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Abstract

The cereal cyst nematode *Heterodera avenae* is considered one of the major disease agents of wheat throughout the world reporting in most wheat–growing regions in the world and inducing loss in wheat yield ranging between 19-86%. The relationship between different *Heterodera avenae* densities either initial population (Pi) or final population (Pf) and wheat growth parameters was studied under greenhouse and field conditions. The length of wheat plants and grain yield were plotted against nematode densities for depicting linear and quadratic regressions. The nematode damage threshold (DT): minimum nematode density that inhibits plant growth and yield was estimated depending on quadratic regression. Expected losses in the grain yield were calculated based on quadratic equations. Under green house test, DT was 1.9 J₂/1g soil for grain yield, whereas it was 2.5 J₂/1g soil for plant length and 3.9 J₂ for grain yield, under fieldconditions. Expected loss in the grain yield was estimated by 80% at Pi of 8J₂/1g soil in green house test and by 79.9% at Pi of 9 J₂/1g soil in field test. Both length of plant and grain yield of two wheat cultivars viz. Gemmeza-12 and Misr-3 were negatively correlated with *H. avenae* final population (Pf): number of brown cyst in the soil at harvest. For cv. Gemmeza-12, expected loss in grain yield ranged between 8.7% at 40 cyststo 90.6% at 400 cyst /100g soil. For cv. Misr-3, expected loss in grain yield ranged between 3.1% at 25 cysts to 78.4% at 160 cysts /100g soil. Combination between the two methods of yield loss determination (based on Pi and on Pf) may be more better for obtaining a precise prediction of the crop loss, subsequently choosing the best appropriate strategy of nematode managements.

Key words: wheat, Heterodera avenae, damage threshold, grain yield.

Introductions

Wheat, *Triticum aestivum* L. is one of the most important sources of food worldwide, particularly in poor countries. Since it has been the staple food of about 35% of worldwide population (Oerke *et al.*, 1999; Shewry and Hey, 2015), forming an essential part of their food of low cost protein, lipids and amino acid. In Egypt, wheat is cultivated under irrigation throughout the country but most intensively in northern and middle provinces. It occupies about one-third of total winter crop area (about 1.38 million hectors) subjected to increase due to its importance in food security. The cereal cyst nematode, *Heterodera avenae* is considered one of the major disease agents of wheat throughout the world reporting in most wheat growing regions in the world (Meagher, 1977; Sikora, 1988; Evans and Rowe, 1998; Nicol *et al.*, 2003; McDonald and Nicol, 2005; Nicol and Rivoal, 2008; Smiley and Nicol, 2009; Qiao *et al.*, 2016) inducing loss in wheat yield ranged between 19-86% in different regions (Holgado *et al.*, 2003; Smiley *et al.*, 2005; Namouchi-Kachouri *et al.*, 2008; Peng *et al.*, 2007; Hassan *et al.*, 2010; Korayem and Mohamed, 2015). The loss in wheat yield was found to be correlated with the nematode population density, environmental and genotypic factors (Smiley and Nicol, 2009). It was ranged from 32.4% to 66.5% at initial nematode population (Pi) varying from 4.6 to 10.6 eggs/ml soil (Mathur *et al.*, 1986), from 16% to 40% at Pi varying from 5 J₂/ml soil (Baklawa *et al.*, 2012a). The grain yield was also reduced by 26% at Pi of 10.6 eggs + J₂ and 64 % at Pi of 25.3 eggs + J₂/g soil



(Namouchi-Kachouri, 2008). Economic damage threshold of *Heterodera avenae* was estimated by 20 eggs + J_2/g soil (Gill and Swarup, 1971), by 6 eggs/g soil (Swarup and Sosa-Moss, 1990) and by 7 J_2/g soil (Al-Hazmi *et al.*, 1999). Because management of the wheat cysts nematode is expensive involving growth of alternative crops and/or nematicides usage, information on damage thresholds and damage functions are a pre-requisite for making decisions on the nematode management.

Studying the relationship between a wide range of nematode initial population densities in soil (Pi) and growth parameters of host crop is very important in estimating the nematode threshold levels that is damage threshold (DT); the nematode population density at which a detectable yield loss occurs and economic threshold (ET); the nematode density at which cost of yield loss is equal to the cost of nematode control. Such information are basic to implementation of the most appropriate control strategies (Greco and Di Vito, 2009). Obtaining a wide range of white cysts nematode Heterodera avenae initial soil population densities in naturally infested fields is not always available. Correlating the crop yield with the final nematode population (Pf) at harvest is useful and may be a good method for estimating the yield loss in severely infested yields. Although the cereal cysts nematode Heterodera avenae has been reported in some localities of Egypt associated with wheat (Ibrahim et al., 1986; Baklawa et al., 2012b; Korayem and Mohamed, 2015), studies on estimation of yield loss in wheat due to Heterodera avenae is still neglected in Egypt, especially under natural infestation.

Therefore, the objectives of the present investigation were 1) to determine the relationship of different initial population densities of *Heterodera avenae* to wheat growthparameters under artificial and natural infestation, 2) to correlate growth and yield of two wheat genotypes with different final nematode population densities at harvest.

Materials and Methods

Nematode population and extraction:

Population of *Heterodera avenae* was collected from a field naturally infected with nematodes. This population was previously classified as pathotype Ha-13 (Baklawa *et al.*, 2012a). Nematode cysts were extracted from soil according to methods of Shephered (1970), dried at room temperature $(20\pm2^{\circ}C)$ and kept at 7°C until further use. Ten cysts were squashed according to Seinhorst and Den Ouden, (1966) and total numbers of eggs and juveniles (J₂) were counted under stereo microscope.

Plant Materials: Seeds of two wheat cultivars i.e.

Misr-3 and Gemmeza-12 were obtained from the Field Crop Research Institute, Ministry of Agriculture, Egypt.

Relationship between different nematode initial population densities and wheat growth parameters:

Greenhouse Test: The experiment was done in plastic pots 15 cm diameter containing 2 Kg sterilized loamy soil. Cysts of *Heterodera avenae* were added to the pots to give initial population density (Pi) of 0, 1, 2, 3, 4, 5, 6, 7, 8 eggs +J₂/1g soil. Each pot was planted with five plants of wheats cv. Misr-3. Five pots were used for each population density. Pots were arranged on greenhouse, fertilized with ammonium nitrate (1.5 g/kg soil) and watered as necessary with tap water. At harvest, length of plants and grain yield/pot were determined.

Field Test: The experiment was conducted in a field naturally infested with Heterodera avenae located in the 6th October Farm at Ismailia province, irrigated by central pivot irrigation system. Seeds of wheat Misr-3 were grown in 11/12/2017 growing season. About 35 days after wheat germination, 55 micro plots of 100×100cm were randomly selected for assaying the nematode initial population (Pi). Then three samples (soil with its wheat roots) were collected approximately 15cm deep using a trowel. Soil and roots were processed for the second stage juveniles (J2) analysis. J_2s were extracted from soil by sieving method (Cobb, 1918) and from roots by maceration method (Fallis, 1943). All micro plots received the recommended dose from N, P and K. At harvest, the average of plant length and yield were determined per each micro plot.

Relationship between different *Heterodera avenae* final population densities and wheat growth parameters:

Two experiments were carried out for determining the relationship of Heterodera avenae final population to wheat growth and yield. Both experiments were conducted in a naturally infested area located in Ismailia province during 2018-2019 growing season. First experiment was done on wheat cv. Gemmeza-12, planted in 10/11/2018, whereas the second was done on wheat cv. Misr-3 planted in 1/12/2018. About 60 days after seed germination (at the beginning of appearance of infection symptoms), 100 micro plots of $1m^2$ each with different infection symptoms were randomly selected for sampling (50 micro plots for cv. Gemmeza-12 and 50 micro plots from area of wheat cv. Misr-3). At harvest, three samples were collected from each micro plot and processed for brown cysts analysis as previously mentioned, average of plant length and grain yield per 1 m^2 were determined.

Statistical analysis: Regression analysis was used to relate the plant length and grain yield (dependent variable)

to initial or final numbers of nematodes (independent variable) in each experiment. The numbers of nematode cysts at harvest (final population) were transformed to $\log_{10}(x+1)$. The quadratic regressions were depicted, then the nematode damage thresholds (DT); minimum nematode density that inhibit plant growth and yield were estimated from quadratic curves; as the point at which plant length and/or grain yield begin to decrease compared to the point at zero nematodes. Expected losses in wheat yield at different nematode population were calculated from quadratic equations.

Relationship between nematode initial population



Fig. 1: Relationship between Heterodera avenae initial population density and plant length of wheat cv. Misr-3 under greenhouse condition.



Fig. 2: Relationship between Heterodera avenae initial population density and grain yield of wheat cv. Misr-3 undergreenhouse conditions.

at different <i>Heterodera avenae</i> initial population densities (Pi) under greenhouse and field conditions.							
Pi Eggs +J ₂ /1g soil	% Expected loss*						
	Plant length		Grain yield				
	GT	FT	GT	FT			
0	-	-	-	-			
1	-	-	-	-			
2	0.7	0.3	-	-			
3	4.5	2.2	1.4	-			
			1				

Results

densities (Pi) and growth parameters of wheat.

Table 1: Expected loss in cv. Misr-3 wheat growth parameters

D:	% Expected loss*					
Pi Eggs +J ₂ /1g soil	Plant length		Grain yield			
	GT	FT	GT	FT		
0	-	-	-	-		
1	-	-	-	-		
2	0.7	0.3	-	-		
3	4.5	2.2	1.4	-		
4	10.5	5.3	9.7	1.8		
5	18.7	9.5	21.6	10.6		
6	29.3	14.9	37.3	22.9		
7	42.0	21.5	56.8	38.5		
8	57.1	29.3	80.0	57.5		
9	74.1	38.2	-	79.9		
10	93.9	48.3	-	-		
* Based on the quadratic equations. (GT = Greenhouse test; FT = Field test)						

The relationship between Pi of Heterodera avenae and both plant length and grain yield was adequately described by quadratic regressions either under greenhouse or field conditions.

Greenhouse test: Data presented in fig. 1-2 indicated the plant growth parameters were correlated with Pi $(R^2 = 0.92$ for plant length and $R^2 = 0.70$ for grain vield). As Pi increased the plant length and grain vield decreased. Damage threshold (DT) were derived from quadratics curves, it was $1.9 J_2/1g$ soil for plant length and 2.7 J₂/1g soil for grain yield. Based on quadratic equations, the expected loss in plant length ranged between 0.7% and 93.9% at 2 J_2 and 10 $J_2/1g$ soil respectively and ranged between 1.4% to 80.0% at 3J₂ and 8J₂/1g soil for grain yield respectively (Table1).



Fig. 3: Relationship between Heterodera avenae initial population density and plant length of wheat cv. Misr-3 underfield conditions.



Fig. 4: Relationship between *Heterodera avenae* initial population density and grain yield of wheat cv. Misr-3 under field conditions.

Field test: A negative correlation between Pi and plant length (Fig. 3 with $R^2 = 0.81$) and grain yield (Fig. 4 with $R^2 = 0.62$) was also obtained. DT was 2.5 J₂/1g soil for plant length and it was 3.9 J₂/1g soil for grain yield. The expected loss in plant length ranged between 0.3% at 2 J₂ and 48.3% at 10 J₂/1g soil, whereas it ranged between 1.8% at 4 J₂ and 79.9% at 9 J₂/1g soil, for grain yield (Table 1).

Relationship of final population densities of *Heterodera avenae* and growth parameters of two wheat cultivars.

Relationship of final population densities of *Heterodera avenae* (number of brown cysts in soil at harvest) and growth parameters of two Gemmeza-12



Fig. 5: Relationship between *Heterodera avenae* final population density and plant length of wheat cv. Gemmeza-12 under field conditions.

Table 2: Expected loss in growth parameters of two wheat cultivars at different nematode final population densities (Pf) under field conditions.

PF	% Expected loss*					
No. of cyts/100g	cv. Gemmeza-12		cv. Misr-3			
Soil (log x+1)	PL	GY	PL	GY		
Zero	-	-	-	-		
1.0	-	-	-	-		
1.2	-	-	-	-		
1.3	0.7	-	-	-		
1.4	4.4	-	3.4	3.1		
1.6	13.2	8.7	17.1	16.9		
1.8	24.0	20.3	34.1	34.1		
2.0	36.9	34.3	54.5	54.5		
2.2	51.9	50.7	78.2	78.4		
2.4	68.8	69.5	95.1	-		
2.6	87.8	90.6	-	-		
* Based on the quadratic equations.						

and Misr-3 wheat cultivars were also adequately described by quadratic regressions. As to wheat cv. Gemmeza-12, both length of plants and grain yield were negatively correlated with the number of cysts in soil with $R^2 = 0.80$ for plant length (Fig. 5) and $R^2 = 0.86$ for grain yield (Fig. 6). The expected loss in plant length ranged between 0.7% at 20 cysts/100g soil and 87.8% at 400 cysts/100g soil, whereas expected loss in grain yield ranged 8.7% at 40 cysts to 90.6% at 400 cysts/100g soil (Table 2).

As to wheat cv. Misr-3 both plant length and grain yield were also negatively correlated with the number of



Fig. 6: Relationship between *Heterodera avenae* final population density and grain yield of wheat cv. Gemmeza-12 under field conditions.



Fig. 7: Relationship between *Heterodera avenae* final population density and plant length of wheat cv. Misr-3 under field conditions.

cysts in soil with $R^2 = 0.91$ for plant length (Fig. 7) and $R^2 = 0.91$ for grain yield (Fig. 8). As the number of cysts increased the plant length and grain yield decreased. Based on quadratic curves. The expected loss in plant length ranged between 3.4% at 25 cysts/100g soil to 95.1% at 255 cysts/100g soil, while it ranged between 3.1% at 25 cysts to 78.4% at 160 cysts/100g soil for grain yield (Table 2).



Fig. 8: Relationship between *Heterodera avenae* final population density and grain yield of wheat cv. Misr-3 under field conditions.

Fig 1: Linear regression: y=93-5.9x, $R^2=0.81$ Quadratic regression: $y=84.1+1.6x-0.95x^2$, $R^2=0.92$ DT = 1.9 J₂/g soil Fig 2: Linear regression: y=8.77-0.77x, $R^2=0.60$ Quadratic regression: $y=7.4+0.38x-0.14x^2$, $R^2=0.70$ DT = 2.7 J₂/g soil Fig 3: Linear regression: y=74.1-3.1x, $R^2=0.74$ Quadratic regression: $y=68.3+0.7x-0.4x^2$, $R^2=0.81$ DT = 2.5 J₂/g soil Fig 4: Linear regression: y=600.8-50.3x, $R^2=0.53$ Quadratic regression: $y=479.6+30.3x-8.1x^2$, $R^2=0.62$ DT = 3.9 J₂/g soil Fig 5: Linear regression: y=88.5-18.6x, $R^2=0.52$ Quadratic regression: $y=72.1-23.5x-18.4x^2$, $R^2=0.80$ Fig 6: Linear regression: $y=518.6+215.9x-152.5x^2$, $R^2=0.86$ Fig 7: Linear regression: y=75.6-17.3x, $R^2=0.50$

Fig 7: Linear regression: y=75.6-17.3x, $R^{2}=0.50$ Quadratic regression: $y=58.7+32.6x-24.3x^{2}$, $R^{2}=0.91$ Fig 8: Linear regression: y=783.3-179.7x, $R^{2}=0.5$ Quadratic regression: $y=607.2+341.2x-253.4x^{2}$, $R^{2}=0.91$

Discussions

The nematode damage to host plant depends on nematode population density in the soil (Seinhorst, 1965). Our study indicated that the relationship between wheat growth (plant length and grain yield) and Heterodera avenae inoculums density was adequately described by quadratic regressions and a negative correlation between them was detected. Thus, it was possible to estimate damage thresholds (DT) of the nematode to wheat growth from these polynomial regressions. DT (minimum nematode initial population in soil, which inhibit wheat grain yield) was lower in case of pot experiment (2.7 J_2 / 1g soil) than in field experiment $(3.9 J_2/1g \text{ soil})$. Decrease in DT of nematode on wheat grown in sterilized soil (pot experiment) may be due to absence of nematodeantagonistic microorganisms, which are in association with the rhizosphere of wheat grown in the natural soil (xenic soil), these antagonists play an important role in suppression of nematodes (Korayem et al., 2017 & 2019). A significant negative correlation between initial population densities of Heterodera avenae and wheat growth were also reported by many investigators (Mathur

et al., 1986; Romero et al., 1988; Rammah, 1994; Zancada and Althofer, 1994; Al-Hazmi et al., 1999; Namouchi-Kachouri et al., 2008). They concluded that losses in grain yield of wheat were mainly due to the reduction of number of spikes and weight of grains/spike. Expected loss in grain yield of wheat cv. Misr-3 caused by Heterodera avenae ranged between 1.4% to 80% at $2 \text{ to } 8 \text{ J}_{2}/1 \text{ g soil, under greenhouse conditions and between}$ 1.8% to 79.9% at 4-9 $J_2/1g$ soil, under field conditions. Same trend was obtained by other investigators in different countries. In Morocco Heterodera avenae caused 40-50% grain yield loss on wheat (Rammah, 1994) and up to 90% in Spain (Romero et al., 1988). Loss in wheat grain yield was also ranged from 32.4% to 66.5% at Pi ranged from 4.6-10.6 eggs/ml soil on India (Mathur et al., 1986) and ranged from 26% to 64% at Pi ranged from 10.6-25.3 eggs + J₂/g soil, in Tunisia (Namouchi-Kachori et al., 2008. Economic damage threshold of Heterodera avenae was estimated by 20 eggs + J_{2}/g soil (Gill and Swarup, 1971) and by 7 J₂/g soil (Al-Hazmi et al., 1999). Differences in wheat responses to Heterodera avenae (damage thresholds) may be attributed mainly to biotic and abiotic factors in different regions. These factors include climatic conditions, soil type and nutrients, wheat genotype, nematode pathotype and presence of other pathogens (Simon, 1980; Rivoal and Cook, 1993; Nicol et al., 2003). In most cases, estimation of the nematode initial density in soil is based on the number of brown cysts in soil before planting without take into consideration the viability of these cysts. Also many of hatching juveniles (j_2) may be a subject of attack by many antagonistic micro organisms in soil Therefore, such data can be used as general guidelines and cannot be depended upon to offer precise predication of crop losses on the basis of initial infestation. Hence, studying the relationship between different final Heterodera avenae population densities (Pf) at harvest (number of cysts/100g soil) and the grain yield of wheat may be a good method for determining the damage of nematodes on wheat.

Combination between the two methods of determination of yield loss (based on Pi and on Pf) may be more useful for obtaining a precise prediction of the crop loss, subsequently choosing the best appropriate strategy of nematode management.

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