GROWTH, YIELD AND CHEMICAL COMPOSITION OF SUNFLOWER SEEDS IN SOIL INFESTED WITH DIFFERENT POPULATION DENSITIES OF ROOT-KNOT NEMATODE

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Summary. The effect of the root-knot nematode, *Meloidogyne incognita*, on the growth and yield of sunflower grown in two different geographical regions was investigated in microplots at initial population densities of 0, 10, 100, 1000, 10000 and 20000 eggs and juveniles/kg soil. Tolerance limits of sunflower growth and yield, as well as the chemical composition of sunflower seeds, including oil, protein, carbohydrate, phenolic compound contents and fatty acids composition, were estimated. Tolerance limits (*T*) for fresh shoot and seed weights were 110 and 400 eggs and juveniles of *M. incognita*/kg soil, respectively, at Kafr-Kandeel region, and 105 and 153 eggs and juveniles/kg soil, respectively, at Kafr-Elsheikh region. Seed oil content (seed quality) and protein content in oil cakes (meal) were decreased by nematode infection, and the reduction was greater with increasing nematode inoculum. The fatty acids (oil quality) were not affected by nematode infection.

Keywords: *Helianthus annuus*, fatty acids, *Meloidogyne incognita*, oil content, protein content, tolerance limit.

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in the world used in the human diet while its oil cakes are used as livestock food and soil amendment. Also, sunflower oil is a rich source of vitamins E, A, D, K and flavour substances and has other uses in medicine and the wood industry. In many parts of the world, as well as in Egypt, several nematodes attack sunflower but the most severe damage is caused by the root-knot nematodes *Meloidogyne* spp. (Rich and Green, 1981; Sasanelli and Di Vito, 1992; Sasanelli and D'Addabbo, 1993; Sasanelli *et al*., 1992; Di Vito *et al*., 1996; Radwan *et al*., 2004; Abdul Rehman and Hafeez Ullah., 2006; Korayem *et al*., 2006; Youssef *et al*., 2008). Since the discovery of root-knot nematodes infecting plant hosts in Egypt (Tarjan, 1964), many studies have been conducted on their identification, distribution, occurrence and control methods (Oteifa *et al*., 1970; Elgindi and Moussa, 1979; Ibrahim, 1985; Ibrahim *et al*., 1986). However, studies on the relationship between initial nematode densities (per plant) and sunflower yield to estimate damage threshold level and the expected yield losses at different nematode population levels are still lacking. Such information is basic to recommending practical and economic means of nematode control (McSorley and Duncan, 1995).

Therefore, the objectives of the present work were (*i*) to relate the growth and yield of sunflower cv. Sakha 102 to initial population densities of *Meloidogyne incognita* (Kofoid *et* White) Chitw. for estimation of the tolerance limit, and (*ii*) to study the effect of the nematode infection on chemical composition of the seeds and oil quality of sunflower grown in two different geographical regions.

MATERIALS AND METHODS

Two experiments were conducted during the 2007 summer season. The first experiment was carried out in a clay loam soil (37.7% clay, 51.7% silt and 10.6% sand) at Kafr-Kandeel region, south of Giza governorate, where the daily mean temperature (daytime and night) was ca 27 °C from May to August. The second experiment was conducted in a clay sand soil (55% clay, 40% sand and 5% silt) at Kafr-Elsheikh governorate, in northern Egypt, with a mean daily temperature of ca 23 °C during the same period.

Ninety bottomless cylinders (35 cm diameter × 45 cm deep) made from fiberglass were used for each experiment and were buried to 40 cm depth in the field soil. Each cylinder was considered as a microplot.

Preparation of the nematode inoculum. The root-knot nematode population was identified as *Meloidogyne incognita* according to the morphological characters described by Eisenback *et al*. (1981) and was reared on tomato (*Solanum lycopersicum* L. cv. Super Marmande) grown in a greenhouse at 22-29 °C. Tomato roots infested with the nematodes were finely chopped, and the numbers of eggs and juveniles were estimated by processing ten root samples of 10 g each with 1% aqueous solution of sodium hypochlorite (Hussey and Barker 1973). The chopped roots were then thoroughly mixed with 50 kg of sterilized sand and used as inoculum. Appropriate amounts of this inoculum, containing 350 eggs and juveniles/g, were thoroughly mixed with the soil of each microplot (35 kg) to give nematode densities of 0, 10, 100, 1000, 10000 or 20000 eggs and juveniles per kg soil. The microplots were arranged in a randomized block design, with 15 replicates per inoculum level.

Four seeds of sunflower cv. Sakha 102, were sown in each microplot on May 3, 2007 at Kafr-Kandeel region and on May 25, 2007 at Kafr-Elsheikh region. Plants were thinned to one per microplot seven days after emergence. Microplots were weeded by hand and plants were not sprayed with any chemicals as they were not attacked by insects or fungi. Plants were harvested on 3 and 25 August for the first and second region, respectively. Then, plant height, fresh shoot weight and seed dry weight were recorded per plant. Root galling (0-10 scale) was estimated according to Barker (1978).

Oil content determination. To asses the effect of the nematode infection on seed quality, the oil content of the seeds was determined according to the procedure reported by the American Association of Analytical Chemists (A.O.A.C., 1990).

Protein, carbohydrates and phenolic compounds determination. The protein content of the sunflower meal (oil cakes) was determined according to A.O.A.C. (1990). Also, total carbohydrates and total soluble carbohydrates, expressed as glucose in the oil cakes, were determined colorimetrically according to Smith *et al*. (1956). Total phenolic compounds in the oil cakes were determined according to the method described by Snell and Snell (1953).

Fatty acids determination and identification. The fatty acid composition of the oil and their relative proportions were determined quantitatively by gas liquid chromatography of the methyl esters using a Hewlett Packard HP 6890 series GC system instrument equipped with a flame ionization detector. The capillary column was an HP–INNOW AX with polyethylene glycol of length 30 cm, diameter 530 µm and film thickness 1 µm. Two injections were made from each sample. The operating conditions were: initial temperature 120 °C, final temperature 240 °C and detector temperature 300 °C.; the nitrogen, hydrogen and air rates were 30, 30 and 300 ml/min, respectively. Methyl esters of fatty acids were prepared from an aliquot of total lipid with 5% HCl in anhydrous methanol (w/w) according to the method of Fedak and De La Roche (1977). Identification of the fatty acids on the chromatogram was made by comparing the retention times of the lipid methyl esters with those of known mixtures of methyl esters run on the same column under the same conditions. The fatty acid composition was expressed as area percentage of all methyl esters present.

Statistical analysis. Data were subjected to analysis of variance and means were compared by least significance differences and Duncan's multiple range test at the 5% level. Growth and seed yield of sunflower (g/plant)

Table I. Effect of different initial population densities of *Meloidogyne incognita* on the growth and yield of sunflower (cv. Sakha 102) grown in the Kafr-Kandeel region.

Nematode density/kg soil	Plant height (cm)	Reduction %	Fresh shoot weight (g) per plant	Reduction (%)	Dry seed weight (g) per plant	Reduction (%)	Root gall index
0	185[a]	0.0[b]	450	0.0	38.1	0.0	0[c]
10	185	0.0	450	0.0	39.0	0.1-	1
100	186	0.0	452	0.0	38.2	0.0	3
1000	179	3.2	422	6.2	37.0	2.9	5
10000	173	6.5	395	12.2	31.0	18.6	8
20000	168	9.2	365	18.9	27.0	29.1	10
LSD	4.90		26.96		3.95		2.5

[a]Data are averages of 15 replicates.
[b]Percentage reduction in comparison to non-inoculated control.
[c]Root-gall index according to a 0-10 scale (0 = no galls, 10 = 100% of roots galled).

Table II. Effect of different initial population densities of *M. incognita* on the growth and yield of sunflower (cv. Sakha 102) grown in the Kafr-Elsheikh region.

Nematode density/kg soil	Plant height (cm)	Reduction (%)	Fresh shoot weight (g) per plant	Reduction (%)	Dry seed weight (g) per plant	Reduction (%)	Root gall index
0	175[a]	0.0[b]	470	0.0	69.4	0.0	0[c]
10	176	0.0	471	0.0	70.1	0.0	1
100	175	0.0	472	0.0	69.5	0.0	4
1000	166	5.1	445	5.3	64.8	6.6	6
10000	162	7.4	404	14.0	55.5	20.0	10
20000	158	9.7	372	20.9	47.5	31.6	10
LSD	4.0		26.8		4.35		3.70

[a]Data are averages of 15 replicates.
[b]Percentage reduction in comparison to non-inoculated control.
[c]Root-gall index according to a 0-10 scale (0 = no galls, 10 = 100% of roots galled).

were plotted against nematode population densities to depict regression lines, for estimating tolerance limits and/or damage threshold levels.

RESULTS

Effect of M. incognita on sunflower growth and yield. The effect of initial densities of *M. incognita* on plant height, fresh shoot weights and dry seed yield are presented in Tables I and II. Data indicated that the nematode negatively affected growth and seed yield of sunflower both in Kafr-Kandeel (KK) and in Kafr-Elsheikh (KE). At the KK region, a significant reduction of 6.2%, 12.2% and 18.9% in the fresh shoot weight was observed at the densities of 1,000; 10,000 and 20,000 nematodes/kg soil, while both plant height and dry seed yield were significantly reduced (P = 0.05) by 6.5% and 18.4% at 10,000 nematodes/kg soil and by 9.2% and 28.9% at 20,000, nematodes/kg soil, respectively (Table I). The reductions in plant growth and seed yield increased with greater nematode densities. Also, root-gall indices increased with increasing nematode inoculum, with 80% of the roots galled at 10,000 nematodes/kg soil and 100% at 20,000 nematodes/kg soil. At the KE region, significant (P = 0.05) reductions of plant height (5.1%), fresh shoot weight (5.3%) and dry seed weight (6.6%) occurred at 1,000 nematode/kg soil and these reductions gradually increased with increasing nematode inoculum, with the greatest reductions in plant height, fresh shoot weight and dry seeds of 9.7%, 20.9% and 31.6% occurring at 20,000 nematodes/kg soil, respectively (Table II). The root-gall indices also gradually increased with increasing nematode inoculum, and were 1% at 10 nematodes/kg soil and 100% at 20,000 nematodes/kg soil.

Linear regressions of both fresh shoot weight and seed weight against nematode densities are shown in Figs 1 and 2. Negative and significant correlations were found between nematode densities and both shoot and seed weights, with correlation coefficients (r) of -0.886 and -0.835, respectively, in the experiment at KK region (Fig. 1) and -0.875 and -0.864, respectively, at KE region (Fig. 2).

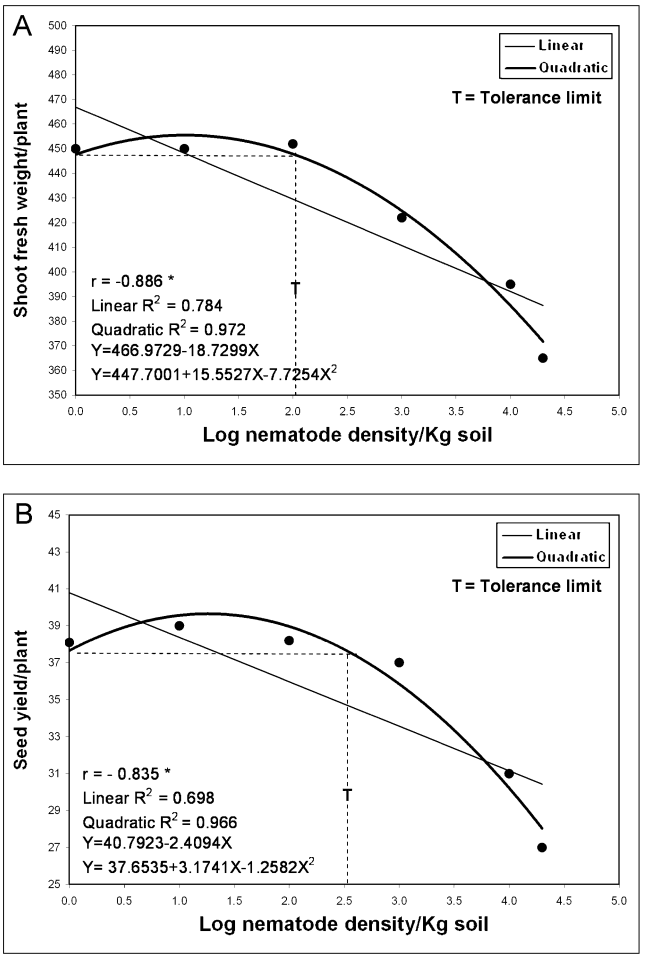

Fig. 1. Linear and quadratic relationships between initial population densities of *Meloidogyne incognita* and shoot fresh weight (A) and seed yield (B) of sunflower cv. Sakha 102 grown in Kafr Kandeel region.

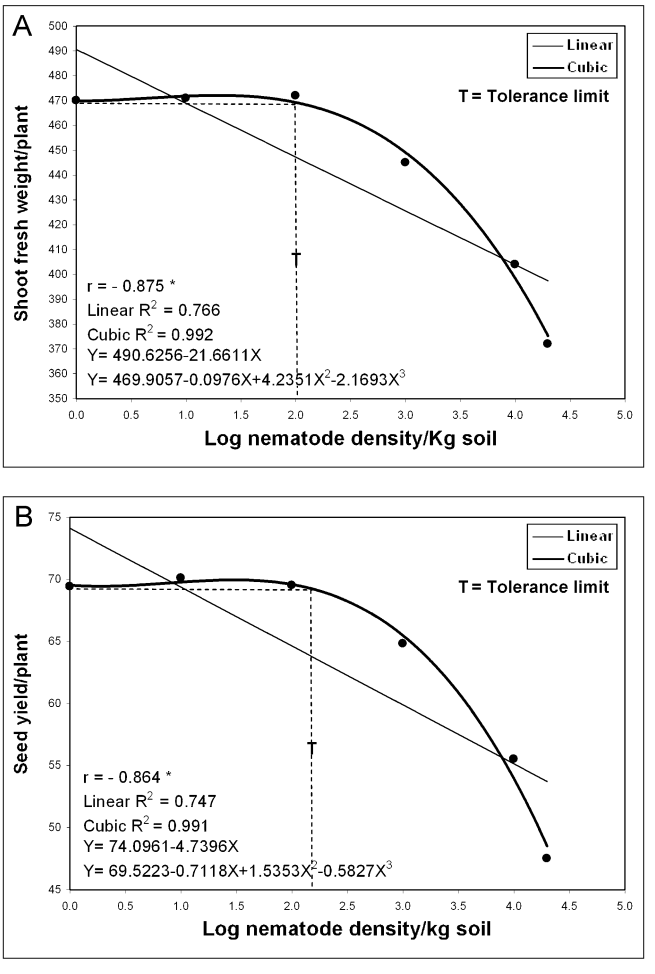

Fig. 2. Linear and cubic relationships between initial population densities of *M. incognita* and shoot fresh weight (A) and seed yield (B) of sunflower cv. Sakha 102 grown in Kafr-Elsheikh region.

Table III. Chemical composition of seeds of sunflower plants infected by different population densities of *M. incognita* at two different regions.

Nematode density/kg	Oil content (%)		Protein (%)		Total carbohydrates (%)		Soluble carbohydrates (%)		Total phenolic compounds (%)	
	KK	KE	KK	KE	KK	KE	KK	KE	KK	KE
0	43.2	33.5	57.3	66.2	19.4	28.50	4.3	3.12	0.62	0.73
10	43.0	33.5	56.9	65.5	20.8	27.34	4.3	3.11	0.74	0.72
100	42.3	33.3	55.2	64.0	21.9	27.18	4.5	3.12	0.74	0.71
1000	40.2	33.2	54.7	58.2	22.3	27.17	4.61	3.11	0.70	0.71
10000	39.1	32.1	54.6	54.9	22.3	27.16	4.73	3.12	0.68	0.70
20000	38.1	31.9	54.5	57.7	24.3	26.75	5.05	3.15	0.67	0.71
LSD 5%	1.01	0.32	2.14	2.01	0.94	0.67	0.12	NS	0.05	NS
T value at P = 0.05	8.699 S		2.776 S		7.727 S		12.511 S		1.131 NS	

KK = Kafr-Kandeel region; KE = Kafr-Elsheikh region; S = significant; NS = non-significant.

Our data could not be fitted satisfactorily to Seinhorst's exponential model (Seinhorst, 1965, 1998), probably because of the insufficient number of different levels of nematode density. The relation between nematode densities and both fresh shoot weight and seed yield weight, at least for the range of nematode levels we used, was well described by a quadratic curve (Fig. 1) and even better by a cubic regression (Fig. 2). These polynomial curves were used to estimate tolerance limit (T) and/or damage threshold and indicated that T was 110 and 400 nematodes/kg soil for fresh shoot and dry seed weights, respectively, for plants grown in KK region (Fig. 1), while T was 105 and 153 nematodes/kg soil for the fresh shoot and seed weights, respectively, at the KE region (Fig. 2).

Effect of nematodes on the chemical composition of seeds. Oil, protein, carbohydrate and phenolic compound contents are presented in Table III. Data indicated that the oil content of the sunflower seeds was greater in the KK region than in the KE region. In both regions, seed oil contents were gradually reduced as the nematode inoculum increased and the reduction was more at KK region than at KE region. The observed reductions in oil content were 9.5% and 11.1% at 10,000 and 20,000 nematodes/kg soil, respectively, at KK region, and 4.2% and 4.8% at the KE region.

The protein content of oil cakes from plants grown in the KE region was more than that of oil cakes from the KK region, but gradually decreased with increasing nematode inoculum in both regions, with the reduction being greater in the KE region than the KK region. At the highest nematode density (20,000/kg soil), the reductions were 4.9% and 17.4% for the KK and KE regions, respectively.

Total carbohydrates and soluble carbohydrates were also influenced by nematode infestation. They tended to increase with increasing nematode inoculum at the KK region, but at the KE region the total carbohydrates tended to decrease with increasing nematode inoculum, while soluble carbohydrates were not affected by nematodes.

Total phenolic compounds increased following nematode infection compared with uninfected plants grown in the KK region. In the KE region, total phenolic compounds were not significantly influenced by nematode inoculum level.

Effect of nematodes on fatty acid composition. Sunflower seed oil consisted of seven fatty acids (data not reported), four of them unsaturated (C18:1, C18:2, C18:3 and C20:1) and the rest saturated (C16:0, C18:0, and C20:0). Oleic C18:1 and linoleic C18:2 acids are the predominant unsaturated fatty acids, whereas palmitic acid (C16.0) was the most abundant saturated fatty acid.

Although statistical analysis could not be performed, due to the limited numbers of determinations, it would

appear that the oil obtained from KK region was characterized by lower oleic acid content (32.52%) and higher linolenic acid content (3.04%) compared with oil obtained from KE region, which had high oleic acid content (50.46%) and low linolenic acid content (0.47%). Also, oleic acid increased in the oil obtained from infected plants compared with that obtained from healthy plants in both regions while, on the contrary, linolenic acid decreased in the oil from infected plants compared with that from healthy plants, and this reduction was more pronounced in seed from the KK region. However, increasing the nematode density did not seem to affect the oil composition in either locality (data not reported).

DISCUSSION

The results of our study demonstrated that sunflower cv. Sakha 102 is susceptible to the root-knot nematode *M. incognita*, which negatively affected its growth and yield at both regions, although to a different extent. Greater reduction in plant growth and seed yield occurred at the KE region than at the KK region. These differences may be due to the differences in the biotic and abiotic factors prevalent in the two localities, since soil fertility, structure, temperature and microbial activity have great influences on both sunflower growth (Karunajeewa *et al*., 1989) and the severity of symptoms from nematode attack (Stirling, 1991).

In our study, tolerance limits of sunflower to the nematode were 110 and 400 eggs and juveniles per kg soil for shoot and dry seed weights, respectively, at the KK region and 105 and 153 nematodes/kg soil, respectively, at the KE region. Sasanelli and Di Vito (1992) estimated tolerance limits of sunflower to *M. incognita* of 1850 eggs and juveniles per kg soil for top weight in a pot experiment, while Di Vito *et al*. (1996) found that in Italy the tolerance limit of sunflower grown in microplots to *M. javanica* (Treub) Chitw. was 740 eggs and juveniles per kg soil for seed yield. The observed differences could be due to different environmental conditions in Egypt and Italy, the use of different cultivars or differences of environment between pot and microplot experiments.

Significant reductions in seed yield occurred at an inoculum level of 1,000 nematodes/kg soil at KE region (6.6%) and at an inoculum of 10,000 nematode/kg soil at KK (18.4%) region. Whether or not these yield reductions have economic significance was not assessed and, therefore, more studies are necessary to estimate the economic threshold under Egyptian agro-economic conditions to serve as a basis for management of the nematode.

Data also showed that the chemical composition of the seeds differed according to regions. This may be due to the differences in environmental conditions, as the chemical composition of sunflower seeds depends on weather, soil and how the crop is grown (Senkoylu and Dale 1999). Oil and protein contents of the seeds decreased with increasing nematode infection. Similar results were obtained by Di Vito *et al*. (1996) and Prasad and Narayana (1999), who found that oil and protein contents of sunflower seeds were reduced by *M. incognita* infection. Also, Sivakumar and Seshadri (1971) found that a reduction in oil content of castor infected with *Rotylenchulus reniformis* Linford *et* Oliveria increased with increasing nematode inoculum.

Total carbohydrates were also influenced by nematode infestation but did not show a constant trend, as they increased in the infested plants in KK region but decreased in KE region. Total phenolic compounds increased in the infested plants grown in KK region and were not affected in plants grown in KE region. These differences may be due to the differences in the climatic conditions prevalent in the two regions, as KK region is located in the south and is near to the desert, while KE region is located in the north and in the Nile Delta, where mean daily temperature is lower. Also, the thermal stress in KK may have induced plants to produce more phenolic compounds as a defence reaction against both environmental stress and nematode infection.

The unsaturated fatty acids differed according to both nematode infection and locality. The oil produced in the KK region was characterized by lower oleic and higher linolenic acid than that of the KE region. These differences may also be due to differences in the environmental conditions (El-Nikeety 1981). Nagoa and Yamazaki (1983) reported that there was a strong correlation between oleic or linolenic acid contents and temperature during maturation of sunflower seeds. Although the oil content of the seeds decreased at all levels of nematode inoculum, the fatty acid composition of the oil (quality) seem to be improved as oleic acid increased and linolenic acid decreased, making the oil more suitable for frying and more resistant to autoxidation (Shahidi, 1999; Weber, 1981).

In general our findings indicate that the growth, yield, oil and protein contents of seeds of sunflower plants can be severely damaged if the crop is established in root-knot nematode infested soil and, therefore, appropriate management measures should be adopted to control the nematode.

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