Middle East Journal of Agriculture Research Volume : 09 | Issue : 02| April-June | 2020
EISSN: 2706-7955 ISSN: 2077-4605 Pages: 377-387
DOI: 10.36632/mejar/2020.9.2.29

Field evaluation of some organic and inorganic fertilizers for management of *Heterodera avenae* infecting wheat

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Received: 15 March 2020 / Accepted 20 May 2020 / Publication date: 30 May 2020

ABSTRACT

Two field trials were conducted in a wheat farm naturally infested with the wheat cyst nematode *Heterodera avenae*. The first trial was done to evaluate humic and fulvic acids as organic fertilizers, NPK and ammonium nitrate as inorganic fertilizers for their potential to manage *H. avenae* infecting wheat, in comparison with oxamyl (a chemical nematicide). The second trial was done to evaluate over rates of nitrogen (urea 46%) for their suppressive effect against *H. avenae*. Results indicated that all organic and inorganic fertilizers plus oxamyl clearly improved growth parameters of wheat and reduced nematode reproduction, except of NPK treatment. Plant growth and grain yield were greater by fulvic acid and humic acid, as compared with ammonium nitrate and oxamyl. Grain yield weight/1m^2 increased by 37.5%, 20.3%, 18.8% and 18.8% at fulvic acid, humic acid, ammonium nitrate and oxamyl, over control, respectively. The rates of nematode reproduction were reduced by all treatments except of NPK one, they reduced by 23.9%, 20.6%, 16.0% and 13.7% at oxamyl, ammonium nitrate, fulvic acid and humic acid, respectively, however no significant differences between them were detected. Results also showed that all urea rates increased wheat growth parameters and reduced nematode development and reproduction. Greater grain yield (615 g/1m^2) and harvest index (0.420) were obtained at 24 N rate. All nitrogen rates reduced both the 2-nd stage juveniles in soil 30 days after urea application and the rate of nematode reproduction. Greater reductions in J_2 and rate of nematode reproduction 49% and 40.9% were occurred at 48 N rate, respectively.

Keywords: Ammonium nitrate, NPK, urea, humic acid, fulvic acid, *Heterodera avenae*, wheat.

Introduction

Wheat is the most important cereal crop in Egypt. It is the principal source of bread which form the essential part of Egyptian food of low cost protein, lipids and amino acids. It is cultivated under irrigation, throughout most country but most intensively in Northern and Middle Egypt. The cultivated area was about 1.38 million hectares in 2018 growing season producing about 8.7 million MT (Anonymous, 2018). However, Egypt is the world's largest wheat importer in the world (FOA, 2012). Increasing yield of wheat production is possible through the recognition of phytonematodes affecting wheat productivity and application of suitable methods of their management.

The cereal cyst nematode (*Heterodera avenae* Woll.) is considered one of the most important nematode pests of wheat in the world (Smiley *et al*., 2005; Smiley, 2009: Smiley and Nicol, 2009). It has been reported in most countries of Europe and Middle East as well as Australia, Canada, China, India, Japan, New Zealand, Peru, South Africa, USA and countries in North Africa and Western Asia (Meagher, 1977; McDonald and Nicol, 2005; Qiao *et al*., 2016). The yield losses of wheat due to this nematodes were ranged between 15-92% depending on nematode density, the environmental factors and sampling protocols (Nicol and Rivoal 2008; Smiley and Nicol, 2009). The annual loss was equivalent to 70 US$ million in Australia, 4.5 US$ million in Europe, 9.6 US$ million in India (CAP International, 1999), and 3.4 US$ million in the Pacific Northwest region of USA (Smiely, 2009).

In Egypt *H. avenae* was first reported on wheat in Beheira province, northwest Egypt (Ibrahim *et al.,* 1986), Recently *H. avenae* was detected on wheat in Ismailia province northeast Egypt causing loss in grain yield estimated by 40% at initial nematode population density of 20J_2/1m1 soil in pot

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experiment (Baklawa *et al.,* 2012), and by 21.6% at 7 J_2/1g soil under natural infestation (Korayem and Mohamed, 2015).

Accordingly, controlling of this nematode is of considerable importance to improve the production and livelihood of the farming communities, especially in cropping system frequently suffer moisture or draught stress (Yadav *et al*., 2002). Strategies that have been used to manage cereal cyst nematodes include chemicals, cultivar resistance, biological control and cultural practices.

The use of chemical nematicides provided effective control against the cereal cyst nematode in many wheat-growing regions of the world especially under severe infestation conditions (Gurner *et al*., 1980; Brown, 1982; Shahina and Maqbool 1991; Dababat *et al*., 2015b). However, in the present day the cost and environmental concerns associated with these chemicals do not make them a realist economic option for most wheat growers (Dababat and Fourie, 2018).

Cultivar resistance is considered one of the best means for controlling phytonematodes, it requires no additional equipments or cost. It has been utilized on farms in some wheat-growing areas (Rivoal and Nicol, 2009; Smiley *et al*., 2013; Dababat *et al*., 2014; Cui *et al*., 2016; Pariyar *et al*., 2016b), but resistant cultivars are not always available. Besides applying of these cultivars needs an accurate knowledge of the virulence spectrum of the target nematode species and pathotypes since the cereal cyst nematodes have a number of pathotypes and intraspecific forms (Andersen and Andersen, 1982; Riggs and Schmitt, 1988; Müller, 1992). So the presence of mixture of parasitic phenotypes in the cereal cyst nematodes adversely affect the use of resistant cultivars and reduce their longevity (Riggs and Schuster, 1998). Moreover the use of resistance, especially derived from single dominant gene may cause disequilibrium in biological communities and possibly ecological replacement with other nematodes such as *Pratylenchu s*(Lasserre et al., 1994; Turner and Rowe, 2006).

The use of biological antagonists for controlling cyst nematodes is still considered promising method. Nematophagous fungi such as *Nematophthora gynophila Trichoderma longibrachiatum* and *pochonia chlamydosporium* were found to be effective for cyst nematode management (Kerry *et al.,* 1982b; Ismail *et al*., 2001; Holgado and Crump, 2003; Zhang *et al*. 2014; Baheti *et al*., 2017). However the use of these antagonists for cyst nematode management has never been commercially feasible .Biological control is an environmentally sensitive, and its ultimate use relies greatly on the agroecology of the cropping systems for persistence and effectiveness (Nicol and Rivoal, 2008). Also it is difficult to generate nematode-suppressive soils to control the cereal cyst nematode populations under economic damage thresholds (Dababat and Fourie, 2018).

Culture practices represent efficient methods in managing *H.avenae* and may be for other cyst nematodes. They include crop rotation clean fallow, summer ploughing and application of organic and inorganic soil amendments. The crop rotation is considered a good management tactic in terms of effectiveness and economics. Use of grass-free rotations using non-host crops or resistant cultivars are very useful for keeping *H.avenae* population below damaging levels. (Handa *et al*., 1976; Fisher and Hancock, 1991; Nombela *et al.,* 1998; Elkcioglu *et al*., 2004), but the effectiveness depends on the population density, ecological factors and the percentage of non host in the rotation. Besides, in many areas, rotation is not acceptable by growers because the alternate crops are not a profitable or because they require changes in machinery or practices that make them more expensive to grow (Riggs and Schuster, 1998). Deep ploughings during host summer months can reduce population densities of wheat cyst nematodes (Mathur *et al*., 1987), but it is not always economically and environmentally sound (Dababat *et al*., 2015b). Addition of soil amendments such as organic and inorganic fertilizers may be a good alternative strategy for managing phytonematodes and increasing the crop productivity. Effect of these fertilizers on nematode reproduction and the plant growth has been reported by many investigators (Swarup, 1986; Brown, 1987; Mathur *et al*., 1991; Sharma and Khan 1995; Akhtar *et al*., 1998; Maareg *et al*., 2000; Trifonova, 2001; Kheir *et al*., 2009; Farahat *et al*., 2010; Jothi and Poornima, 2017). The objective of the present research, was to evaluate some of organic and inorganic fertilizers as alternative methods for managing the wheat cyst nematode *Heterodera avenae* and their effect on wheat productivity under field condition.

Materials and Methods

Two experiments were conducted in a farm of loamy sand texture naturally infested with the cereal cyst nematode, *Heterodera avenae*, located in the 6th October Farm at Ismailia Province, Egypt. The farm is irrigated by central Pivot irrigation system. The first experiment was conducted to study the effect of some organic and chemical fertilizers, compared to a nematicide (oxamyl 24% L) on the nematode reproduction and wheat growth parameters. Area of the experiment was divided into three main plots of 60 m^2. Each main plot was also divided into six micro plots of 10 m^2 each. Three soil samples was obtained from each micro plot, and processed for *H.avenae* cysts analysis, according to method of Shephered (1970). Ten cysts were squashed according to method of Senihorst and Den Ouden (1966), and the total number of eggs + juveniles (J_2) per cyst was counted. In 20-11-2018 seeds of wheat (*Tritucum aestivum* L) cv. Misr-1 were planted. About 50 days after seed germination (in 10-1-2019) each main plot separately received six treatments as follows:

1- Humic acid at rate of 0.65 g/1m^2
2- Fulvic acid at rate of 0.65 g/1m^2
3- Ammonium nitrate (33.5% N) at rate of 66 g/1m^2
4- NPK (20-20-20) at rate of 0.6 m1/1m^2
5- Oxamyl (Vydate 24%) at rate of 6.0 m/1L water
6- Control (without treatments).

Humic and fulvic acids, NPK and oxamyl were dissolved in water and applied as foliar spraying, except of ammonium nitrate was applied as manual application. All treatments were arranged in a randomized block design. Shortly before harvest (in 20-4-2019) a composed sample was obtained from each replicate (micro plot) and processed for cyst analysis as previously mentioned.

The second experiment was carried out to evaluate different rates from nitrogen in the form of urea (46% N) for their suppressive effect against *H.avenae* reproduction and wheat productivity. The land was divided into three main plots of 50m^2, each one was divided into five microplots of 10m^2. Seeds of wheat cv. Misr-1 were planted in 1-12-2018. About 35 days after seed germination and before treatments (in 13-1-2019) a composed soil and root sample was collected from each microplot to assay the nematode initial population (Pi): the second stage juveniles. Then each main plot separately received the following nitrogen rates: 0, 12, 24, 36 and 48 g N/1m^2. Treatments were arranged in randomized block design with three replicates.

About 30 days after nitrogen application (in 12-2-2019), soil and root samples were collected from each replicate to assay the nematode population (J_2) in soil and roots. Juveniles were extracted from soil according to Christe and Perry (1951), and from roots by warring blender. At harvest (5-5-2019) a composed soil samples were collected from each replicate and processed for cyst analysis. Cysts were extracted from soils and their content from eggs+ J_2 were counted as previously mentioned.

Wheat growth parameters and yield:

At harvest the plant growth i.e. plant length (cm), weight of grain yield/ spike, grain yield per 1m^2, dry straw yield (g/m^2) were determined. The harvest index (HI) of treatments (the grain weight / total weight of grain + dry straw) was also calculated.

Statistical analysis:

Data from each experiment were subjected to analysis of variance (ANOVA), using SPSS statistical software. Comparison of means was done by use of Duncan's Multiple Range Test at 5% probability level.

Results

Effect of fertilizers on wheat growth parameters:

Effect of organic, inorganic fertilizers and oxamyl on wheat growth and yield is presented in Table (1). Data indicated that all treatments significantly increased growth and yield except of NPK treatment compared with control. Greater plant length and grain yield/spike were obtained by fulvic acid treatment (85.4 cm and 1.60g) followed by humic acid (81.6 cm and 1.49g), ammonium nitrate (81.2 cm and 1.45g) and oxamyl (80.0 cm and 1.45g), with corresponding increase over control of 13.9% and 18.5%,8.8% and 10.4%,8.3% and 7.4%,6.7% and 7.4% respectively. Greater weight of grain yield/1m^2 was occurred also at fulvic acid (440g) followed by humic acid (410g), and both of ammonium nitrate and oxamyl (380g), with corresponding % increase over control of 37.5%, 28.1%, 18.8% and 18.8% respectively. Same trend was also observed in case of the weight of dry straw and harvest index (HI). Greater % increases in dry straw weight and HI (17.1% and 0.50) were obtained by fulvic acid followed by humic acid (12.0% and 0.49), ammonium nitrate (9.3% and 0.48) and oxamyl (8.0% and 0.48), respectively compared to control. It was also observed that grain yield/1m^2 occurred at ammonium nitrate and oxamyl were equal.

Table 1: Effect of organic and chemical fertilizers and Oxamyl on growth and yield of wheat naturally infected with *Heterodera avenae*.

Treatments	Plant height (cm)	Inc. (%)	Grain yield per spike (g)	Inc. (%)	Grain yield (g)/m^2	Inc. %	Dry straw (g/1m^2)	Inc. %	HI
NPK	77.0a	2.7	1.40bc	3.7	500c	4.2	580c	3.6	0.46
Ammonium nitrate	81.2b	8.3	1.45b	7.4	575b	19.8	615b	9.8	0.48
Humic acid	81.6b	8.8	1.49ab	10.4	600 a	25.0	620a	10.7	0.49
Fulvic acid	85.4a	13.9	1.60a	18.5	615a	28.1	605a	8.0	0.50
Oxamyl	80.0b	6.7	1.45b	7.4	570b	18.8	610b	8.9	0.48
Control	75.0	-	1.35c	-	480c	-	560c	-	0.46

Data are average of three replicates.
Inc.: % increase over control.
HI: Harvest index (grain yield / total grain yield + dry straw)
Means in a column followed by the same letter are not significantly different according to Duncan's New Multiple Rang Test ($P< 0.05$)

Effect of fertilizers on *H. avenae* reproduction:

Effect of organic and inorganic fertilizers and oxamyl on nematode reproduction is presented in Table (2). A significant reductions in both nematode final population (no. cysts in soil at harvest) and rate of reproduction (Pf/Pi) were obtained by all treatments except of NPK, compared with control. Greater reduction in nematode final population (27.3%) occurred at oxamyl treatment followed by ammonium nitrate (19.2%), humic acid (17.3%) and fulvic acid (15.4%) compared to control. Minimum rate of reproduction occurred at oxamyl (2.33) followed by ammonium nitrate (2.43), fulvic acid (2.57) and humic acid (2.64) , with corresponding reduction of 23.9%, 20.6%, 16.0% and 13.7%, respectively compared to control. However no significant differences in the nematode final population and reproduction were found between them.

Table 2: Effect of organic and chemical fertilizers and oxamyl on *H. avenae* reproduction

Treatments	Nematode population			Rate of reproduction (Pf/Pi)	Decrease/ increase over control (%)
	Pi	Pf	Increase/decrease over control (%)		
NPK	9.8	27.0a	+ 3.8	3.21 a	+ 4.9
Ammonium nitrate	10.1	21.0b	- 19.2	2.43 b	- 20.6
Humic acid	9.5	21.5b	- 17.3	2.64 b	- 13.7
Fulvic acid	10.0	22.0b	- 15.4	2.57 b	- 16.0
Oxamyl	10.0	20.0b	- 27.3	2.33 b	- 23.9
Control	9.9	26.0a	-	3.06 a	-

Data are average of three replicates.
Pi: nematode initial population (no. cysts /100g soil) before planting, each cyst contained 60 eggs + J_2 in average.
Pf: nematode final population (no. cysts / 100g soil) at harvest, each cyst contained 70 eggs +j_2 in average.

Effect of over rates from nitrogen (urea) on wheat growth parameters:

Data presented in Table (3) showed that all nitrogen rates significantly increased the length of wheat plants, compared to control (zero N). The plant length increased with increasing N rates, as greater plant length (64.0 cm) was obtained by 48 N rate, and the lowest (57.6 cm) occurred at zero N (control). Data also indicated that all N rates significantly increased both grain yield per spike and grain yield /1m^{2}compared to control. Greater % increases in the grain/spike and grain yield /1m^2 (23.3% and 20.55%) occurred at 24 N rate, respectively. The dry straw also increased with increasing

N rates, as greater dry straw (890g) occurred at 48N dose, and lesser one(700g) occurred at zero rate , however, greater HI (0.42) occurred only at 24 N dose and lesser HI (0.40) occurred at the greater N rate (48 N).

Table 3: Effect of over nitrogen rates on wheat growth parameters

Nitrogen rates (g/1m²)	Plant height (cm)	Inc. (%)	Grain yield per spike (g)	Inc. (%)	Grain yield (g/m²)	Inc. (%)	Dry straw (g/1m²)	Inc. (%)	HI
0	57.6c	-	1.20c	-	490c	-	700b	-	0.411
12	62.0b	7.6	1.35b	12.5	607a	19.56	850a	21.4	0.415
24	62.5b	8.5	1.48a	23.3	615a	20.55	855a	22.1	0.420
36	62.9b	9.2	1.47a	22.5	608a	20.16	860a	22.9	0.414
48	64.0a	11.1	1.36b	13.3	595b	19.96	890a	27.1	0.400

Data are average of three replicates.
Means in a column followed by the same letter are not significantly different according to Duncan's New Multiple Rang Test ($P< 0.05$)
Inc.: % increase over control.
HI: Harvest index (grain yield / total grain yield + dry straw)

Effect over nitrogen rates on *H.avenae* infestation and reproduction:

Data presented in Table (4) showed that population and reproduction decreased with increasing N rate. A significant reduction in number of juveniles (J_2) in soil after 30 days from urea application occurred at all N rates, compared to control (zero N). Greater reduction in J_2 (49%) occurred at high 48 N rate , and lesser reduction (28%) occurred at 12 N rate. Also significant reductions in the nematode final population (no. cysts in soil at harvest) occurred at all N rates compared to control as greater reduction (40.0%) occurred at 48 N rate, followed by (37.1%) at 36 N rate, 34.3% at 24 N rate and 31.4% at 12 N rate. Rate of nematode reproduction also significantly decreased by all N rates compared to control, it was 1.91, 2.03, 2.30 and 2.40 at 48, 36, 24 and 12 N rates respectively with corresponding % reduction of 40.9%, 37.2%, 28.8% and 25.7%, respectively.

Table 4: Effect of over nitrogen doses on the wheat cyst nematode *H.avenae* reproduction.

Nitrogen rate (g/1m²)	Nematode population					Rate of reproduction (Pf/Pi)	Reduction (%)
	Pi	J_2	Reduction (%)	Pf	Reduction (%)		
0	650	100a	-	35a	-	3.23a	-
12	600	72b	28	24b	31.4	2.40b	25.7
24	660	60b	40	23b	34.3	2.30b	28.8
36	650	52b	48	22b	37.1	2.03b	37.2
48	660	51b	49	21b	40.0	1.91b	40.9

Data are average of three replicates.
Pi: initial nematode population (J_2/100g soil) before planting,
J_2: number of juveniles in 100g soil, 30 days after nitrogen application.
Pf: nematode final population (no. cysts / 100g soil) at harvest, each cyst contained 60 eggs +j_2.

Discussion

Effects of humic acid and fulivc aicd as organic fertilizers, ammonium nitrate and NPK as inorganic fertilizers on the growth parameters of wheat infected with the cereal cyst nematode *H.avenae* compared to oxamyl (a chemical nematicide) were studied under field conditions. Our results showed that all fertilizers improved wheat growth parameters and diminished nematode reproduction except of NPK one. Greater increases in wheat growth parameters were obtained by fulvic and huic acids, and lesser ones occurred at ammonium nitrate and oxamyl treatmetns. The beneficial effects of fulvic and humic acids on wheat growth may be attributed to their positive and important role in plant nutrition balance. Sangeetha *et al*., (2006) reported that humic materials have two direct and indirect effects on physiological and biochemical processes in plant and on physical, chemical and biological properties of soil. These beneficial effects of humic substances on plant growth have been well documented by many investigators, including stimulation of plant metabolism, increased enzyme activity, increased bioavailability and uptake of nutrients (Mylonas and McCants, 1980; Xudan 1986; Yu *et al*., 1998; Mackowiak *et al*., 2001; Atiyeh, 2002; Dursun *et al*., 2002; Nardi *et al*., 2002 & 2009; Serenella *et al*, 2002; Pilanal and Kaplan 2003; Chen *et al*. 2004; Turkmen, 2004; Jifon and Lester, 2009 Ye *et al*., 2009; Hartz and Bottoms, 2010; Parandian and Samavat 2012; Canellas and Olivares, 2014; Priya *et al*., 2014; Canellas *et al*., 2015; D'Addabbo *et al*., 2019).

H. avenae reproduction was also inhibited by applying humic and fulvic acids. The inhibitory effect of humic substances against plant parasitic nematodes has been reported by many investigators (Daneel *et al*., 2000; McBride *et al*., 2000; Saravanapriya and Subramanian, 2007; Jothi and Poornima 2017; Seenivasan and Senthilnathan, 2018).

The nematicidal efficacy of both fulvic and humic acids could be due to active compounds present in them such as carboxyl and phenolic compounds which are toxic to nematodes (Chitwood, 2002). Moreover, humic substances were classified as biostimulants, fertilizing, plant defence enhancement, increase of soil beneficial microflora, and as inducer of systemic resistance of plants (Sun *et al*., 2004; Yildirim, 2007; El-Ghamry *et al*., 2009; Cordeiro *et al*., 2011; Ibrahim *et al*., 2013; D'Addabbo *et al*., 2019). It means that antioxidants such as superoxide dismutase, catalase, reactive oxygen species and ascorbic acid in plant treated with humic substances be increased, these antioxidants may play an indirect role in nematode suppression (Arrigoni *et al*., 1979; Hassan, 1999; Korayem *et al*., 2012).

Application of different rates of urea (46%) improved wheat growth parameters and suppressed nematode reproduction. The dual effect of urea in improving plant growth and suppressing nematode infestation has been documented by many investigators (Rodriguez-kabana and king, 1980; Huebner *et al*., 1983; Mathur *et al*., 1991; Trifonova, 2001; Agu, 2002; Bamel *et al*., 2003; Moussa *et al*., 2006; El-Sherif *et al*., 2008; Ismail and Mohamed, 2012; Wei *et al*., 2012; Al-Hazmi and Dawabah, 2014; Osman *et al*. 2014). Suppression effect of urea against plant parasitic nematodes may be due to the compound is readily converted to ammonia by urease present in soil, a necessary conversion if urea is to be effective both as fertilizer and as a nematicide(Mojtaedi and Lownsbery, 1976; Huebner *et al.*, 1983; Rodriguez-Kabana, 1986). Urea is a good nematicide when applied at levels in excess of 300kg N/ha. However high levels of urea result in accumulation of nitrite in soil such compound is toxic to nematodes and plants at high levels of accumulation.

The integration of different methods for managing the cereal cyst nematodes was archived by many investigators (Handa *et al*., 1975; Trudgill, 1987; Mangat *et al*., 1988; Mathur *et al*., 1991; Pankaj *et al*., 2002). They reported that integration between cultural practices (such as summer ploughing , crop rotation, nitrogen fertilizers and chemical nematicides has given promising results for increasing crop yield and reducing nematode population. True integration of different controlling measures as nematicides, organic manures, nitrogenous fertilizers, biological antagonists and crop rotation including non-host plants or resistant cultivars, may be the best strategy for keeping nematode population densities under economic damage (Trudgill *et al*., 1992).

Conclusion

The wheat cyst nematode *H. avenae* is considered one of the most important nematode pathogen of wheat causing serious losses in the yield. So its control is of great importance, especially in the countries which wheat is often one of the major food staple. These nematodes present difficult problems in their management, since adult females lay down the eggs inside their bodies which upon death become cysts of hard protective wall. The eggs inside cysts may remain quiescent for many years. Also these nematodes are variable within species, and may undergo antibiosis to aid in their survival in the absence of their hosts. Good management strategy should be aimed at decreasing and maintain nematode population densities under economic damage and must be effective over a period of years or usable year after year. The present results give an approach to control the wheat cyst nematode *H.avenae* using organic ferlilizers (humic and fulvic acids) that are safer than nematicides. These results should be considered when designing an integrated nematode management program for wheat cyst nematodes.

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