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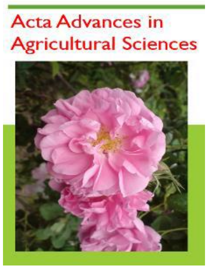

Research Article

Influence of inoculation levels of *Heterodera zeae* on the two nematode populations behavior and the host growth response in Egypt

Ahmed El-Sayed Ismail [1*] and Abbas Mohamed Kheir [2]

[1] *Plant Pathology Dept., Nematology Lab., National Research Center, Cairo, Egypt.*
[2] *Agricultural Zoology & Nematology Dept., Faculty of Agriculture, Cairo University, Egypt.*

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* Corresponding Author;
E. Mail: iismail2002@yahoo.co.uk and president@nrc.sci.eg

ABSTRACT

Different inoculation levels of two Egyptian populations namely, Belbais and Giza of the corn cyst nematode (CCN), *Heterodera zeae* infecting Giza 2 corn had almost similar trend of development and multiplication under greenhouse conditions. As the initial level of CCN increased, the total nematode population also increased; whereas, there was a negative relation between the initial inoculation and the rate of reproduction of the nematode. It is worthy to notice, in general, that Belbais population was the main reproductive population; producing the highest numbers of white females and brown cysts and achieved higher rate of build-up than Giza population did. Also, the plant growth parameters as influenced by different inoculation levels of both isolates of CCN were discussed.

Introduction

The reproductive potential of a nematode species on a host plant is depends on the population types of this species. This nematode has been regarded as one of the most noxious nematodes of corn in several parts of the world. In Egypt, the corn cyst nematode (CCN), *Heterodera zeae* has been recorded in different localities by Aboul-Eid and Ghorab, 1981, Ismail, 1985, Abadir, 1986, Moussa et al., 1988, Ismail et al., 1993 and Ismail 2009. Influence of different cyst nematode species inoculum levels on plant growth and nematode reproduction was studied by many researchers (Rao and Peachy, 1965 on *H. rostochiensis*, Gill and Swarup, 1973 on *H. avenae*, Sharma and Sethi, 1975 on *H.cajani*, Maas and Brinkman, 1977 on *H. avenae* and Griffin, 1988 on *H. schachtii*). Also, some studies were carried out on the impact of different inoculum levels on development and reproduction of *H. zeae* all over the world. In 1978, Ghorab demonstrated that the inoculum levels of 100 or 500 cysts of *H. zeae* / plant affected corn height; while levels of 100, 250, 500 or 1000 cysts / plant decreased the fresh and dry weights. He found also, that 2500 cysts / plant decreased the percentage of phosphorus and potassium in leaves of the nematode infected plants, while no change in percentages of N, Ca and Mg were observed. Moreover, no influence on N, P, K, Ca or Mg was detected in the 800 cysts treatment. Srivastava and

Sethi (1984) found that an initial inoculum of 100 juveniles of *H. zeae* / kg of soil for Pusa population and 1000 juveniles for Udaipur population significantly reduced corn growth in comparison to non-inoculated check plants. Ismail (1985) stated that the highest rate of cyst increase was obtained by 200 cysts / plant which increased by 2.32 folds. In (1986) Abadir, studied the influence of inoculums levels on behavior of four populations of *H. zeae* on corn cv. Giza 2. She found that Tanta population was the most reproductive isolate, producing the highest number of cysts and had achieved the highest rate of reproduction when plants were inoculated with low levels, i.e. 100, 500 or 1000 of mixture of eggs and juveniles / plant. While an inoculums of 2000, it came after Belbais population which recorded the highest number of cysts and highest rate of reproduction. When plant growth was assessed under the nematode infection, Belbais population suppressed the plant growth much more than other populations did. As yet limited studies on the effect of different isolates on the development and reproduction of the corn cyst nematode (CCN), *H. zeae* on maize have been conducted in Egypt (Abadir, 1986). So, our study was carried out to elucidate the behavior of two Egyptian populations viz. Belbais and Giza populations of *H. zeae* under different inoculum levels**.**

Materials and Methods

The influence of different inoculums levels of two Egyptian populations of *H. zeae* namely, Belbais population obtained from Belbais district, Sharkia Governorate and Giza population obtained from Giza district, Giza Governorate on development and reproduction of the nematode and, in turn, their behaviors on the plant growth were studied. Inoculation levels of mixture of both eggs and juveniles were 0, 100, 500, 1000, 2000 and 4000 eggs and juveniles of either Belbais or Giza populations were used to inoculate fourteen days-old Giza 2 corn seedlings grown in 10 cm d. clay pots filled with sterilized sandy loam soil (1:1). We used Giza 2 corn in this study because it was rated as a highly susceptible host to *H. zeae* (Ismail and Hassabo, 1995). Inocula of both populations were obtained from stock cultures propagated on Giza 2 corn in the greenhouse at 30±5ºC and prepared by crushing cysts by a homogenizer according to the technique of Ismail (1985), and then added in holes around the root systems. Treatments of each population were replicated four times as well as those kept without inoculation to serve as control. All pots were arranged in a randomized block design on a greenhouse bench at approximately of 30±5ºC. All the plants received the same treatments of watering and nutrition. Ninety days from inoculation time, the test was terminated and the plants were uprooted and data on the plant growth, nematode populations in soil and roots were recorded.

Bioassay of nematode and plant growth

The soil of each sample was mixed thoroughly after taking off the plant, and divided equally to subsamples. One part was processed for extraction of *H. zeae* juveniles by the sieving and decanting technique (Barker, 1985).While, the other part of soil was processed for cyst extraction by using a Fenwick can apparatus (Fenwick, 1940).Cysts were separated from debris and other organic materials by using a very fine drawing brush (Ismail, 1985), then the collected cysts were counted for each pot. Roots of the plants were cut off, weighed, measured and stained in hot acid fuchsin - lactophenol and then cleared with plain lactophenol (Taylor and Sasser 1978). Numbers of the developmental stages (D.S.), white females (W.F.) and brown cysts (B.C.) per root system were counted. Shoot system of each plant was also weighed and measured.

Statistical analysis

Data were subjected to analysis of variance by the Least Significant Differences (LSD) according to Gomez and Gomez, 1984.

Results

Six inoculum levels, approximately, 0, 100, 500, 1000, 2000 and 4000 of both eggs and juveniles of two isolates namely, Belbais and Giza populations of the corn cyst nematode, *H. zeae* were used to inoculate Giza 2 corn seedlings. The experiment was conducted to find out the influence of inoculation levels on behavior of two populations of *H. zeae* and also, the pathological differences possibly existed between the above mentioned nematode populations in relation to the inoculation levels. Data presented in Tables (1 and 2) show that the average numbers of the nematode developmental stages, white females, brown cysts and the nematode final populations varied according to the isolate type and the inoculation level.

Between the previous two isolates, there were conspicuous differences in numbers of nematode developmental stages depending on the inoculation levels used. For instance, the highest number of such stage (734 D.S. / plant) was obtained with the inoculum level of 1000 eggs and juveniles of Belbais population, while it was (422 D.S. / plant) with the level of 2000 in case of Giza isolate. Amongst the inoculation levels of Belbais population there were significant ($P \leq 0.05$) difference in numbers of the D.S. (Table 1). On the other hand, no significant differences amongst the inoculation level on numbers of such stages in case of Giza population (Table 2). With respect to white female and brown cyst numbers, they were also affected by the inoculation levels. A positive relationship was observed between the numbers of such stages of both nematode isolates and the initial used. Thus, by increasing the initial inoculum, the resultant number of W.F. or B.C. increased. Exceptionally, the white female in case of Giza population increased by increasing the initials up to 2000 eggs and juveniles then a further increase of the initials to 4000 resulted in a sharp decline in white female number (14 W.F. / plant). Although, a remarkable effect of the inoculum level was noticed on the number of egg / cyst in case of Belbais population (Table 1) there was no effect of such factor in case of Giza population (Table 2). In other words, there was a negative relation between the initial inoculation and the number of eggs / cyst of Belbais population, as the maximum number (289 egg / cyst) was obtained by the lowest initial population level, while it reduced (207 egg / cyst) by using 4000 eggs and juveniles / plant. Data on the total final populations and rates of build-up under the effect of difference inoculum levels are presented in Table 1 and 2. The behavior of the nematode final population and multiplication of both isolate were almost similar. As the initial level of *H. zeae* increased, the final total population also increased. On the other hand, there was a negative relation between the initial inoculation and the rate of build-up of the nematode. It reached its maximum by the lowest initial population level (100 eggs and juveniles / plant), whereas it decreased by using 4000 eggs and juveniles / plant (Tables 1 and 2). It is worthy to notice, in general, that Belbais isolate was the most reproductive population; producing the highest numbers of W.F., B.C. and achieved the highest rate of build-up than Giza population did.

The influence of the previously mentioned inoculation levels of each of the two populations of the CCN on the growth of Giza 2 corn was also estimated. Data on the plant growth on the basis of length and weight of both shoots and roots of the plants were listed in tables 3 and 4.It is obviously noticed that, the nematode of each tested population could suppress shoot and root growth of corn plants. Generally, both length and weight of either shoots or roots of all the inoculated treatments were markedly less than those of non- inoculated. The amount of damage occurred in both roots and shoots was greatly correlated with the initials used.

Effect of different inoculation levels on Giza 2 corn inoculated by Belbais population of H. zeae:

Table 1. *Effect of inoculum levels of H. zeae (Belbais population) on the nematode development and reproduction.*

Inoculum level (eggs and juveniles)	Average number of nematode / plant						Rate of build-up
	In Soil	In Root			Eggs / cyst	Total*	
	Juveniles	D.S.	White female	Brown cyst			
100	0	137	15	4	289	1308	13.08
500	0	297	33	6	281	2016	4.03
1000	0	734	80	8	267	2951	2.95
2000	0	550	85	16	219	4139	2.07
4000	1814	295	100	47	207	11938	2.99
LSD 5%		151	19	5	37	1232	

D.S. = Developmental stages (including 3rd and 4th stages).
*Total= juveniles in soil + D. S. + white females + (Brown cysts × eggs / cyst).

Table 2. *Effect of inoculum levels of H. zeae (Giza population) on the nematode development and reproduction.*

Inoculum level (eggs and juveniles)	Average number of nematode / plant						Rate of build-up
	In Soil	In Root			Eggs / cyst	Total*	
	Juveniles	D.S.	White female	Brown cyst			
100	0	318	10	3	187	889	8.9
500	135	379	28	7	187	1851	3.7
1000	705	371	46	9	187	2805	2.8
2000	248	422	51	13	187	3152	1.6
4000	790	308	14	27	187	6161	1.5
LSD 5%		149	16	7		1321	

D.S. = Developmental stages (including 3rd and 4th stages).
*Total= juveniles in soil + D. S. + white females + (Brown cysts × eggs / cyst).

The influence of Belbais population densities on Giza 2 corn (Table 3) revealed that the nematode caused, in most cases, significant (P≥0.05) reduction in shoot and root lengths as well as in shoot and root weights. However, the root parameters were highly affected with the nematode infection more than those of the shoot. By increasing the inoculation levels, the percentage of reduction increased, until the 2000 level after which it decreased (Table 3). Thus, the mostly pronounced percentage of reductions were obtained by the level of 2000 as they were 13, 25, 23.2, 24.1, 38.2 and 40% for hoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight, respectively.

Table 3. *Growth response of Giza 2 corn as influenced by different inoculum levels of H. zeae (Belbais population).*

Inoculum level (eggs and juveniles)	Shoot						Root					
	Length (cm)	R.%	Fresh weight (g)	R.%	Dry weight (g)	R.%	Length (cm)	R.%	Fresh weight (g)	R.%	Dry weight (g)	R.%
0	81.0	--	34.0	--	9.5	--	24.5	--	21.2	--	7.0	--
100	78.0	3.7	32.0	5.9	8.8	7.4	21.0	14.3	16.4	22.7	5.5	21.4
500	75.6	6.7	30.3	10.9	8.8	7.4	20.8	15.1	14.8	30.2	5.3	24.3
1000	72.0	11.1	29.3	13.8	8.8	7.4	19.6	20.0	13.7	35.4	4.3	38.6
2000	70.2	13.0	25.5	25.0	7.3	23.2	18.6	24.1	13.1	38.2	4.2	40.0
4000	71.7	11.5	28.6	15.9	7.3	23.2	18.8	23.3	13.6	35.9	4.4	37.1
LSD 5%	4.7		4.6		1.4		2.9		4.6		1.6	

R. = Reduction %.

Effect of different inoculation levels on Giza 2 corn inoculated by Giza population of H. zeae:

Inoculation levels of the Giza population on Giza 2 corn caused non-significant (P≥0.05) reduction in the shoot length; whereas they caused significant (P≥0.05) reduction in both shoot fresh and dry weights (Table 4). As for the root length and root fresh weight, the nematode caused non-significant reduction while, it caused significant (P≥0.05) reduction in case of root weight. However, the percentage of reduction proportionally correlated with inoculation levels. The highest percentage of reduction in all growth parameters were obtained by the 4000 eggs and juveniles level as they were 10.8, 21.5, 25.2, 11.1, 19.6 and 55.2 % for shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight; respectively(Table 4).

Comparatively, it could be observed that the critical damage occurred in the plants by Belbais population was obtained at using the 2000 eggs and juveniles; whereas, it was needed to inoculate 4000 eggs and juveniles of Giza population to plants to obtain such effect.

Discussion

Nematode biotic factors are important in determining the reproductive potential of a species. One of which is the inoculation level that used as an inocula to a host. When different levels of either Belbais or Giza populations of *H. zeae* were used as initial inoculations to Giza 2 corn, the resultant final populations were positively correlated to the initials. Namely, as the initial population increased, the final population of the nematode also increased. Likely, the rates of nematode multiplication of both populations seemed to be greatly governed by the inoculation levels. However, there was a negative relation between the initial inoculation and the rate of build-up of the nematode. Belbais isolate was the more reproductive than Giza isolate . Many investigators had obtained similar results (Rao and Peachy , 1965; Sharma and Sethi, 1975; Maas and Brinkman, 1977; Aboul-Eid and Ghorab, 1981; Srivastava and Sethi, 1984; Ismail, 1985; Abadir, 1986; Dhawan and Nagesh, 1987; Griffin, 1988 and Shahina and Maqbool, 1990).

When plant growth was assessed under the infection by the previously mentioned inoculation levels, the amount of growth suppression was greatly varied according to population and inoculation levels. The maximum damage occurred by an initial infectation of 2000 eggs and juveniles / plant for the Belbais population, while it was by 4000 eggs and juveniles / Plant for the Giza population. Therefore, influence of inoculation level on nematode reproductivity and virulence is also governed by the population type. Relatively, smaller numbers of the most virulent population can get greater and reproduces rapidly causing effective damage to the host. On the other hand, it is needed a relatively, higher initials of both eggs and juveniles of the less virulent population to obtain a similar effect. Damage caused by the nematode could be attributed to modification of plant physiological functions like photosynthesis, transpiration and mineral uptake following infection as reported by Heald and Jenkins (1964), Jenkins and Malek (1966) and Wallace (1971).However, considerable damage occurs only when the population density exceeds a certain limit (Ismail 1985).

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