Comparative study on the integrated application of environmental friendly compounds and a chemical- nematicide in controlling root knot nematode Meloidogyne incognita infecting sunflower plants: a field study

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Abstract: A field study was set up at Kafre – Hakim village, Giza, Governorate, Egypt, to compare the protective value of applying environmental friendly compounds with a known chemical nematicide, oxamyl, for controlling the nematode Meloidogyne incognita infecting sunflower cv. Sakha 53 and their effects on crop production and oil contents. These compounds were 1) furfural,a natural nematicide, 2) amino green 11, commercial formula of amino acids, 3) NPK, an inorganic fertilizer. Data revealed that all the tested treatments resulted in variable significant decrease in root knot disease incidence associated with improvement in crop yield except the plants treated with furfural plus amino green 11 that showed a significant decrease in percentage of oil content. A significant decrease in shoot weight was observed by applying the combined treatment of furfural plus NPK. Moreover, oxamyl plus amino green 11 resulted in –12% reductions in shoot weight. From the present study it was concluded that better plant growth characters of sunflower e.g. shoot length, root weight, % oil content and reduction of the nematode root galls, egg masses and second stage larvae could be achieved by a single rather than combined treatments. This is to avoid signs of phytotoxicity that might occur such as decreasing the oil content, an observation that needs further investigation.

Keywords: Root-knot nematode, meloidogyne incognita, amino acids, furfural, oxamyl, eggplant

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1 Introduction

Sunflower (*Helianhus annuals* L.) is an important oil crop in Egypt. Root knot nematode of the genus Meloidogyne are recognized as the major cause of decreasing crop productivity after seed germination (Ploeg and Staplean, 2001; Mokabel, 2007; Abd-elgawad, 2014).

Although chemical nematicides effectively control parasitic nematodes, they are being blamed due to their environmental toxicity (Abad et al., 2008). It is therefore important to identify safe and effective control strategies that have low toxicity to crops, humans and animals.

An alternative to application of nematicides, different natural occurring compounds has been tried. Furfural (2-Furancarboxaldehyde) is a naturally occurring compound, present in essential oils. The efficacy of furfural as a natural nematicide has been recorded (Stephan et al., 2001; Steyn, 2006; and El-Mougy et al., 2008). Abdel-Kader et al. (2015) reported that the applied treatments of furfural reduced the number of Meloidogyne incognita juveniles

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in soil, galls and egg masses in roots of cucumber plants compared to untreated control under plastic house conditions. Residues of furfural have not been detected in plant tissue or soil after multiple applications, because the compound has a short half-life. It is degraded to carbon dioxide and acetic acid (Burger, 2005a).

Another alternative strategy in controlling nematodes is the use of amino acids. Some amino acids reduced the nemic disease like root knot of vegetables. Also, promising results were obtained when some amino acids were used in treating seeds or seedlings of soybean against M. incognita (Osman and Viglierchro, 1981).

Kim and Whang (2012) examined the control effects of amino acids extracted from chicken feathers on Meloidogyne incognita. They investigated the lethal effects of second stage juveniles for 19 types of amino acids. As a result, five amino acids, L-asparagine, L-aspartic acid, L-methionine, L- tyrosine and L- cystyne showed mortality rates more than 50%.

B-amino butyric acid (BABA) and ϒ-amino butyric acid (GABA), non-protein amino acids have induced resistance against broad range of disease – causing organisms including fungi, bacteria, virus and nematodes (Osman et al., 2008; Francis et al., 2009).

The approach of using inorganic fertilizers such as NPK to diminish nematode and maximize plant growth has been reported by (Ameen et al., 2013; Osman et al., 2015).

Integrated application of either natural nematicides or chemical nematicides with organic fertilizesr or commercial product of amino acids did not receive much attention in the available literature. Therefore, this study was designed to compare: the protective value of application of furfural natural nematicide, oxamyl a chemical nematicide in single or combined integrated regime with either NPK, an inorganic fertilizer, or the commercial product amino green11 in controlling root knot nematode Meloidogyne incognita infecting sunflower and crop production under field conditions.

2 Materials and Methods

This field experiment was conducted at Kafre-Hakim village, Giza Governorate, Egypt during the period of September 2015- January 2016. The experimental field was divided into plots each comprising rows of 6 m long and 50 cm apart and the distance between each plant was 50 cm. The experiment was set up in a completely randomized block design with 9 treatments designed as shown in Table 1 with 60 replicate (plants) for each treatment. Concentration, method and time of application and source are shown in Table 1. The normal cultural practices of growing sunflower plants were applied.

Table 1 Designed treatments for controlling the root-knot nematode Meloidogyne incognita infecting sunflower cv. Sakh 53 under field conditions

Treatments	Concentration	Method of applicationSingle treatment	Time of application	Source
1-Untreated Control				
2-Furfural*	7 mL L^{-1}	Soil drench	3 days before planting	National Research centre
3-NPK**	20 unit for each	Soil treatment	At planting	Hamza company
4-Amino green 11***	6 mL L^{-1}	Foliar spraying	3 weeks after planting	Dishner Company
5-Oxamyl	6 cm L^{-1} 24% liquid	Foliar spraying	Two times. The first one week after plants emerged and the second 15 days from the first spraying	Ministry of Agriculture
		Combined treatment: Methods & time of application: as individual treatments		
6- Furfural+ Amino green ll	7- Furfural +NPK			
8- Oxamyl +NPK	9- Oxamyl+ Amino green ll			

Note: * Commercial product, containing 90% furfural in liquid formulation; ** Commercial product containing nitrogen, phosphorous & potassium; *** Commercial product containing 20% amino acids, Fe 2.9%, Zn 1.4% and Mn 0.7%.

Seeds of sunflower were sown in each row at the beginning of September 2015. Initial population densities of Meloidogyne incognita were determined prior the sowing time from 250 g samples of well mixed soil from each row according to (Barker, 1985). Four months later, at harvest, five plants were chosen at random from every row were hand harvested for yield estimation, and the following data were recorded: plant height, shoot weight, disc weight, number of root galls and egg masses in 5 g roots. The final nematode soil population was extracted

as previously. The second stage juveniles ($j_{2)}$ were counted and average of three counts was taken to determine the final population densities of M. incognita in soil and expressed as juveniles/250 g soil. Data was subjected to analyses of variance and means were compared statistically according to Duncan (1951). A Percentage nematode reduction in soil was determined according to Henderson and Tilton formula (Puntener, 1981) as follows:

$$\text{Nematode reduction (\%)} = [1-(\text{PTA}/\text{PTB} \times \text{PCB}/\text{PCA})] \times 100$$

where, PTA = population in the treated plot after application; PTB = population in the treated plot before application; PCB = population in the check plot before application and PCA = population in the check plot after application.

Oil content determination:

To assess 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 (AOAC, 1990).

3 Results and Discussion

3.1 Effects on nematode population

Data presented in Table 2 revealed that all the tested treatments resulted in variable significant decrease in root knot nematode M. incognita in soil and roots of sunflower plants as compared to control untreated plants. Moreover, the yield of the crop indicated variable significant improvements in all the treatments except for furfural plus amino green 11 treatments.

The results indicated that application of furfural alone produced the greatest percentage reduction 40.0%, 68.0% in M.incognita root galls and egg masses respectively as compared to control untreated. Moreover, furfural combined with NPK treatments indicated the highest reduction 72.4% in M.incognita $j_2$in soil compared to control untreated plants. Although the combined treatment of furfural plus amino green 11 resulted in the lowest percentage decrease in M. incognita j_2 in soil yet, the suppression in j_2 in soil was obviously clear in amino green 11 single treatment. The percentage decreases in soil j_2 were 29.6% and 56.3% respectively as compared to control. As for the application of the recommended dose of oxamyl as single treatment the data indicated the second percentage reduction by 64.3% in M. incognita $j_2$in soil compared to control. Concerning the NPK treatment the data revealed percentage reduction by 46.6%, 32.0%, and 38.8% in M.incognita j_2 in soil, root galls and egg masses respectively as compared to control.

Table 2 Effects of Furfural natural nematicide, NPK, Amino green ll and oxamyl on sunflower plants cv. Sakha 53 infected with the root knot nematode *Meloidogyne incognita* under field conditions

Treatments	*No. of J_2 in 250 g soil			*No. of root galls in 5 g roots		*No. of egg masses in 5 g roots		Disc weight		Plant heights		Shoot weights		Oil percent, %
	Intial pop.	Final pop.	% ** Red	*No.	% **Red.	*No.	% **Red	(g)	% *** Inc	cm	% *** Inc.	(g)	***% change	
Control	220	463 a	-	75	-	72a	-	291h	-	157d	-	642f	-	25.9
Furfural	230	248 e	50.4	45 c	40	23d	68	733b	151.8	218ab	38.9	1066a	158.6	31.2
NPK	245	275 d	46.6	51 bc	32	44be	38.8	441g	51.5	220a	40.1	870d	35.5	31.3
Amino green ll	250	230 e	56.3	65 ab	13.3	50b	30.5	779a	167.7	216ab	37.6	1020c	86.9	31.2
Oxaml	280	210 f	64.3	63 ab	16	55b	23.6	723b	148.4	195b	24.2	1035b	110.3	28
Furfural+Aminogreenll	210	311 c	29.6	55 bc	26.6	43bc	40.2	493f	69.4	202bc	28.7	1018c	83.8	23.1
Furfural+NPK	365	212 f	72.4	58 bc	22.6	33cd	54.1	523e	79.7	188bc	19.7	625g	–5.6	28.2
Oxamyl+NPK	360	349 b	53.9	64 ab	14.7	53b	26.3	622d	113.7	180c	14.6	820e	27.7	28
Oxamyl+Aminogreen ll	285	278 d	53.6	59 bc	21.3	48b	33.3	656c	125.4	175c	11.5	565h	–12	29.6

Note: Each value represent mean of five replicates; *No. = Number; **% Red. = Reduction; ***% inc. = Increase; ***%Change of control. Means followed by some letter (s) within a column are not significantly (Poo5) different according to Duncan's Multiple range test. Initial pop. = Initial population; Final pop. = Final population.

The data also indicated insignificant differences between either oxamyl combined with NPK, or amino green 11 treatments in suppressing the above mentioned nematode reproductive parameters. Application of oxamyl plus NPK combined treatment suppressed nematode multiplication by 53.9%, 14.7%, and 26.3% for M.incognita j_2 in soil, root galls and egg masses respectively as compared to control untreated.

Application of combined treatment of oxamyl plus amino green 11 exhibited 53.6, 21.3% and 33.3% reduction in M.incognita j_2 in soil, root galls and egg masses respectively as compared to control. Amino green 11 resulted in the lowest percentage reduction 13.3% in M. incognita root galls.

3.2 Sunflower plant growth and yield production

All treatments showed variable improvement in the disc weight, plant height, shoot weight and percentage oil content except for furfural combined with NPK treatment and oxamyl combined with amino green 11 which exhibited percentage reduction by –5.6% and –12.0% in shoot weight respectively as compared to control. Moreover, furfural combined with amino green 11 treatments, showed significant decrease in oil content by 23.1% compared to control untreated Table 2. Application of furfural alone resulted in percentage increase by 151.8%, 38.9%, 158.6%, and 31.2% in disc weight, plant height, shoot weight and oil content respectively as compared to control. The inorganic fertilizer NPK alone treatment produced 51.5%, 40.1%, 35.5% and 31.3% percentage increase in disc weight, plant height, shoot weight and oil percent respectively as compared to control untreated, Table 2.

Amino green 11 alone application exhibited the highest percentage increase 167.7%, 37.6%, 86.9% and 31.2% in disc weight, plant height, shoot weight and oil content respectively compared to control, Table 2. Moreover, data indicated insignificant differences in either oxamyl plus NPK or oxamyl plus amino green 11 treatments in increasing percentage oil content. It was increased by 28.0% and 29.6% respectively as compared to control untreated Table 2.

The results of the present study revealed that application of furfural single treatment produced significant reductions in the number of root galls, egg masses and M. incognita j_2 infecting sunflower plants. At the same time significant increase in yield production was recorded. These results agreed with the findings of Stephan et al. 2001, El-Mougy et al. 2008 and Abdel-Kader et al. 2015. In fact, many researchers conducted laboratory and field trials concerning the efficacy of furfural against soil microorganism (Rodreges, 2005a; Burger, 2005a; and El-Mougy et al., 2012). They recorded that furfural acts as natural bactericide, fungicide, and nematicide. It interacts directly with the cuticle of the nematode, stripping the protective layers which results in the cuticle swelling and disintegration and subsequently nematode death.

On application of the chemical fertilizers NPK alone the results exhibited significant decrease in the nematode reproduction, associated with a significant increase in plant growth and percentage oil content. These results are in agreement with the findings of (Osman et al., 2015; Farahat et al., 2012; and Ameen et al., 2013). They suggested that nitrogen present in the fertilizers was utilized in protein synthesis, while potassium and phosphorus are involved in many cellular functions belonging to production of energy, thus increasing plant tolerance to pathogenic organisms and development of disease. Moreover, the dual effects of NPK in improving plant growth and suppression of nematode population have been documented by Sinah and Neog (2003).

Although combination of furfural with NPK produced remarkable effects on reducing juveniles of M. incognita in soil by 72.4%, increase in disc weight and increase in oil content by 79.7% and 28.2% respectively, the lowest increase in plant height associated with significant decrease in shoot weight were observed. They were 19.7% and –5.6% respectively compared to control untreated.

On the other hand, combined treatment of furfural plus amino green 11 exhibited the lowest percentage reduction in juveniles in soil and decrease in oil content by 29.6% and 23.1% respectively compared to control untreated. Moreover, chemical nematicide oxamyl plus amino green 11 treatments resulted in a 12% decrease in shoot weight and 29.6% increase in oil content compared with control untreated.

The mechanism of the observed phytotoxicity in this experiment may be explained in the light of the fact that a synergistic toxic action of both treatments of furfural plus NPK or amino green 11 and oxamyl plus amino green 11 treatments at the applied dose levels. It could be assumed that the observed side effects on the plant growth and percentage oil content might not have occurred if the doses of the combination were reduced.

Application of amino green 11 alone produced a positive response in suppressing nematode reproduction and enhancement of plant growth and oil content. These results agreed with the findings of Hague et al. (2014) on their study on the effect of amino acids on root knot nematode M. javanica infecting tomato plants. They recorded that all the six amino acids viz; DL-methionine, DL- valine, DL- serine, DL- phenylalanine, L- proline and L- histidine showed significant response on plant growth characters with corresponding reduction in the number of galls, adult females, egg masses and juvenile stages within the tested plants. The highest plant growth and maximum reduction of galling indices of tomato were recorded in the DL- phenylalanine treated plants followed by L- proline and L- histidine. Reddy et al. (1975), reported that DL- amino acids had some contact toxicity in vitro to the second stage juveniles of M.incognita. Decrease in survival of J_2 juveniles was thought to be due to their lethal effects as many of the DL- amino acids like Dl- methionine and DL- phenylalanine as well as L-proline and L-histidine were found to be lethal to J_2 juveniles (Osman, 1993). Accumulation of different amino acids and enzyme activation in nematode- inoculated plants playa specific and vital role against nematode hatching, survival and gall formation as reported by (Prelman and Lu, 2000; Mohanty et al., 2001). The findings of these authors indicate that amino acids are lethal to the survival of J_2 juveniles of Meloidogyne spp.to varying degrees at different concentrations.

The present study showed significant percentage reduction in nematode reproduction parameters associated with a significant increase in plant growth response by using oxamyl alone treatment as a chemical nematicide. This is not surprising on the light of the previous finding of Osman et al. (2016) on their studies on the control of Meloidogyne incognita on potato plant. They recorded significant decrease in nematode reproduction parameters associated with a significant increase in potato yield by application of oxamyl. In view of adverse effects of the chemical nematicides on the environment, the alternative bio-control elicitors must be considered.

4 Conclusion

The present study revealed that better plant growth characters of sunflower e.g. shoot length, root weight, disc weight, oil content and reduction of root galls, egg masses and second stage juveniles' number of M.incognita could be achieved by single application of furfural as a natural nematicide, NPK as an inorganic fertilizer and the commercial product amino green 11. Application of these compounds might be more economical and less toxic to the environment. However, further research to determine the best dose-response of these compounds on sunflower and other economic crops is required.

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