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Yield and oil quality of sunflower infected with the root-knot nematode, *Meloidogyne arenaria*

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Abstract: Relationship between growth, yield, chemical composition of sunflower seeds, oil quality and the root gall index (GI) caused by the root- knot nematode, *Meloidogyne arenaria* was studied in sandy soil under field and drip irrigation condition. Both growth and seed yield were negatively affected by nematode infection. A significant reduction in seeds yield 35.4%, 42.0% and 43.4% was obtained at 3,4 and 5 GI, respectively. Seed content from protein, N,P, Mn and Zn decreased with increasing nematode damage (GI), in opposition to carbohydrates and Fe content which increased with increasing nematode damage, while oil and K contents were not affected. Sunflower seed oil consisted of six fatty acids, four of them were saturated (myristic, palmitic, stearic and arachidic) and two unsaturated (oleic and linoleic). The unsaturated fatty acids were predominant, as they formed about 91% from total fatty acids, while saturated formed about 9%. The poly unsaturated linoleic acid alone formed about 62% from total fatty acids in the oil. The saturated fatty acids, myristic and palmitic increased in oil of seeds of plant infected with nematodes, while stearic acid decreased. The polyunsaturated fatty acid (linoleic) increased in oil seeds of infected plant with nematodes, while oleic acid decreased compared with those in oil of healthy plants. Tocopherol (Vitamen E) concentration was more in oil of the infected plants with nematodes than that in oil of healthy sunflower plants.

Keywords: Sunflower, yield, oil quality , tocopherol, root knot nematode.

Introduction

Sunflower, *Helianthus annuus L.*, is considered one of the most important oil crops in the world, as its seeds contain 32% to 45% edible oil[1]. This oil is rich in poly unsaturated fatty acids which form a vital part of the human diet[2]. Also sunflower oil is a source of vitamin E (tocopherol) which has a protective effect against cardio-vascular diseases and cancer[3]. Sunflower oil has also other uses in medicine and wood industry; moreover its oil- cakes are used in livestock food and as soil amendment.

In Egypt as well as in many parts of the world, sunflower plants are infected by many plant parasitic nematodes. Among of them, the root knot nematode species are the most important nematodes affecting sunflower causing substantial damage to its growth and yield. Losses in yield of sunflower infected with the root- knot nematodes ranged from 16.4% to 100% according to nematode species and population density[4,5,6,7,8,9,10].

The effect of the root-knot nematode *M. arenaria* on sunflower is laking in Egypt especially in sandy soil irrigated by drip irrigation; moreover the effect of nematodes on saturated and unsaturated fatty acids, (essential fatty acids) and on the vitamin E (tocopherol) is not evident. Therefore the objectives of the present work were to study (i) the relation between sunflower yield and damage caused by *M. arenaria*, (ii) the effect of

nematode infection on chemical composition of sunflower seeds, on saturated and unsaturated fatty acids and on vitamin E content of oil (oil quality).

Materials and Methods

The experiment was conducted during 2014 season in sandy soil naturally infected with the root- knot nematode, *Meloidogyae arenaria*, and irrigated by drip irrigation system at the National Research Centre Experimental Station, Noubaria, Egypt. The soil was well prepared for planting, then seeds of sunflower cv. Sakha 53 were planted in 25-5-2014 (Summer season). Plants were thinned to two plants per each dropper, about two weeks after germination. Chemical fertilizers, N, P and K were applied according to the recommended rates for sunflower plants. At harvest in 11-9-2014, more than eighty plants were randomly selected and processed to assay the nematode damage and to record weights of fresh heads (discs) and dry seeds per plant.

Nematode damage assaying*:* Roots of the selective plants were processed to assay the nematode damage as root gall index (GI) as follows: Fifteen plant roots were categorized as GI=1 (no galls), sixteen as GI=2 (1-25% root galling), seventeen as GI=3 (26-50%), seventeen as GI=4 (51-75%), fifteen as GI=5 (more than 75% root galling) according to[11].

Protein, carbohydrates and oil content determination: The content of crude protein in sunflower seeds was determined according to[12], total carbohydrates were determined according to[13], the oil content was done according to[14].

Mineral elements determination: Macro-elements N, P, and K and micro-elements Fe, Mn and Zn contents in sunflower seeds were determined according to method of[15].

Fatty acids determination and identification: The fatty acids composition of the oil and their relative proportions were determined quantitively by using gas chromatography of the methyl esters GC: Perkin Elmer Auto System XL, equipped with flame ionization detector FID and fused silica capillary column DB-5 (60m x 0.32mm i.d). Two injections were made from each sample. Oven temperature was maintained initially at 150°C and programmed from 150 to 240°C at rate 3°c/min held at 240°C for 30min. The injector temperature was 230°C and detector temperature was 250°C, the carrier gas was helium, flow rate 1ml/ min. Methyl esters of fatty acids were prepared from an aliquot of total lipid with 5% HCL in anhydrous methanel (w/w) according to method of[16]. 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 percent.

Tocopherol determination: HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA).

Statistical analysis: Data were subjected to analysis of variance and comparison between means was made using the least significant difference (LSD) test at P= 0.05. Also, fresh heads and dry seeds weights were plotted against nematode damage (GI) to depict both linear and quadratic regression lines, to calculate the regression equations and the correlation coefficients.

Results

Relationship between *M. arenaria* root- galling and growth and yield of sunflower:

Data presented in Figures (1&2) and Table (1) indicated that the fresh weight of heads and dry weight of seeds were negatively affected with nematode infection. A negative and significant correlation was found between nematode damage (GI) and dry seed yield as correlation coefficient (r) was -0.91 (Fig.1). The observed reductions in seed yield 35.4%, 42.0% and 43.4% were occurred at GI 3,4 and 5 while the expected reductions were 31.5%, 42.0% and 49.04%, respectively (Table 1). However correlation between GI and the fresh weight of heads, was negative (Fig. 2) but it was not significant at P= 0.05 (Table 1).

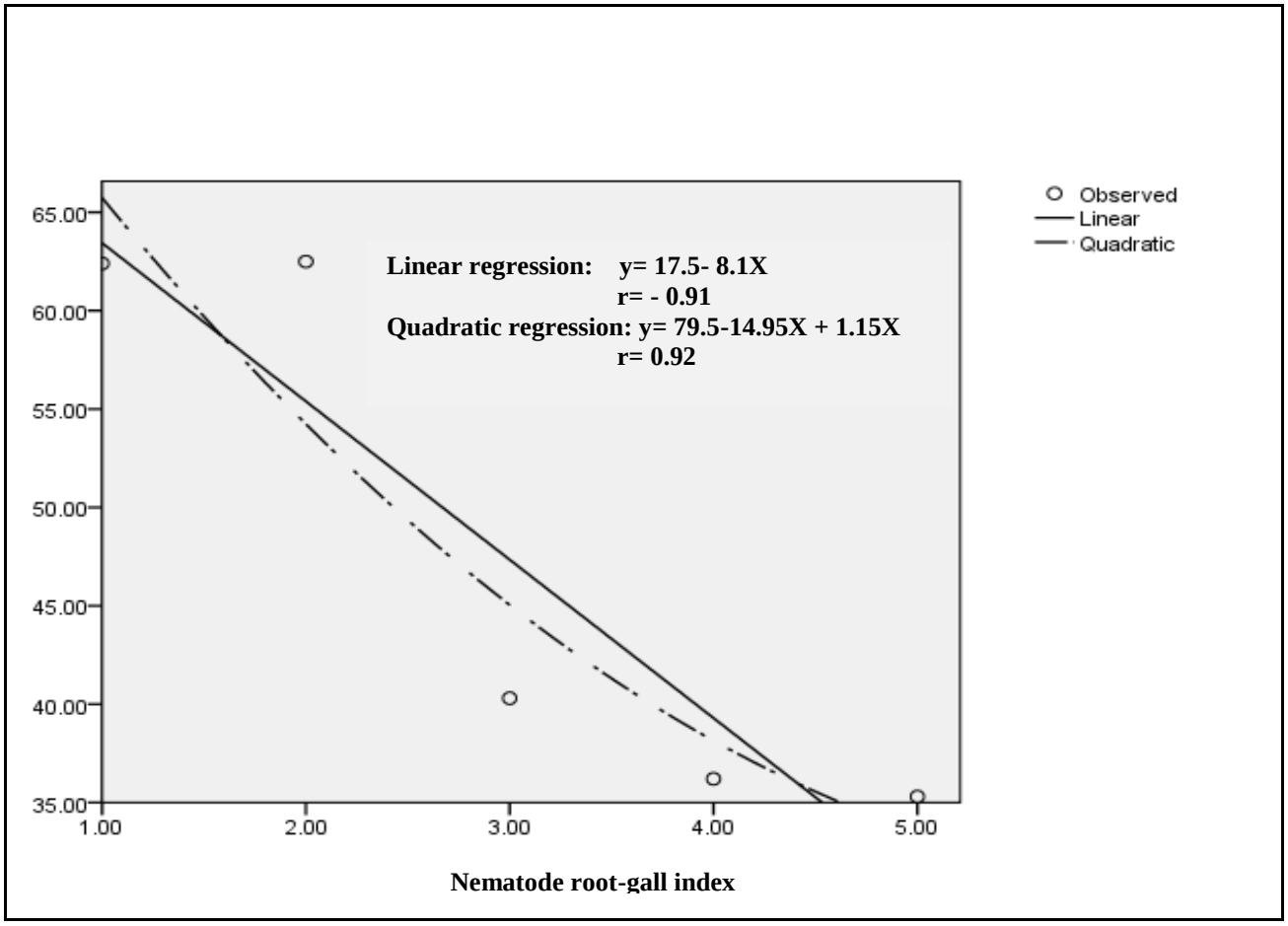

Fig 1: Relationship between the root gall index and the dry seed weight of sunflower infected with *M. arenaria*

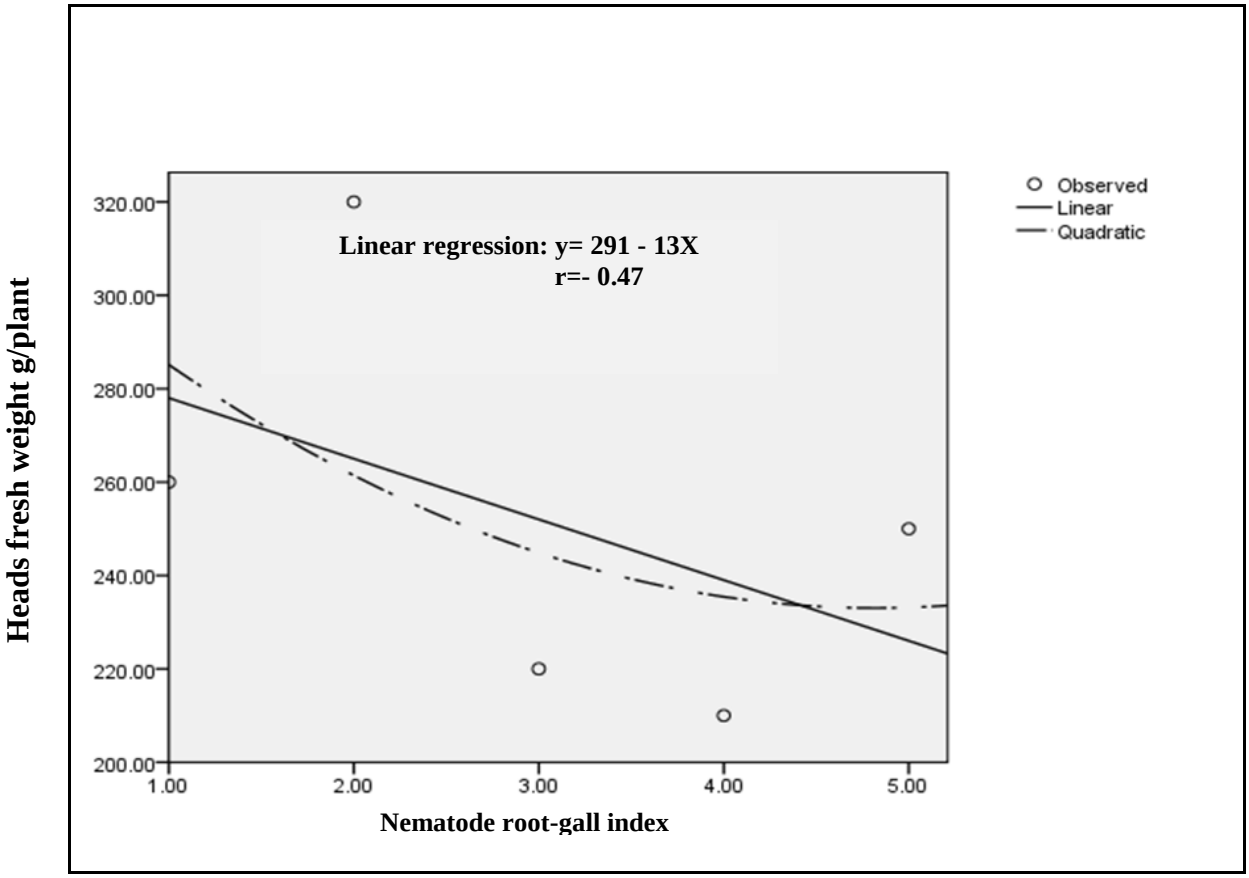

Fig 2: Relationship between the root-gall index and heads fresh weight of sunflower infected with *M. arenaria*

Table 1: Relationship between nematode damage (root galling) and growth and yield of sunflower.

Nematode root gall index (GI)	Fresh head (disc) weight		Dry seed weight g/ plant			
	g/ plant	red%	observed	red %	expected*	red/%
1	260	-	62.4	-	65.7	-
2	320	-	62.5	-	54.2	17.5
3	220	15.4	40.3	35.4	45.0	31.5
4	210	19.2	36.2	42.0	38.1	42.0
5	250	3.8	35.3	43.4	33.5	49.0
LSD (0.05)	NS		21.3	21.3		

NS = Not significant at P = 0.05
*= Calculated from the quadratic equation in figure 1.
red = Reduction.

Effect of nematodes on the chemical composition of seeds:

Oil, crude protein, and total carbohydrates contents are presented in Table (2). Data indicated that crude protein content was negatively affected by nematode infection, as it significantly (P=0.05) reduced by 19.5% and 20.2% at 4 and 5 GI, respectively. However, carbohydrates content was positively affected by nematode infestation, as it increased by 17.3% at 5 GI, while no significant effect of nematodes on oil content was obtained. As for macro – and micro-elements contents in seeds, it was found that N, P, Mn and Zn decreased with increasing nematode damage (GI). The highest reduction in N, P, Mn and Zn contents was 21.0%, 35.2%, 15.7% and 20.0%, respectively at 5 root gall index. However, it was observed that Fe content increased with increasing nematode infection, while K content was not affected (Table 3).

Table 2: Effect of *Meloidogyne arenaria* infection on oil, crude protein and total carbohydrates content of sunflower seeds.

Nematode gall index (GI)	Oil content %	Increase %	Crude protein %	Reduction %	Total carbohydrates %	Increase %
1	41.9	-	17.0	-	33.6	-
2	39.9	-	17.0	-	33.2	-
3	42.7	1.9	16.30	4.1	34.2	1.8
4	42.5	2.4	13.69	19.5	39.3	16.96
5	43.0	2.6	13.56	20.2	39.4	17.3
LSD 0.05	**NS**		**2.55**		**5.8**	

Table 3: Effect of nematodes on macro- and micro-elements content in sunflower seeds.

Nematode gall index (GI)	Macro-elements (%)			Micro-elements (ppm)		
	N	P	K	Mn	Zn	Fe
1	2.72	0.91	1.0	17.2	55.9	155
2	2.75	1.0	0.94	15.7	53.5	177
3	2.52	0.60	1.2	15.3	48.0	143
4	2.19	0.60	1.2	14.8	44.4	190
5	2.15	0.59	0.94	14.5	44.7	17.0
LSD 0.05	0.49	0.25	NS	3.8	8.1	

NS= Not significant (P= 0.05).

Effect of nematodes on fatty acids composition:

Data in Table (4) indicated that sunflower seed oil consisted of six fatty acids, four of them are saturated, myristic acid (C14:0), palmitic acid (C16: 0), stearic acid (C 18: 0) and arachidic acid (C20: 0) and two unsaturated oleic acid (C18: 1) and linoleic acid (C18:2). The unsaturated fatty acids (oleic and linoleic) were predominant forming about 91% of fatty acids content, whereas the saturated acids formed about 9%. It was found that 56.7% from the fatty acids was identified as polyunsaturated linoleic acid (C18: 2), 34.06% as oleic acid (C18:1) and 6.03% as palmitic acid (C16:0). Amongst saturated acids, it was observed that both myristic and palmitic acids increased in oil of the infected plant by 12.5% and 6.8%, respectively, compared with their content in the healthy plants, converse stearic acid decreased in infected plants by 16.2 %. Arachidic acid was not influenced by nematode infection. As to unsaturated acids, it was observed that oleic acid decreased in the oil obtained from infected plants by 31.0% while linoleic acid increased in the infected plants by 18.7% , also it was observed that SFA/UFA was 0.1 in oil of both infected and non- infected plants, wearous PUFA/ SFA increased from 6.1 in the healthy plants to 7.3 in the infected plants.

Table 4: Effect of *Meloidogyne arenaria* on fatty acids composition of sunflower seed oil.

Fatty acid	*Percentages*			
	Non- Infected plants	Infected plants	Increase/ decrease%	*Average*
Myristic	0.08	0.09	+12.5	*0.085*
Palmitic	6.03	6.44	+6.8	*6.24*
Stearic	2.97	2.49	-16.2	*2.73*
Arachidic	0.16	0.16	-	*0.16*
Total (SFA)	9.24	9.18		*9.21*
Oleic	34.06	23.5	-31.0	*28.76*
Linoleic	56.7	67.32	+18.7	*62.01*
Total (UFA)	90.76	90.82		*90.79*
SFA/ UFA	0.10	0.10		
PUFA/SFA	*6.1*	*7.3*		

SFA= Saturated fatty acids, UFA = Unsaturated fatty acids,
PUFA= Poly unsaturated fatty acids.

Effect of nematodes on tocopherol:

α and γ tocopherol (vitamin E) content in sunflower oil is illustrated in Figs. (4&5) and summarized in table (5). Data indicated that both α and γ tocopherols in the oil extracted from sunflower infected with nematodes was more than those in oil of healthy plants. As the concentration of α and γ tocopherols in oil of infected plants was 46.12 and 2.21, while they were 26.38 and 1.99 (mg/kg) in oil of healthy plants, respectively (table.5).

Sample 1:

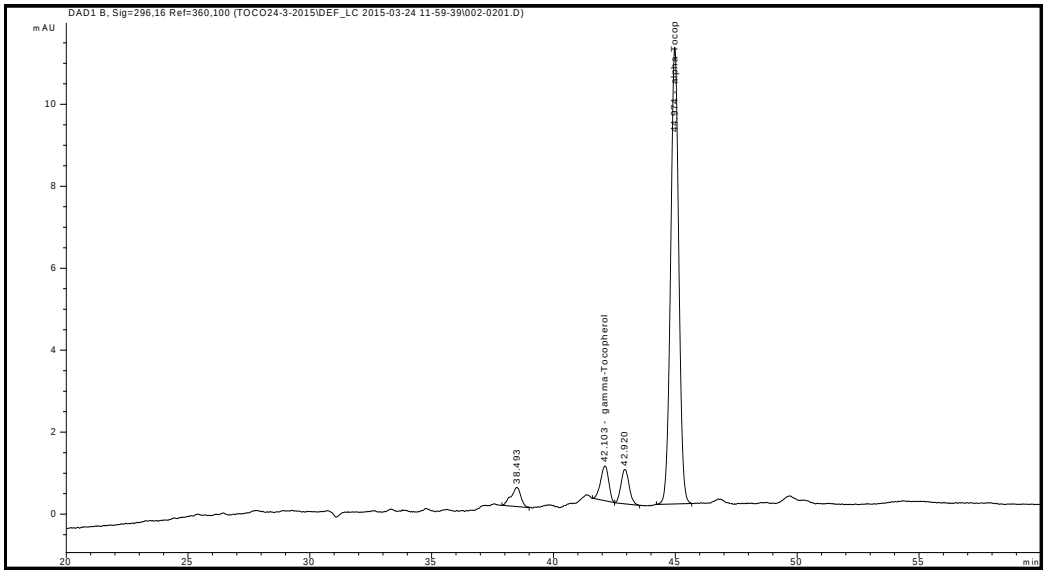

Fig. 3: α and γ tocopherols content in seed oil of sunflower not infected with the root-knot nematode.

Sample 5:

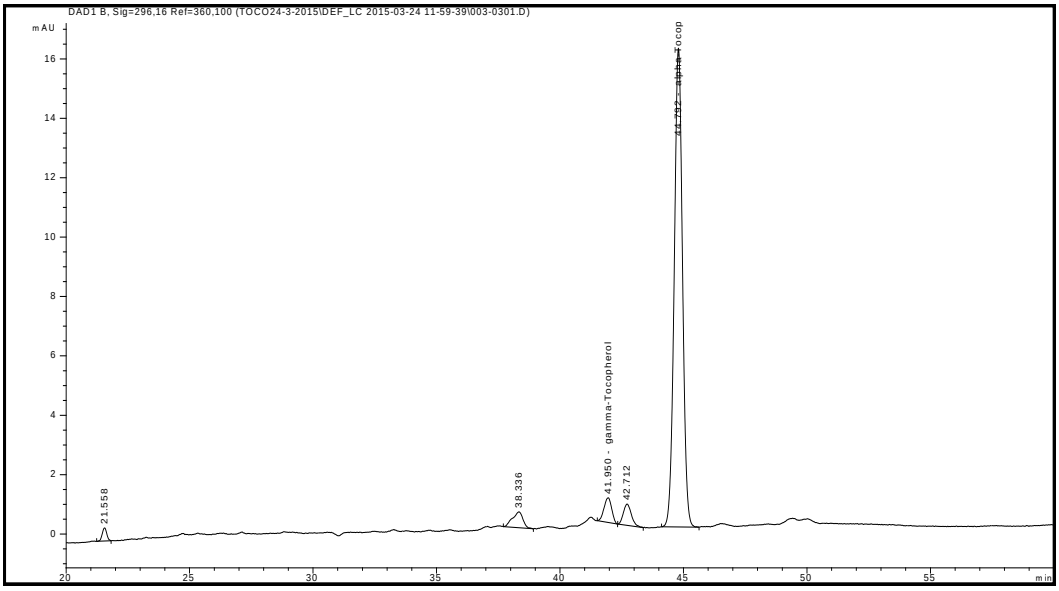

Fig. 4: α and γ tocopherols content in seed oil of sunflower infected with the root-knot nematode.

Table 5. Tocopherol concentration in oil of the seeds of sunflower plants infected and non-infected with nematodes.

Treatment	Concentration of tocopherols (mg/kg)	
	α –tocopherol	**γ -tocopherol**
Healthy plants	26.38	1.99
Infected plants	46.12	2.21

Discussion

Results of our study suggested that the root – knot nematode, *Meloidogyne arenaria* infects sunflower cv. Sakha 53 causing a significant reduction in its yield. The observed reduction in dry seed yield was 43.4% when sunflower plants were severely damaged by nematodes (GI= 5), whereas the expected reduction was 49.0% at same nematode damage. Reduction in sunflower yield was also reported in case of *M. incognita* and *M. javanica*, as the yield was reduced by 31.6% when plants were inoculated with *M.incognita* $2j_2$/g soil[10], while it increased by 16.4% when the infected plants with *M incognita* were treated by nematicide carbofuran[8]. Also, a dramatic loss in yield of sunflower was found when plants were infected with *M javanica*, as no yield was obtained when plants were infected with $64j_2$/g soil[7], while the yield increased by 78.3% when the infected plants were treated by phenamiphos nematicide[4].

Data also showed that chemical composition of sunflower seeds were influenced by nematode infection. The content of crude protein was decreased in seeds of infected plants. This reduction in crude protein content of infected sunflower seeds was also reported by[7,9,10]. Similar results were also found in case of peanut and common bean seeds infected with *M.arenaria*[17,18]. On the contrary, total carbohydrates content was increased in seeds of sunflower infected with nematodes. Increasing of carbohydrates was also reported in seeds of sunflower, potato tubers, peanut and common bean infected with root- knot nematodes[10,17,18,19]. The mineral element N, P, Mn and Zn content was found to be reduced in seeds of infected plants. A similar trend was found in cabbage leaves and common bean seeds infected with root-knot nematodes[18,20]. Although the oil content in sunflower seeds was not affected by nematode infection, both fatty acid composition and vitamin E in oil were differed, as the polyunsaturated fatty acid (linoleic) content increased in oil of infected plants compared with that in healthy ones. This increase in the essential fatty acid (C18: 2) makes oil more suitable for using in coking and in fresh salad but less suitable for frying[21]. On the other hand, increasing of tocopherol in oil makes it more resistant to autoxidation, as it prevents the rancidity of oil during storage, thus increasing its shelf-life[22,23]**.** In general our findings indicated that the growth and yield of sunflower is severely damaged by root knot nematode, *M. arenaria*, also the chemical composition of seed (seed quality) as well as fatty acids composition and vitamin E (oil quality) possibly be affected.

References

1. Hill A. F., ed. (1952). Economtic Botany. Second Edition by the Mc Graw- Hill Book Company, Inc. New York. 730 pp
2. Jeffcoat R. (1979). The Biosynthesis of Unsaturated Fatty Acids and Its Control in Mammalian Liver, Essay Biochem., 5: 1-36
3. Rader J.I., C.M. Weaver, L. Patrascu, L.H. Ali, G. Angyal. (1997). Food chem. 58(4): 373
4. Rich J. R. and V.E. Green (1981). Influence of *Meloidogyne javanica* on growth and yield of oil seed sunflower. Nematropica, 11 (1): 11-16
5. Sasanelli N. and M. Di Vito (1992). The effect of *Meloidogyne incognita* on growth of sunflower in pots. *Nematol. Medit*, 20: 9-12.
6. Sasanelli N., N. Vovlas and T. D' Addabbo. (1992). Influence of *Meloidegyne javanica* on growth of sunflower. Afro. Asian Journal of Nematology, 2: 84-88
7. Di Vito M., G. Zaccheo, C. Della Gatta and F. Catalano (1996). Relationship between initial population densities of *Meloidogyne javanica* and yield of sunflower in micro plots, Nematologia Mediterranean, 24: 109-112
8. Devappa V., K. Krishnappa and B. M. R. Reddy (1998). Estimation of avoidable losses in yield due to root – knot nematode, *Meloidegyne incognita* in sunflower .Indian J. Nematol. 28: 95-96

9. Prasad D. and R. Narayana (1999). Effect of *Meloidogyne incognita* Race- 1 on the oil content of sunflower. Annals of Plant Protection Sciences, 7: 116-117
10. Korayem A.M., Mona G. Daweed and M.M. M. Mohamed (2009). Growth, yield and chemical composition of sunflower seeds in soil infested with different population densities of root – knot nematode. *Nematol. Medit.*, 37: 187-192
11. Barker K. R. (Chariman) (1978). Determining nematode population responses to control agents. Pp. 114-125. In: Methods for evaluating plant fungicides, nematicides and bactericides, E. I. Zehr. ed, Am. Phytopathol. Soc., St Paul, Minn, 141 pp
12. A.O.A.C. (1990). Official methods of analysis. 20th Edition. Association of Official Analytical Chemists. Arlington, Virginia, USA
13. Smith F., M.A. Gilles, J.K. Hamilton and P.A Godees. (1956). Colorimetric method for determining of sugar related substances. Analytical Chemistry, 28: 350
14. A.O.C.S. (1982). Official and Tentative Methods of American Oil Chemists Society,35 East Walker Drive, Chicago, Illinois, USA
15. Cottenie A., L. Verloo, L. Kiens, G. Velghe and R. Camerlynch. (1982). Chemical analysis of plants and soils. Laboratory of Analytical and Agrochemistry. State Univ. Ghent, Belgium
16. Fedak G., and I. De La Roche. (1977). Lipid and Fatty acid composition of barley kernels. Canadian Journal of Plant science, 57: 257
17. Korayem A.M. and Mohamed M.M.M. Bondok (2013). Damage threshold of root-knot nematode *Meloidogyne arenaria* on peanut in relation to date of planting and irrigation system. Canadian Jour. Plant protection, 1 (3): 115-122
18. Korayem A.M., M.M.M. Mohamed and S.M. El. Ashry. (2015). Damage threshold of *Meloidogyne arenaria* to common bean influenced by dates of planting. Pakistan Journal of Nematology, 33 (1): 87-92
19. Korayem A.M., M.M.M. Mohamed and S. D. Abou Hussein (2012). Damage threshold of root- knot nematode *Meloidegyne arenaria* to potatoes grown in naturally and artificially infected fields and its effect on some tubers properties. Jour. Appl. Sci. Research, 8 (3): 1443-1452
20. Korayem A. M., E. M.A. Noweer and M.M.M. Mohamed (2008). Thershold population of *Meloidogyne* species causing damage to some vegetable crops under certain conditions in Egypt. Egypt. Journ. Agronematol., 6 (2): 217-227
21. El- Nikeety M.M. A. (1981). Chemical and physical changes during oxidation and interesterification of vegetable oils. Ph. D. Thesis, Faculty of Agric., Cairo University, Egypt
22. Blekas G., M. Tsimidou, D. Boskou. (1995). Food chem. 52:289
23. Manzi P., G. Panfifi, M. Esti, L. Pizzoferrato. (1998). J. Sci. Food. Agric., 77:115

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