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PHYTONEMATODES ASSOCIATING WHEAT IN NORTH EASTERN EGYPT AND PATHOGENICITY OF HETERODERA AVENAE ON CERTAIN CEREAL CULTIVARS

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ABSTRACT

A survey of plant-parasitic nematodes (PPNs) associating wheat in Ismailia governorate, north eastern Egypt was carried-out during 2016/2017 and 2017/2018 growing seasons. Results showed that seven PPNS genera and/or species were found associating wheat roots. These genera and/or species, in descending order of frequency, were: *Tylenchorhynchus* spp. (14%), *Helicotylenchus* spp. (10%), *Heterodera avenae* (8%), *Ditylenchus* spp. (5%), *Meloidogyne javanica* (4%), *Pratylenchus* spp. and *Xiphinema* spp. (3%, each). Two screening pot experiments, in two consecutive growing seasons (2016/2017 and 2017/2018) were carried out to determine the host suitability of 15 Egyptian cereal cultivars including bread wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and oat (*Avena sativa*) to the cereal cyst nematode, *Heterodera avenae*, under greenhouse conditions. Results of both experiments were very similar and showed that oat cv. Baladi and wheat cv. Giza 171 were highly susceptible, while wheat cvs. Masr 1, Masr 2, Masr 3, Sakha 95, Seds 1, Seds 12, and Shandawel 1 were susceptible. However, barley cvs. Giza 135, Giza 123, Giza 124, and Giza 125 were found to be moderately resistant to the tested nematode. *H. avenae* suppressed ($P \leq 0.05$) the dry weights of roots, shoots and spikes of the inoculated plants, compared to the non-inoculated checks. Another greenhouse pot experiment was carried out during the wheat-growing season 2018/2019 to determine the effect of different initial population densities (Pi) of *H. avenae* on the growth parameters of wheat cv. Giza 171 and on nematode reproduction. Results showed that as the nematode Pi increased, both the wheat growth parameters and the nematode reproduction factor (Rf) were decreased ($P \leq 0.05$).

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INTRODUCTION

Wheat, *Triticum aestivum* L., is one of the most important food crops all over the world. The crop has been cultivated for thousands of years in Europe, West Asia, and North Africa. It is grown on 20% of the global cultivated land area and is considered as the main food resource for 40% of the world's population (Braun *et al.*, 2010). In Egypt, the total wheat-cultivated area reached about 1.31 million ha with an annual production of

about 8.55 million tons and an average yield of 6.5 tons/ha (MALR, 2019).

Unlike, cereal production is greatly affected by many biotic and abiotic factors, including plant parasitic nematodes, whereas more than 33 plant-parasitic nematode species were found associated with wheat (Ibrahim, 2002). Cereal cyst nematode, *Heterodera avenae* Woll., is found to be the most economically important root pathogen on wheat (Dawabah *et al.*,

2010; Smiley and Nicol, 2009) and was firstly reported in Egypt by Ibrahim *et al.* (1986).

Cereal cyst nematode, *H. avenae*, causing severe economic yield losses, particularly in the areas where dry land and cereal monoculture systems are practiced (Al-Hazmi and Dawabah, 2009; Ibrahim *et al.*, 1999; Nicol *et al.*, 2003). Wheat grain yield losses due to *H. avenae* infection have been estimated to reach up to 15-20 in Pakistan (Maqbool, 1988), 40-90% in Saudi Arabia (Ibrahim *et al.*, 1999) and 25-50% in Australia (Nicol, 2002). As money wise, Barker *et al.* (1998) have reported that *H. avenae* caused losses of about \$78 billion to wheat worldwide.

The objectives of this study were to; 1- survey the plant-parasitic nematodes associated with wheat in Ismailia governorate, north eastern Egypt, 2- Screening different local Egyptian wheat, barley, and oat cultivars against the cereal cyst nematode, *H. avenae*, 3- Determining the effect of increasing population densities (Pi) of *H. avenae* on the growth parameters of the susceptible wheat cultivar "Giza171" and on the nematode reproduction.

MATERIALS AND METHODS

Survey study

A total of 240 soil and rhizosphere soil samples were collected from five different wheat localities in Ismailia governorate, north eastern Egypt. Samples were collected during the early vegetative growth of wheat, as well as flowering and pre-mature stages. Samples were kept in plastic bags, labelled and transported to our laboratory, where they were stored in a refrigerator at 5 °C until they were processed.

Nematode extraction and identification

Soil samples were mixed thoroughly and a weight of 250 g sub-sample from each sample was rendered for nematode extraction by sieving and decanting method according to Christie and Perry (1951). Root samples showing disease symptoms were cut into small pieces and incubated in Petri dishes containing distilled water for 2-3 days at room temperature to extract any migratory endo-parasitic nematodes (Young, 1954). Identification of nematode genera and/or species was performed, based on the morphology of adult and larval forms (Golden, 1971).

For the cyst nematode (*Heterodera*) extraction, a subsample of 100 g soil was air-dried at room

temperature and was processed for cyst extraction using sieving and floatation method described by Shepherd (1970). *Heterodera* species were identified based on the morphological features of vulvar cone structures of the brown cysts (Handoo, 2002).

Adult females of root-knot nematodes (*Meloidogyne* spp.) were picked-out from the infected roots whenever found and the perennial pattern of these females was cut-off and prepared for examination as described by Hunt and Handoo (2009). *Meloidogyne* species were identified based on the morphological features of the perennial pattern (Hunt and Handoo, 2009).

Nematode communities were analyzed using; frequency of occurrence (F.O %), population density (PD) and prominence value (PV) (Norton, 1979) where:

$$\text{Frequency of occurrence (F.O \%)} = \frac{\text{Number of samples containing a genus}}{\text{Total number of collecting samples}} \times 100$$

$$\text{Population Density (P.D.)} = \frac{\text{Total number of individuals of a genus}}{\text{Number of samples containing this genus}}$$

$$\text{Prominence value (PV)} = \text{nematode density} \sqrt{\text{frequency.}}$$

Greenhouse Experiments

1- Screening test

Wheat (*T. aestivum*) cvs. Masr 1, Masr 2, Masr 3, Seds 1, Seds 12, Seds 13, Sakha 95, Giza 171, Gemiza 11, and Shandwel 1, barley (*Hordeum vulgare* L.) cvs. Giza 135, Giza 123, Giza 124, and Giza 125, and oat (*Avena sativa* L.) cv. Baladi was screened for resistance and/or susceptibility to *H. avenae* in two greenhouse pot experiments. Seeds of the tested cereal cultivars were obtained from Field Crops Research Institute, Agricultural Research Center, Egypt.

Heterodera avenae-infected wheat plants were collected at the end of the growing season from a heavily infested wheat field in Al-Kassasen region, Ismailia governorate. Newly formed brown cysts on the roots of these plants were picked-up and stored in steam-sterilized dry sand. In the second season, cysts were extracted from the sand using the sieving and floatation method (Shepherd, 1970), and crushed in sterilized water to obtain eggs and newly-hatched second-stage larvae (J₂).

Seeds of wheat, barley, and oat cultivars were sown in plastic pots (4 kg soil) filled with steam-sterilized sand and clay mixture (4:1). Seven days after emergence,

plants were thinned to three seedlings/pot, and the soil of each pot was infested with *H. avenae* inoculum at 20000 eggs + J₂/pot pipetted in three holes around the base of each seedling at a depth of 5-10 cm. Plants were watered and fertilized with water-soluble N-P-K (20-20-20) fertilizer as needed. All treatments were replicated five times, and the pots were arranged in a complete randomized design (CRD) in the greenhouse at 20-28 °C at Ismailia Agricultural Research Station during the wheat growing season (2016/2017). The experiment was repeated typically in the second growing season (2017/2018). At the end of each experiment, plants were gently re-potted, and the roots were washed free of soil. Nematode cysts were picked-out and/or extracted from the roots and potting soil and crushed in a suitable volume of water to release eggs and J₂s. J₂s in the potting soil was also extracted and counted. Final nematode population (Pf) = no. eggs + J₂/pot was determined, and the nematode reproduction factor (Rf) was calculated where $Rf = Pf \div Pi$. The tested cultivars were rated on a 0-5 scale, based on the nematode reproduction factor. Plants with Rf = 0 were considered as resistant, Rf = 0.1-0.5 moderately resistant, Rf = 0.6-1.0 moderately susceptible, Rf = 1.1-5.0 susceptible and Rf > 5 highly susceptible (Ibrahim *et al.*, 2012). The dry weight of roots, shoots and spikes were also determined to reflex the effect of *H. avenae* on the tested cereal cultivars.

2- The effect of increasing population densities (Pi) of *H. avenae* on the growth parameters of wheat cv. Giza 171 and on the nematode reproduction

Twenty plastic pots (4kg soil) were filled with steam-sterilized sand and clay mixture (4:1) and seeded with the grains of wheat cv. Giza 171 (a highly susceptible cultivar to *H. avenae*). Seven days after emergence, plants were thinned to three seedlings/pot and inoculated with the designated Pi's (50000, 100000 and 150000 eggs + J₂/pot). Non-inoculated plants served as controls. Treatments were replicated five times. Pots were arranged in a complete randomized design (CRD) in the greenhouse during the 2018/2019 growing season, and the pots were watered and fertilized as needed.

One hundred and twenty days after nematode inoculation, the experiment was terminated. Plants were re-potted, and the roots were washed free of soil. Dry weights of roots, shoots and spikes and nematode reproduction factor were determined as mentioned earlier.

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) using MSTAT-C program version 2.10. Means were compared by Duncan's multiple range test at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Plant-parasitic nematodes associated with wheat plants in Ismailia governorate, north eastern Egypt

Data listed in Table (1) showed that seven nematode genera and/or species were found in association with wheat plants in Ismailia governorate. Frequency of nematode occurrence ranged from 3% for *Pratylenchus* spp. and *Xiphinema* spp. to 14% for *Tylenchorhynchus* spp. The highest population density was also recorded for *Tylenchorhynchus* spp., followed by *Helicotylenchus* spp. (180 and 120 individuals/250 g soil, respectively). Whereas the lowest population densities were recorded for *Xiphinema* spp. and *Pratylenchus* spp. (20 and 40 individuals/250 g soil, respectively). According to the prominence values (PV), *Tylenchorhynchus* spp. and *Helicotylenchus* spp. were found to be the most prominent in wheat fields in Ismailia governorate (PV= 67.35 and 37.95, respectively). These results are in coincidence with those previously reported by Korayem *et al.* (2019) who indicated that there were 14 nematode genera and species associating wheat plants in 12 governorates in northern and mid Egypt. They also concluded that *Tylenchorhynchus* spp., *Pratylenchus* spp., *Helicotylenchus* spp., and *Heterodera* spp. had the highest frequencies and population densities on wheat plants. This could be attributed to the host preference of wheat and those nematodes in the surveyed governorates, and also to similarity of the ecological and environmental conditions in the north and mid of Egypt.

M. javanica was previously found attacking the roots of the Egyptian wheat cvs. Giza 155 and Giza 157 causing significant losses of wheat root and shoot dry weights by Ibrahim *et al.* (1988). *H. avenae* has been firstly reported attacking wheat in Egypt in Rosetta, Behera governorate, north of Egypt (Ibrahim *et al.*, 1986). Twenty six years later, *H. avenae* has been reported from Ismailia governorate, Egypt (Baklawa *et al.*, 2012). *H. avenae* is one of the most damaging nematode pests of wheat in the Arab world (Ibrahim *et al.*, 1999; Namouchi-Kachouri *et al.*, 2007). Therefore, there is a strong need to study the effects of this nematode species on wheat under the Egyptian field conditions.

Table 1. Frequency of occurrence (F.O %), population density (PD) and prominence value (PV) of plant-parasitic nematode genera and species associated with wheat in Ismailia Governorate during the growing seasons 2016/2017 and 2017/2018.

Nematode	Frequency of occurrence (FO%) ¹	Population density (PD) ²	Prominence value (PV) ³
<i>Ditylenchus</i> spp.	5	60	13.42
<i>Helicotylenchus</i> spp.	10	120	37.95
<i>Heterodera avenae</i>	8	80	22.63
<i>Meloidogyne javanica</i>	4	60	12.00
<i>Pratylenchus</i> spp.	3	40	6.93
<i>Tylenchorhynchus</i> spp.	14	180	67.35
<i>Xiphinema</i> spp.	3	20	3.46

¹Frequency of occurrence (FO %) = (number of samples containing a genus and/or species / total samples collected) × 100.

²Population Density (PD) = number of nematodes per 250g soil for a genus and/or species/ number of samples containing this genus and or species. ³Prominence value (PV)= density $\sqrt{\text{frequency}}$.

Reaction of wheat, barley, and oat cultivars to *Heterodera avenae* under greenhouse conditions

Most of the tested Egyptian wheat cultivars herein were

found to be susceptible to *H. avenae* (Table 2). Moreover, all of these susceptible cultivars were assessed as intolerant.

Table 2. The reaction of certain wheat, barley, and oat cultivars to the cereal cyst nematode, *H. avenae* under greenhouse conditions in two successive seasons 2016-17 and 2017-18.

Plant Cultivar	Season 2016/17		Season 2017/18	
	(RF)*	Reaction**	(RF)*	Reaction**
<i>Wheat, T. aestivum</i>				
Masr1	4.70	S	4.30	S
Masr 2	4.40	S	4.10	S
Masr3	1.90	S	2.10	S
Sakha95	1.80	S	1.60	S
Giza171	5.10	H.S	5.10	H.S
Gemiza11	1.00	M.S	1.00	M.S
Shandwel1	2.60	S	2.40	S
Seds1	1.20	S	1.30	S
Seds12	4.40	S	4.30	S
Seds13	1.00	M.S	1.20	S
<i>Barley, H. vulgare</i>				
Giza135	0.22	M.R	0.25	M.R
Giza 123	0.30	M.R	0.30	M.R
Giza124	0.28	M.R	0.22	M.R
Giza125	0.48	M.R	0.49	M.R
<i>Oat, A. sativa</i>				
Baladi	5.70	H.S	5.90	H.S

Means are average of 5 replicates. *Rf= Nematode reproduction factor = Pf/Pi (15000 eggs +)2s/pot).

**HS= highly susceptible, S= susceptible, MS= moderately susceptible and MR= moderately resistant, based on a 0-5 scale where; plants with Rf = 0 were considered as resistant, Rf = 0.1-0.5 moderately resistant, Rf = 0.6-1.0 moderately susceptible, Rf= 1.1-5.0 susceptible and Rf > 5 highly susceptible.

According to the nematode reproduction factor (Rf) on the tested cereal cultivars, the cultivars were categorized into three groups as follows: the highly susceptible group (Rf > 5.0) included oat cv. Baladi, and wheat cv. Giza 171, the susceptible group (Rf = 1.1- 5.0) included wheat cvs. Masr 1, Masr 2, Masr 3, Sakha 95, Seds 1, Seds 12 and Shandwel 1, the moderately susceptible group (Rf = 0.6-1.0) included wheat cvs. Gemiza 11, and Seds 13, and the moderately resistant group (Rf= 0.0-0.5) included all the barley cvs. Giza135, Giza 123, Giza 124, and Giza 125. Baklawa (2013) already reported that wheat cultivars Seds 1 and Sakha 95 were susceptible to *H. avenae*. The effects of *H. avenae* on the tested wheat, barley, and oat cultivars in the two successive seasons

(2016/17 and 2017/18) are shown in Tables (3 and 4). There were significant reductions ($P \leq 0.05$) in the dry weights of roots, shoots and spikes of *H. avenae*-infected plants, compared to the non-infected ones. Oat cv. Baladi, wheat cvs. Baladi, Giza 171 and Masr 1 showed the highest reductions of spike weight in both seasons. In previous studies, *H. avenae* significantly reduced the spike weight, root dry weight and shoot dry weight of different wheat cultivars under greenhouse conditions (Al-Hazmi *et al.*, 1999; Baklawa *et al.*, 2012). Other studies also showed the ability of *H. avenae* to adversely affecting the growth of wheat under field conditions (Ibrahim *et al.*, 1999; Korayem and Mohamed, 2015; Namouchi-Kachouri *et al.*, 2007).

Table 3. Effect of *H. avenae* on the growth of different wheat, barley, and oat cultivars under greenhouse conditions, 2016/2017 growing season.

Plant cultivar	Root dry weight (g)			Shoot dry weight (g)			Spike dry weight (g)		
	CP ¹	IP ²	Red. ³ (%)	CP ¹	IP ²	Red. ³ (%)	CP ¹	IP ²	Red. ³ (%)
<i>Wheat, T. aestivum</i>									
Masr 1	5.0 ab	2.4 b	52	39.6 efg	23.3 f	41	3.0 bcd	1.8 g	40
Masr 2	5.0 ab	2.5 b	50	45.6 abc	27.0 cde	40	3.6 a	2.2 de	38
Masr 3	4.9 ab	2.7 b	45	39.6 efg	25.3 ef	36	2.8 de	1.9 g	32
Sakha 95	4.8 ab	2.8 b	41	46.3 ab	29.3 c	36	2.8 de	1.96 fg	30
Giza 171	4.4 bc	1.9 c	56	47.0 a	25.6 ef	45	3.1 bcd	1.8 g	41
Gemiza 11	3.5 d	2.8 b	20	36.0 h	26.6 de	26	2.7 de	2.2 de	18
Shandwel 1	5.0 ab	2.6 b	48	42.6 cde	26.3 de	38	2.7 e	1.8 g	33
Seds 1	4.4 bc	2.8 b	36	41.3 defg	25.6 ef	38	3.6 a	2.4 d	33
Seds 12	5.3 ab	2.7 b	49	42.6 cde	26.0 de	38	3.3 abc	2.1 ef	36
Seds 13	3.7 cd	2.4 b	35	42.3 cdef	28.3 cd	33	3.6 a	2.7 c	25
<i>Barley, H. vulgare</i>									
Giza 135	5.5 a	5.2 a	5	38.3 gh	37.0 ab	3	3.5 ab	3.2 a	8
Giza 123	5.2 ab	4.8 a	7	44.3 bcd	39.0 a	11	5.2 ab	4.8 a	7
Giza124	5.5 a	5.2 a	5	39.3 efgh	36.0 b	8	5.2 ab	4.8 a	7
Giza125	5.2ab	4.8a	7	39.0 fgh	35.3b	9	5.2 ab	4.8 a	7
<i>Oat, A. sativa</i>									
Baladi	1.6e	0.6 d	62	9.6 i	5.6 g	42	1.5 f	0.5 h	66

Means are average of 5 replicates.

Means followed by the same letter(s) in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

¹CP = Control plant.

²IP= Infected plant.

³Red. % = percentage of reduction = $\frac{CP-IP}{CP} \times 100$

Table 4. Effect of *H. avenae* on the growth of different wheat, barely, and oat cultivars under greenhouse conditions, 2017/2018 growing season.

Plant cultivar	Root dry weight (g)			Shoot dry weight (g)			Spike dry weight (g)		
	CP ¹	IP ²	Red. ³ (%)	CP ¹	IP ²	Red. ³ (%)	CP ¹	IP ²	Red. ³ (%)
<i>Wheat, T. aestivum</i>									
Masr 1	4.1 de	2.2 d	46	37.1 cd	23.8e	35	3.5 bc	1.9 f	45
Masr 2	4.0 de	2.2 d	45	35.3 cdef	24.6 de	30	3.4 bc	1.9 f	44
Masr 3	3.8 e	2.2 d	42	32.6f	26.3 cd	19	3.4 bc	2.3 cd	32
Sakha 95	4.3 de	2.5 bcd	41	33.3 ef	27c	18	2.9 de	1.9 f	34
Giza 171	4.5 cd	2.3 cd	48	38 bc	24 e	36	3.5 bc	1.9 f	45
Gemiza 11	4.2 de	2.8 b	33	34.3 def	29b	15	2.6e	2 ef	23
Shandwel 1	4.1 de	2.3 cd	43	34 ef	27 c	20	3.3 bcd	2.1 de	36
Seds 1	4.3d	2.6 bc	39	36 cde	26.6 c	26	3.6 b	2.4c	33
Seds 12	5.1 ab	2.8 b	45	34 ef	24.6 de	27	3.1 cd	1.9 f	38
Seds 13	4d e	2.6 bc	35	32.3 f	26.6 c	17	3.4 bc	2.3 cd	32
<i>Barley, H. vulgare</i>									
Giza 135	5.0 bc	4.3 a	14	41.3 a	39.6 a	4	3.6 bc	3.4 b	5
Giza 123	5.4 ab	4.2 a	22	41.0 ab	38.0 a	7	4.2 a	3.8 a	9
Giza124	5.2 ab	4.2 a	19	40.6 ab	38.3 a	5	4.2 a	3.9 a	7
Giza125	5.5 a	4.1 a	25	42.0 a	38.0 a	7	4.2 a	3.8 a	9
<i>Oat, A. sativa</i>									
Baladi	1.8 f	0.8 e	55	11.0 g	5.0 f	54	1.0 f	0.5 g	50

Means are average of 5 replicates.

Means followed by the same letter(s) in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

¹CP = Control plant.

²IP= Infected plant.

³Red. % = percentage of reduction = $\frac{CP-IP}{CP} \times 100$

The effects of increasing population densities (Pi) of *H. avenae* on the growth parameters of wheat cv. Giza 171 and on nematode reproduction

As *H. avenae* Pi increased, the dry weights of roots, shoots and spikes, as well as nematode reproduction factor (Rf), were decreased ($P \leq 0.05$) (Table 5). These results are in agreement with previous results of Al-Hazmi *et al.* (1999) who found that increasing initial population densities of *H. avenae* gradually decreased the growth parameters of wheat cv. Yecora Rojo under greenhouse conditions. Suppression of wheat growth was dependent on Pi level even at the lowest Pi (50000 eggs + J₂/pot = 12.5 eggs + J₂/g soil). So, wheat growing wouldn't be profitable at these Pi levels. *H. avenae* negatively affects the general physiology of infected

wheat plants including photosynthesis, mineral uptake, plant canopy temperature, transpiration and plant water content (Al-Yahya *et al.*, 1998) which consequently decline the growth of infected wheat plants. The gradual decrease of *H. avenae* reproduction factor on wheat plants might be related to the greater damage of roots with increasing Pi levels (Al-Hazmi *et al.*, 1999).

This study proves that *H. avenae* is a real threat to the Egyptian cereal cultivars, especially wheat. Therefore, there is an urgent need to develop new resistant cultivars. Besides, effective management programs should be developed to protect the cereal crops which are already insufficient for human and livestock demands in Egypt.

Table 5. Effect of different initial population densities (Pi) of *H. avenae* on the growth of wheat cv. Giza171 and on nematode reproduction, under greenhouse conditions 2018/2019 growing season.

Pi ¹ Eggs + J ₂ /pot	Root dry weight (g)	Red. ² (%)	Shoot dry weight (g)	Red. ² (%)	Spike dry weight (g)	Red. ² (%)	(Rf) ³
50,000	1.40 b	66	7.10 b	79	1.20 b	55	3.3
100,000	0.70 c	83	4.60 c	86	0.63 c	76	2.8
150,000	0.40 d	90	2.00 d	94	0.30 d	88	2.4
Non-infected	4.10 a	-	35.00 a	-	2.70 a	-	-

Means are an average of 5 replicates.

Means followed by the same letter(s) in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

¹Pi= initial population density.

²Red. % = percentage of reduction = $\frac{CP-IP}{CP} \times 100$

³Rf = Reproduction factor = final nematode population (Pf) ÷ Pi.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

All the authors shared all the work through the entire course of study: planning, designing, implementation, data collection, data analysis and writing.

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