of egg masses/root system	E.I	No. of J_2s/250 g soil
1000	32.0^{c}	5.3^{b}	5.3^{b}	12.0^{c}	3.0^{b}	175.0^{b}
2000	40.0^{b}	6.0^{b}	5.6^{b}	20.0^{b}	4.0^{ab}	195.0^{b}
3000	67.0^{a}	7.3^{a}	7.6^{a}	27.0^{a}	5.0^{a}	315.0^{a}
LSD 0.05	5.8	0.94	1.9	5.2	1.1	39.9

Data are average of 3replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). * Gall index (GI) or egg-masses index (EI) was determined according to Sharma et al., (1994).

Plant growth of faba bean as influenced by initial population of *M. incognita*

As shown by the results in Table (4), all the growth parameters of faba bean plants significantly decreased in all nematode-inoculated plants compared to control plants. Plant growth was reduced, albeit not to the same amount with lower initial inoculum level (1000 J_2s). Even still, compared to control plants, there was a notable decrease in plant growth at higher inoculum (3000 J_2s). Data in Fig (1) showed that the correlation between yield (100 seeds) and initial population densities (Pi) of faba bean was negative with a correlation coefficient. Based on the quadratic equation, the estimated loss of faba bean yield severely nematode-infected (pi =3000) was more than the loss obtained from (Pi=1000).

Table 4: Influence of different initial population densities (Pi) of *Meloidogyne incognita* on faba bean cv. Giza 843 growth parameters under greenhouse conditions.

Pi (*M. incognita*)	Shoot length (cm)	R %	Shoot fresh weights (g)	R %	Root fresh weights (g)	R. %	100 Seeds weight (g)	R %	No. of nodules / plant	R %
1000	55[b]	14	36[b]	25	11[b]	21	46[b]	11	103[b]	16
2000	47[c]	26	35[b]	27	10[bc]	28	39[c]	25	95[b]	22
3000	37[d]	42	26[c]	45	8[c]	42	33[d]	36	56[c]	54
Non-inoculated	64[a]	-	48[a]	-	14[a]	-	52[a]	-	123[a]	-
LSD 0.05	5.5	-	5.2	-	2.1	-	3.7	-	8.3	-

Means are the average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test (P ≤ 0.05). R% = Percentage of reduction =cp-ip/cpX100

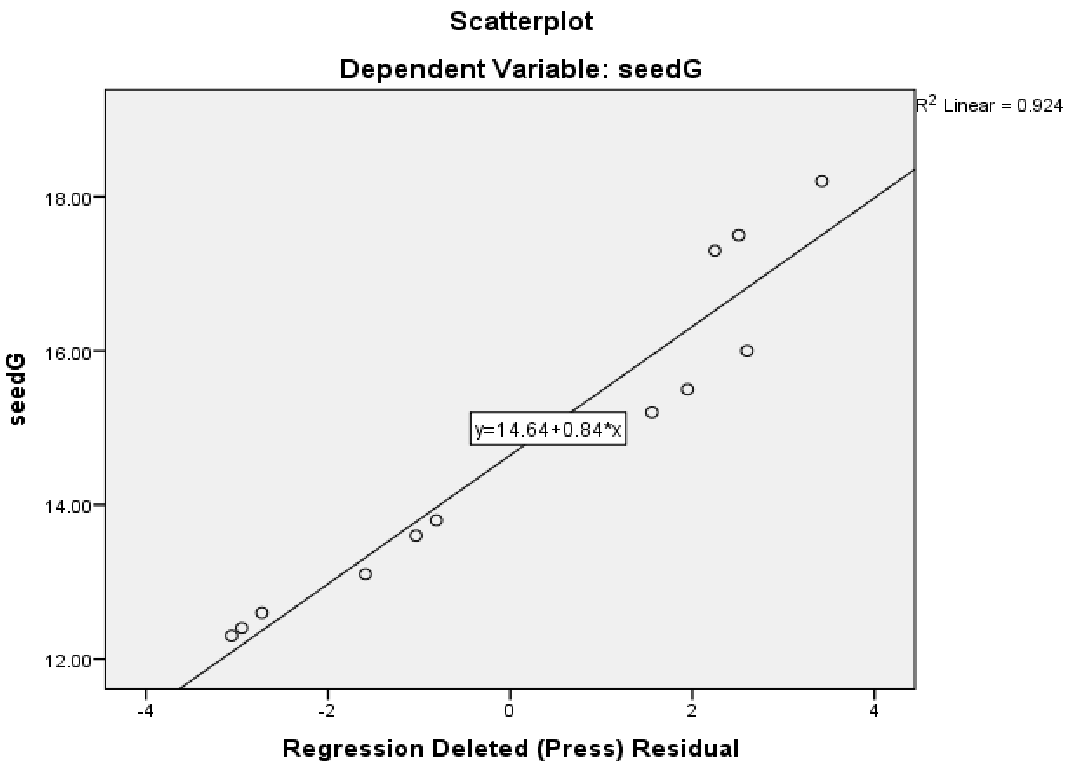

Figure 1: Relationship between initial nematode densities (pi) and faba bean yield.

Influence of certain organic soil amendments alone or in combination on reproduction of *M. incognita*

Data in Table (5) showed that all treatments significantly reduced the number of galls, egg-masses on root and final nematode population (J_2s) in the soil. The nematicide Fosthiazate 10G (Nemathorin) achieved the highest reduction in the percentages of galls (81%), number of egg masses (83.7%), and final nematode population (J_2s) (89.5%). However, the combination of chitosan + chicken manureinduced a reduction in the number of galls (65%), number of egg masses (75.6%), and final nematode population (J_2s) (84%). Meanwhile, a less effect was observed with humic acid that caused reduction in galls (29.7%), egg masses number (37.8 %), and final nematode population (J_2s) (67%).

Table 5: Influence of organic soil amendments alone or in combination on reproduction of *Meloidogyne incognita*, on faba bean.

Treatment	No. of galls/ root system	R %	No. of egg masses/ root system	R %	No. of J_2s/250g soil	R %
Chitosan (A)	55^{bc}	34.5	17^{cd}	54.0	186^{bc}	70.6
Chicken manure (B)	51^{c}	39.0	19^{c}	48.6	176^{bc}	72.0
Humic acid (C)	59^{b}	29.7	23^{b}	37.8	206^{b}	67.0
Chitosan + B	29^{e}	65.0	9^{e}	75.6	100^{d}	84.0
Chitosan +C	43^{d}	48.8	16^{d}	56.7	160^{c}	74.7
Chicken manure + C	44^{d}	47.6	17^{cd}	54.0	166^{c}	73.7
Fosthiazate 10G	16^{f}	81.0	6^{f}	83.7	66^{d}	89.5
Nematode only (control)	84^{a}	--	37^{a}	--	633^{a}	--
LSD 0.05	4.6	--	3.2	--	34	--

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). R% = Percentage of reduction =cp-ip/cpX100

Effects of organic soil amendments alone or in combination on some plant growth parameters

Data analysis for plant growth parameters in Table (6) demonstrated that all treatments achieved significant increases in all plant growth parameters compared to control (nematode only). The greatest increase in shoot fresh weight was recorded with combination of chitosan and chicken manure (128%), followed by a combination of chitosan with humic acid (127%). Concerning the pod number/plant parameter, it was found that the application of chitosan with chicken manure induced significant values, when compared to the control treatment. Likewise, with shoot length, and number of nodules / plant.

Influence of some organic soil amendments on chemical composition of faba bean seeds

Data presented in Table (7) revealed that organic soil amendments had a simulative impact on the chemical constituents in seeds as elements N, P, K, protein, and carbohydrates contents in faba bean seeds, specialty when combined. Combined chitosan with chicken manure achieved superior increase in chemical constituents of faba bean seeds by 97, 103, 25, 89, and 81%, respectively compared with control treatment. In addition, combined chitosan with humic acid with 81, 93, 23, 77, and 72%, respectively.

Table 6: Influence of organic soil amendments alone or in combination on some plant growth parameters on faba bean plants infected with root-knot nematode, *Meloidogyne incognita.*

Treatment	Shoot fresh weights (g)	Inc (%)	Shoot length (cm)	Inc (%)	Pods (No./ plant)	Inc (%)	Root fresh weights (g)	Inc (%)	No. of nodules / plant	Inc (%)
Chitosan (A)	69.6[c]	88	72[bc]	36	17.0[bc]	77	33.0[a]	83	92[c]	100
Chicken manure (B)	76.3[b]	106	74[b]	38	17.0[bc]	77	36.0[a]	100	102[b]	121
Humic acid (C)	69.3[c]	87	68[c]	47	15.0[cd]	56	34.0[a]	88	91[cd]	97
Chitosan + B	84.6[a]	128	87[a]	89	19.0[a]	97	38.0[a]	111	109[a]	136
Chitosan +C	84.0[a]	127	83[a]	80	17.6[ab]	83	38.6[a]	114	99[b]	115
Chicken manure+ C	77.6[b]	109	76[b]	65	17.6[ab]	83	34.0[a]	88	85[d]	84
Fosthiazate 10G	76.0[b]	105	61[d]	32	14.0[d]	45	32.0[a]	77	65[e]	41
Nematode only (control)	37.0[d]	-	46[e]	-	9.6[e]	-	18.0[b]	-	46[f]	-
LSD 0.05	5.92	-	4.2	-	1.6	-	6.6	-	6.5	-

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). Inc (%) in plant growth parameters were counted in comparison with a control treatment.

Table 7: Influence of organic soil amendments alone or in combination on chemical compositions of faba bean seeds.

Treatment	N	Inc (%)	P	Inc (%)	K	Inc (%)	Protein	Inc (%)	Carbo-hydrate	Inc (%)
Chitosan (A)	3.59[c]	64	0.45[de]	50	2.06[c]	19	20.9[c]	60	46.0[d]	48
Chicken manure (B)	3.69[c]	69	0.47[cd]	56	2.11[abc]	22	21.3[bc]	63	47.0[cd]	51
Humic acid (C)	3.26[d]	49	0.44[e]	46	2.07[bc]	19	20.6[c]	58	43.0[e]	38
Chitosan + B	4.30[a]	97	0.61[a]	103	2.16[a]	25	24.6[a]	89	56.3[a]	81
Chitosan + C	3.96[b]	81	0.58[b]	93	2.13[ab]	23	23.0[ab]	77	53.3[b]	72
Chicken manure+ C	3.89[b]	78	0.48[c]	60	2.07[bc]	19	22.0[bc]	69	49.0[c]	58
Fosthiazate 10G	2.60[e]	19	0.41[f]	36	1.96[d]	13	18.0[d]	38	40.0[f]	29
Nematode only (control)	2.18[f]	-	0.30g	-	1.73[e]	-	13.0[e]	-	31.0[g]	-
LSD 0.05	0.199	-	0.028	-	0.065	-	1.87	-	2.17	-

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). Inc (%) in plant growth parameters were counted in comparison with a control treatment.

DISCUSSION

Faba bean is among the most widely cultivated winter-season legumes in the Mediterranean regions, where plant parasitic nematodes are quite common. Newly reclaimed areas in Egypt help the risk of planting crops in light or sandy soils, where RKNs can readily multiply and seriously destroy many yields. Reports regarding the impact of RKNs on the faba bean plant are few Korayem et al. (2018). Obtained results revealed that all the examined cultivars of faba been (Sakha 4, and Giza 843) were susceptible to root-knot nematode, *M. incognita*, while, Giza 716 cultivar was classified as moderately resistant. The present results differed with El-Shafeey et al. (2019) as they reported that faba bean cultivars, Giza 40, Giza 716, Sakha 3 and Sakha 283 were susceptible to root-knot nematode, *M. incognita* infection. While, Soliman (2002) reported that cultivars, Giza 1, Giza 3, and Giza 716 were resistant to root-knot nematode, *M. incognita* and *M. javanica* under greenhouse conditions. Montasser et al. (2017) revealed that root-knot nematodes behavior differed substantially depending on the plant cultivar type and the nematode species that succeeded in establishing and multiplying practically on each cultivar of faba bean.

The influence of different initial inoculum levels of *M. incognita* (1000, 2000, and 3000J_2s) on faba bean susceptible cultivar was studied under greenhouse conditions. This damage was greatly increased with increased inoculum levels of *M. incognita*. It was clear that the nematode had adversely affected the yield of faba bean pods. These results were in line with Korayem et al. (2018) who noticed a significant correlation between the natural *M. arenaria* population levels and the field output of faba bean pods, making it possible to evaluate the significant RKN-pod weight losses. In soil, galls, or egg masses on roots, the correlation coefficient between actual or transformed number of juveniles (J_2s) and pod weight was consistently negative at varying probability levels. All correlation coefficients were significant, demonstrating a decrease in yield with increasing galls, egg masses, or J_2s. Because of the nematodes' broad host range, diversity in the RKN species, and the lack of resistant genotypes of faba bean, caution should be given in selecting rotation crops (Sikora et al., 2018).

As our study showed, that amending soil with chitosan, chicken manure, and humic acid alone or in combination enhanced the growth and growth-yielding attributes of faba bean significantly ($P \leq 0.05$) compared with control treatment and root-knot nematode, *M. incognita* infection has been suppressed in both the soil and root systems. According to obtained data, the effect of the examined materials was more obvious, when combined than as single treatments, which may be explained by the plant residues' synergistic effects. Data obtained revealed that the treatment nematicide, Fosthiazate 10G, the combination of chitosan +chicken manure, combination of chitosan +chicken manure and chitosan+humic achieved reduction in the studied nematode parameters at various degrees. These results are in agreement with El-Sayed and Mahdy (2015) as they demonstrated that different molecular weights of chitosan considerably reduced nematode parameters, such as number of galls, egg masses, and juveniles/250 g soil on tomato plants *in vivo*. At the same time, the results of Asif et al. (2017) cleared the use of chitosan with different agricultural wastes considerably reduced galls, egg masses per plant, as well as number of eggs per egg mass in all the treatments. Many studies have been conducted on chitosan's ability to suppress plant diseases (El-Hadrami et al., 2010). Therefore, the chitosan application appeared to reduce the disease severity, most likely owing to the inhibition of reproduction in nematodes and/or the development of physiological changes in nematodes as well as plants that prevented the nematodes from reproducing successfully (Khalil and Badawy, 2012).

Previous literature revealed that chicken manure application, alone or in combination with bio agents led to a nematicidal impact on root-knot nematode population with melon (Abdel-Dayem et al., 2012), and cowpea (Abdel-Monaim et al., 2018). Poultry manure was the most widely used and generally was the most efficient against RKN among the organic manures. Poultry manure's high nitrogen content primarily uric acid, which is quickly transformed into ammonia may be the reason for its effectiveness (NH_3) (Hue and Silva, 2000). Ammonia is a harmful byproduct formed during the breakdown of organic substances. A higher concentration of NH_3

can have negative effects on soil RKN because nitrogen released in the form of NH_3 enters nematode cell membranes and causes death (Riegel et al., 1996). Poultry manure's low C: N ratio typically accelerates the rate of decomposition, raising the amount of NH_3 in the soil. Degradation rates are higher and nematicidal activities are frequently present in organic materials having a ratio of less than 20:1 C: N (Mcsorley, 2011).

Numerous researchers have documented humic acid's effectiveness against root knot nematodes. Saravanapriya and Subramanian (2007) revealed that adding humic acid to the soil significantly reduced galls, egg masses /plants, and soil juvenile's population (J_2s) of *M. incognita* in tomato. In the same way, Seenivasan and Senthilnathan (2018) demonstrated that *M. incognita* egg incubation in 0.08%–2.0% humic acid decreased hatching by 50%–100% *in vitro*. The effectiveness of nematicides may be possibly ascribed to humic acid's active principles, specifically carboxyl and phenolic groups, as well as alcoholic, hydroxyl, and carbonyl groups (Chitwood, 2002).

Based on obtained data, combination of chitosan with (chicken manure, and humic acid) resulted in the most effective treatments in root-knot nematode control and faba bean growth. This may be to increased availability of nutrient elements such as N, and P treatments in treatments (Alhammad and Seleiman, 2023). The presence of a higher content of available N may have affected and enhanced growth of plant with increased plant defense against attacks of nematode. Therefore, plant growth improved by combined treatments might result from the combination of inhibitory influence on *Meloidogyne* spp. and direct fertilization effect on faba bean plants as demonstrated in previous experiments with melon (Abdel-Dayem et al., 2012), and cowpea (Abdel-Monaim et al., 2018).

Data from the current study demonstrated that all treatments (alone or in combination) significantly increased the content of N, P, K, protein, carbohydrates contents in faba bean seeds compared with control treatment. Combined chitosan with chicken manure, and humic acid achieved superior increase in chemical constituents of faba bean seeds. These results are in line with Mohamed and Gomaa (2005) who reported that adding farmyard manure increased the contents of N, P, K, protein, and carbohydrates in faba bean seeds. This could be owing to the contribution of adding organic soil amendments to reach the state of optimal nutritional balance appropriated for the plant's growth and development as they gave it the nutrients it needed, as evidenced by the important increases in the majority of the factor's indicators (El-Shakry 2005 and AL-Habar et al., 2014). The reason for increasing protein concentration, when using organic fertilizers is the high concentration of nutrient elements that the plant can easily absorb, as well as the ability of humic acids to increase the activity of most enzymes, including proteinase, which converts nitrogen to protein in the plant and accumulates in the seeds during maturation. This is consistent with Jasim and Mhanna (2014).

REFERENCES

Abdel-Monaim, M. F.; Abdel-Baset, S.H. and Wahdan, R. H. (2018). Effectiveness of some organic fertilizers and bio-control agents for controlling root-knot nematodes *Meloidogyne* spp. in cowpea forage (*Vigna unguiculata*) in new valley, Egypt. J. Phytopathol. 46(1): 143-164.

Abdel-Dayem, E. A.; Erriquens, F.; Verrastro, V.; Sasanelli, N.; Mondelli, D. and Cocozza, C. (2012). Nematicidal and fertilizing effects of chicken manure, fresh and composted olive mill wastes on organic melon. Helminthologia, 49: 259-269.

Anonymous, S.A. (1991). A Software Program for the MSTAT-C Design.

Asif, M., Ahmad, F., Tariq, M., Khan, A., Ansari, T., Khan, F., and Siddiqui, A. M. (2017). Potential of chitosan alone and in combination with agricultural wastes against the root-knot nematode, *Meloidogyne incognita* infesting eggplant. J. Plant Prot. Res., *57*(3): 288-295.

AL-Habar, M.T.; Hussein, J.M. and Fathel, F.R.I. (2014). Possibility study of humic acid using as compensation or reduce of chemical fertilizer addition on potato (*Solanum tuberosum* L.). Mesopotamia J. Agric. 42 (1): 68-74.

Alhammad, B. A. and Seleiman, M. F. (2023). Improving plant growth, seed yield, and quality of faba bean by integration of bio-fertilizers with biogas digestate. Agronomy 13(3): 744

A.D.A.S. (1981). The analysis of Agricultural Materials: A manual of the analytical methods used by the Agricultural Development and Advisory Service (ADAS), Ministry of Agricultural, Fisheries and Food, England. Her Majesty's Stationary Office, London. Method 4: Preparation of sample solution of plant material by wet digestion pp. 10-11 and Method 55: N determination in plant material pp. 130 – 131.

Abubakar, U. and Majeed, Q. (2000). Use of Animal manure for the control of root-knot nematodes of cowpea. J. Agric. Environ. 1(1): 29-33.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control. Annu. Rev. Phytopathol. 40(1): 221-249.

Chapman, H.D. and P.F. Pratt (1978). Methods of Analysis for soils, Plant and Waters, Priced Publication 4034. Division of Agricultural Sciences, Univ. of California, USA. Preparation of soil sample, P. 5, P vanadate method pp. 169 – 170 and pH and EC. pp. 233 – 236.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics 11(1):1-42.

Dubois, M.;. Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. and F. Smith, F. (1951). Colorimetric method for determination of sugars and related substances. Analytical Chem. 28: 250 – 256.

Goodey, J.B. (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2. Min. Agric. Fish Ed. London, 47 p.

EL-Shakry, M.F.Z. (2005). Humic materials for agriculture plants. Better Crops. 89 (3):1023-1025.

El-Sayed, S. M., and Mahdy, M. E. (2015). Effect of chitosan on root-knot nematode, *Meloidogyne javanica* on tomato plants. Int. J. Chem.Tech. Res. *7*(4): 1985-1992.

El-Shafeey, E. I.; Abd-El-Hadi, M. A.; Hagag, E. S. and Abu El-Naga, G. S. (2019). Utilization of organic and bio fertilizers against root-knot nematode (*Meloidogyne incognita*) infecting faba bean (*Vicia faba* L.). Bulg. J. Agric. Sci.25(3): 506–513

El-Hadrami, A., Lorne, R.A., El-Hadramim I. and Daayf, F. (2010). Chitosan in plant protection. Marine Drugs 8 (4): 968–987. DOI: 10.3390/md8040968

Jasim, A. H., and Mhanna, Q. L. (2014). Effect of some organic fertilizers treatments on dry seed yield of broad bean (*Vicia faba* L.). Scientific Papers. Series A. Agronomy, Vol. LVII : 218- 222.

Jensen, E. S., Peoples, M. B., and Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. Field Crops Res. 115(3): 203-216.

Hue, N.V., and Silva, J.A. (2000) Organic soil amendments for sustainable agriculture: organic sources of nitrogen, phosphorus, and potassium. In:Silva, J.A. and Uchida RS (eds) Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii at Manoa, Hawaii, pp 133–144.

Korayem, A.M., Mohamed, M.M.M., and El- Ashry, S.M. (2018). Estimation of yield losses in broad bean due to *Meloidogyne arenaria* in Egypt. Pak. J. Nematol. 36: 33-40.

Khalil M.S., and Badawy M.E. 2012. Nematicidal activity of a biopolymer chitosan at different molecular weights against root- knot nematode, *Meloidogyne incognita*. Plant Prot. Sci. 48: 170–178.

Mashela, P. W., Shimelis, H. A., and Mudau, F. N. (2008). Comparison of the efficacy of ground wild cucumber fruits, aldicarb and fenamiphos on suppression of *Meloidogyne incognita* in tomato. J. Phytopathol.156(5): 264-267.

Mohamed, H. A., and Gomaa, A. M. (2005). Faba bean growth and green yield and its quality as influenced by the application of bio-organic farming system. J. Appl. Sci. Res. 1(5): 350-385.

Montasser, S.A., Mahmoud, N.A., El-Mesalamy, A.F. and Abdel-Mageed, M.A.A. (2017). Evaluation of six leguminous crops against the root-knot nematode, *Meloidogyne javanica* infection. Pak. J. Nematol. 35(1): 79-84.

Mcsorley, R. (2011). Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. J. Nematol. 43(2): 69.

Mukhtar, T., Hussain, M. A., Kayani, M. Z.and Aslam, M. N. (2014). Evaluation of resistance to root-knot nematode (*Meloidogyne incognita*) in okra cultivars. Crop Prot. 56: 25-30.

Pakeerathan, K., Mikunthan, G., and Tharshani, N. (2009). Effect of different animal manures on *Meloidogyne incognita* (Kofoid and White) on tomato. World J.Agric. Sci., 5(4):432-435.

Pregl, F. (1945). Quantitative Organic Micro Analysis. 4th Ed. Churchill, London.

Radwan M.A., Farrag S.A.A., Abu-Elamayem M.M., Ahmed N.S. (2012). Extraction, characterization, and nematicidal activity of chitin and chitosan derived from shrimp shell wastes. Biol Fertil Soils 48 (4): 463–468. DOI: https://doi.org/10.1007/s00374-011-0632-7

Riegel, C., Fernandez, F.A., and Noe, J.P. (1996). *Meloidogyne incognita* infested soil amended with chicken litter. J. Nematol. 28:369–378.

Saravanapriya, B. and Subramanian S. (2007). Management of *Meloidogyne incognita* on tomato with humic acid and bioinoculants. Ann. Plant Prot. Sci. 15(1): 195-197.

Seenivasan, N., and Senthilnathan, S. (2018). Effect of humic acid on *Meloidogyne incognita* (Kofoid & White) Chitwood infecting banana (*Musa* spp.). Int. J. Pest Manag., 64(2): 110-118.

Sharma, S. B., Mohiuddin, M., Jain, K. C., and Remanandan, P. (1994). Reaction of pigeonpea cultivars and germplasm accessions to the root-knot nematode, *Meloidogyne javanica*. J. Nematol. 26(4S): 644-652.

Sikora, R.A., Claudius-Cole, B., and Sikora, E.J. (2018). Nematode parasites of food legumes. In: Plant parasitic nematodes in subtropical and tropical agriculture; Sikora, R.A., Coyne, D., Hallmann, J., Timper, P., Eds; CABI International: Boston, USA, pp. 290-345.

Soliman, M. S. I. (2002). Plant-parasitic nematode associated with broad bean (*Vicia faba*) crop in some Egyptian governorates and reaction of some cultivars to root-knot nematode (*Meloidogyne incognita* and *Meloidogyne javanica*). Egypt. J. Appl. Sci. 17: 279-286.

Stoddard, F. L., Nicholas, A. H., Rubiales, D., Thomas, J., and Villegas-Fernández, A. M. (2010). Integrated pest management in faba bean. Field Crops Res.115(3): 308-318.

Youssef, M.M.A. and El-Nagdi, W.M.A. (2004). Assessment of faba bean damage caused by *Meloidogyne incognita* root-knot nematode infection. Pak. J. Nematol. 22: 47 – 53.

الملخص العربى

تأثير الكيتوزان منفردا أو بالاشتراك مع سماد الدجاج وحمض الهيوميك على نيماتودا تعقد الجذور *Meloidogyne incognita* على الفول البلدى

سحر حسن عبدالباسط [١]، سلامة احمد البلاسى [٢] ، محمد إسماعيل احمد [٣]

١-قسم بحوث امراض النبات النيماتودية – معهد بحوث امراض النباتات - مركز البحوث الزراعية – الجيزة – مصر
٢-قسم بحوث امراض البقوليات والاعلاف – معهد بحوث امراض النباتات –مركز البحوث الزراعية – الجيزة – مصر
٣- قسم بحوث امراض البذور – معهد بحوث امراض النباتات –مركز البحوث الزراعية – الجيزة – مصر

تم تقييم حساسية ثلاثة أصناف من الفول البلدى (سخا ٤، جيزة ٧١٦، وجيزه ٨٤٣) تجاه الإصابة بنيماتودا تعقد الجذور *Meloidogyne incognita* وذلك لتحديد مدى ملائمة هذه الأصناف كعوائل للنيماتودا المختبرة تحت ظروف الصوبة. أوضحت النتائج أن الصنفين (جيزة ٨٤٣ وسخا ٤) كانا شديدي القابلية للإصابة بنيماتودا تعقد الجذور *M.incognita*. في حين ان، يمكن اعتبار الصنف جيزة ٧١٦ متوسط المقاومة لتلك النيماتودا. بدراسة تأثير كثافه اللقاح الابتدائي من نيماتودا *M. incognita* على مؤشرات نمو نباتات الفول البلدي "صنف جيزة ٨٤٣" وكذلك على تكاثر النيماتودا. اشارت النتائج ان بزيادة مستويات اللقاح الابتدائي أدى الى زيادة في اعداد كل من الطور اليرقي المعدي في التربة وكذلك كتل البيض على جذور النباتات. وأيضا بزيادة مستويات اللقاح الابتدائي من *M. incognita* انخفضت معايير نمو نباتات الفول بشكل معنوي مقارنة بالنباتات الكنترول (المقارنة). تم تقييم فاعلية استخدام بعض محسنات التربة العضوية (الكيتوزان وسماد الدجاج وحمض الهيوميك) بمفردها أو بالاشتراك مع بعضها البعض مقارنة بالمبيد الكيماوي (فوسثيازات ١٠٪) لمكافحة نيماتودا تعقد الجذور. أدت جميع المعاملات إلى تقليل اعداد العقد الجذرية النيماتودية وكتل البيض على الجذر وكذلك اعداد الطور اليرقي المعدي في التربة بشكل ملحوظ. أدى استخدام مبيد الفوثيازيت (نيماثورين) الى أعلى نسبة انخفاض في اعداد العقد الجذرية النيماتودية (٨١٪) واعداد كتل البيض (٨٣,٧٪) واعداد الطور اليرقي المعدي في التربة بنسبة (٨٩,٥٪). أدى الجمع بين الكيتوزان + سماد الدجاج إلى انخفاض في اعداد العقد الجذرية النيماتودية بنسبة (٦٥٪) واعداد كتل البيض بنسبة (٧٥,٦٪) واعداد الطور اليرقي المعدي في التربة بنسبة (٨٤٪). في حين أن أدى الجمع بين (الكيتوزان + حمض الهيوميك) الى تقليل اعداد العقد النيماتودية (٤٨,٨٪) واعداد كتل البيض (٥٦,٧٪) وعدد الطور اليرقي المعدي في التربة (٧٤,٧٪). أظهرت النتائج أن جميع المعاملات أدت إلى زيادة معنوية في كل من اوزان المجموع الخضري والجذور الطازجة وطول المجموع الخضرى ووزن القرون وعدد العقد الجذرية البكترية لكل نبات مقارنة بالمجموعة المقارنة (النيماتودا فقط). بالإضافة إلى ذلك، أدت جميع المعاملات إلى زيادة محتوى المكونات الكيميائية من العناصر النتروجين والفوسفور والبوتاسيوم وكذلك محتوى البروتين والكربوهيدرات في بذور الفول البلدي.