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POPULATION CHANGES OF WHITE TIP NEMATODE *Aphelenchoides besseyi* ON CERTAIN RICE CULTIVATRS UNDER OUTDOOR CONDITIONS.

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ABSTRACT

Examination of while tip nematode, *Aphelenchoides besseyi* population changes as well as its rate of build-up on four rice cvs. I.e. Giza 171 and 177, Rehoo and Sakha 101 grown in pots (40-cm-diam.) during the rice growing season 2005 under outdoor conditions indicated that nematode population density was significantly different depending on the rice cultivar and its growth stage. Generally, population density of *A. besseyi* remarkably increased during the vegetative stage (tillering), sharply decline in the flowering stage and then increased once again in the harvesting period but not exceeded that of the first growth stage. Among tested rice cultivars, Giza 171 encountered the highest cumulative nematode numbers with value of 194.3 individuals per g. plant sample and rate of build-up amounted to 4.8, whereas Sakha 101 gave the least value of nematode numbers (74.8) with rate of build-up value of 3.93. Regarding nematode population density recovered from soaking one hundred rice grains in water for 48 or 96 hrs, rice cv. Rehoo accomplished the highest numbers of *A. besseyi* (126 or 168), followed by Giza 171 (124.6 or 157) and Giza 177 (99.7 or 130.3), whereas Sakha 101 gave the low average nematode number with values of 77.6 or 107.0 individuals, respectively. In the meantime, rice cvs. Rehoo and Giza 171 achieved the highest levels of nematode infestation with values of 36.25% and 35.2%, respectively, whereas, Sakha 101 also showed the least level of nematode infestation (23.1%).

Keywords: *Aphelenchoides besseyi*, Level of Infestation, Population changes, Rate of build-up, Rice cultivars.

INTRODUCTION

Rice, *Oryza sativa* L. is the stable food for more than two billion people, predominately in Asia, where, more than 90% of the world's rice grown and consumed (Bridge *et al.*, 1990). Rice is also preferred as food by most Egyptians as it contributes about 20%, and is grown in about 1.5 million feddans occupying about 22% of the cultivated area in Egypt (Anonymous, 2003). More than 96% of the Egyptian rice area is cultivated in the Nile-Delta. Productivity of rice in Egypt is 6.38 million tons as the productivity average is 4.24 tons per feddan according to report published by Badawi *et al.* (2005).

Worldwide, rice yield losses due to plant parasitic nematodes are estimated at 10% (Sasser and Freckman, 1987). Moreover, white-tip disease of rice plant caused by a seed-borne nematode, *Aphelenchoides besseyi*, Christie, 1942 has been widely distributed in many rice growing countries in Asia, Tropical America, formerly USSR and Africa (Franklin & Siddiqui, 1972, Fortuner & Williams, 1975 and Ou, 1985). Recently, it was recorded in Egyptian rice fields and seeds (Amin, 2001, Koraim, 2002 and Khalil & El-Sherif 2003). *A. besseyi* caused variable yield losses in different countries ranging from 14.5 to 46.7% in Japan (Nishizawa & Yamamoto, 1951), 40 –

50% in U.S.A. (Atkins & Todd, 1959), 29 to 46% in Taiwan (Hung, 1959), 41 to 71% in formerly USSR (Tikhonova, 1966), about 60% in India (Rao *et al.*, 1985) and about 38.6% in Egypt (El-Sherif *et al.* 2006).

The white-tip nematode, *A. besseyi* behaves as an ectoparasite pest against rice plants. Nematodes usually emerge from the soaked seeds and attack the seedlings as they grow. Moreover, they attack the young leaf surrounded by the innermost leaf sheath during the tillering stage (vegetative stage) entering rice flowers and hibernate beneath seed glumes as adults and fourth stage juveniles. It has a short generation time of 8 – 10 days at 25°C (Hollis and Keoboonrueng, 1984, Chiyonishio and Nakazawa, 1988 and Togashi & Hoshino, 2001). Rice seeds are therefore, the main source of the nematode infestation. Besides, field wastes, related weeds, irrigation water may be involved. Egyptian growers usually use a part of the harvested rice seeds for the following season, thus, the infestation level of white-tip disease increase each year. Moreover, in South Dakahlia governorate, Tahwaai village where paddy areas were cultivated with Giza 171 and Rehoo rice cultivars recorded the highest levels of *A. besseyi* infestations in rice grains as well as straw with values of 68.6% and 72.0%, respectively (Khalil and El-Sherif, 2003).

The purpose of the present work was to determine the population changes of *A. besseyi* on certain rice cvs. i.e. Sakha 101, Giza 177 and 171 and Rehoo grown in pots during the growing season 2005 under outdoor conditions as well as rate of build-up and levels of nematode infestation.

MATERIALS AND METHODS

During a scientific tour to rice fields on August 25th 2004 at Simbellawian district, South of Dakahlia governorate (Nile Delat of A.R. Egypt), symptoms of white-tip leaf (flag leaf) caused by *Aphelenchoides besseyi*, Christie, 1942 were observed based on chlorotic discoloration of the leaf sheath just below the collar of the 3 month-old plants of rice varieties i.e. Sakha 101, Giza 171 & 177 and Rehoo grown in Sobaeen, Borg-El-Nour El-Arab, Tahwaai (A) and Tahwaai (B) villages, respectively. Rice fields of these villages are previously recorded to be infested with this nematode (Khalil and El-Sherif, 2003). Five locations of infested rice plants within each of the four varieties under study were selected and marked by bamboo stakes for collecting their panicles at harvest in order to be used in this study during rice growing season of 2005.

In order to study the population dynamics of *A. besseyi* infesting rice plants and grains of certain cvs i.e. Sakha 101, Giza 171 & 177 and Rehoo, two hundreds of these infested rice seeds of each cultivar were separately broadcasting sown for each plastic tray (100 x 30 x 4 cm). Each plastic tray has small pores within its bottom, which covered with newspaper to prevent clay soil and water. Each tray was filled with sterilized clay soil mixed with five grams of urea plus two grams of zinc sulfate as a fertilizers before sowing rice seeds, then irrigated with enough tap water and covered with plastic sheet for 24 hrs. Each cultivated plastic tray was irrigated with water until seed germination and through the seedbed and nursery period. The initial population of *A. besseyi* per 100 rice grains cultivar was separately

determined by soaking them in a plastic cup 20 cm diam. filled with tapwater for 48 hrs, sieved through 400 mesh (Cobb, 1918), counted and recorded to be 34, 44, 53 and 57 individuals for Sakha 101, Giza 177 & 177 and Rehoo, respectively. After twenty-five days from sowing, rice seedlings/cultivar were transplanted into plastic pot 40 cm. in diam., filled with 20 Kgs sterilized clay soil, previously sterilized with bromide methyl at the rate of 100 gm/m^2. Each plastic pot has three hills with five seedlings each. Eighty plastic pots with 15 rice seedlings each was used in this experiment at the rate of twenty replicates for each cultivar. Pots of each rice cultivar were randomly arranged in outdoor place during the growing season 2005.

One month later, NPK fertilizer was added at the recommended rate/replicate/cultivar, and then 25 grams of rice plant leaves and stems (vegetative stage)/replicate/cultivar at the level of 5 cm height from the plant base was cut and collected in paper bags, brought to the Nematology laboratory and kept in a refrigerator at 4°C for nematode counting. Similar sample per each replicate/cultivar was taken for flag-leaf, stem and at pre-maturity stage of panicles (flowering stage) as well as at harvesting time. These materials were separately cut into about 0.25 cm pieces, mixed and then one gram of each growth stage/cultivar before harvesting of each cultivar was randomly taken and soaked in tap water in a 10-cm-diam. plastic cup. Suspension was separately passed through 60 mesh-sieve 48 hrs after incubation, and the nematodes were concentrated through Cobb's sieve 400 mesh (Cobb, 1918), counted, recorded and their identification confirmed microscopically whereas, at harvest time immediately two sets of one hundred rice grains each per cultivar were separately soaked as previously mentioned for 48 hrs and the other set was soaked for 96 hrs. Nematode extraction was done as previously mentioned. This process was separately repeated four times for each growth stage/cultivar. Nematode extraction in each procedure mentioned above was conducted in darkness at 25 $\pm$ 2°C as this temperature is within the suitable range for extraction of *A. besseyi* from the soaked seeds (Tamura and Kegasawa, 1957).

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) and means were compared by Duncan's multiple at $P < 0.05$ range test (Duncan, 1955).

RESULTS

Data presented in Table (1) show the numbers of *A. besseyi* separately recovered from soaking one gram of plant sample at various growth stages e.g. vegetative (flag leaf + stem), flowering (flag leaf + stem + new panicle) and harvesting (dry flag – leaf + stem) of each rice cultivar tested, i.e. Giza 171 & 177, Rehoo and Sakha 101 for 48 hrs at 25 $\pm$ 2°C, respectively. It is evident that the population density of *A. besseyi* obtained was significantly different depending on the rice cultivar and its growth stage. In general the nematode population density tend to increase within the vegetative stage, sharply decline in the flowering stage and then increase once again in the harvesting stage but not exceeded that of the first stage (Table 1 and Fig. 1). Moreover, the plant sample (one gram) of rice cv. Giza 171 appeared to encounter the highest cumulative numbers of *A. besseyi* with value of 194.3

individuals/g., followed by that of Rehoo (136.6), then Giza 177 (101.2), whereas, Sakha 101 gave the least value of nematode number (74.8 individuals/g. of plant sample) respectively. In addition the average population density of *A. besseyi* at the various growth stages examined showed significant difference ($P < 0.05$). Meanwhile, a significant difference of the cumulative nematode numbers between rice cultivars was also evident. However, there was no significant trend for the average population nematode density/g. of plant sample between the three growth stages for each of rice cultivars under study except that of Sakha 101 in the flowering stage as compared to the other two growth stages.

Table 1: Numbers of *A. besseyi* in one gram of rice plant sample/cultivar at vegetative, flowering and harvesting stages.

Rice cultivar	Population density of *A. besseyi* at various growth stages			Cumulative number of nemas/ 3 g. plant	No. of nemas per g.
	*Vegetative	*Flowering	*Harvesting		
Giza 171	256.0^{a}	133.3^{a}	193.8^{a}	583.0^{A}	194.3^{A}
Giza 177	130.3^{c}	65.5^{c}	107.8^{c}	303.5^{C}	101.2^{C}
Rehoo	199.0^{b}	86.8^{b}	124.0^{b}	409.8^{B}	136.6^{B}
Sakha 101	98.5^{d}	53.3^{c}	72.5^{d}	224.3^{D}	74.8^{D}
**Average	170.9^{A}	84.7^{C}	124.5^{B}		

Each figure represented the average number of *A. besseyi* in four replications.
* Means in each column followed by the same small letter didn't differ at $P < 0.05$.
** Means in each column followed by the same big letter did not differ at $P < 0.05$.

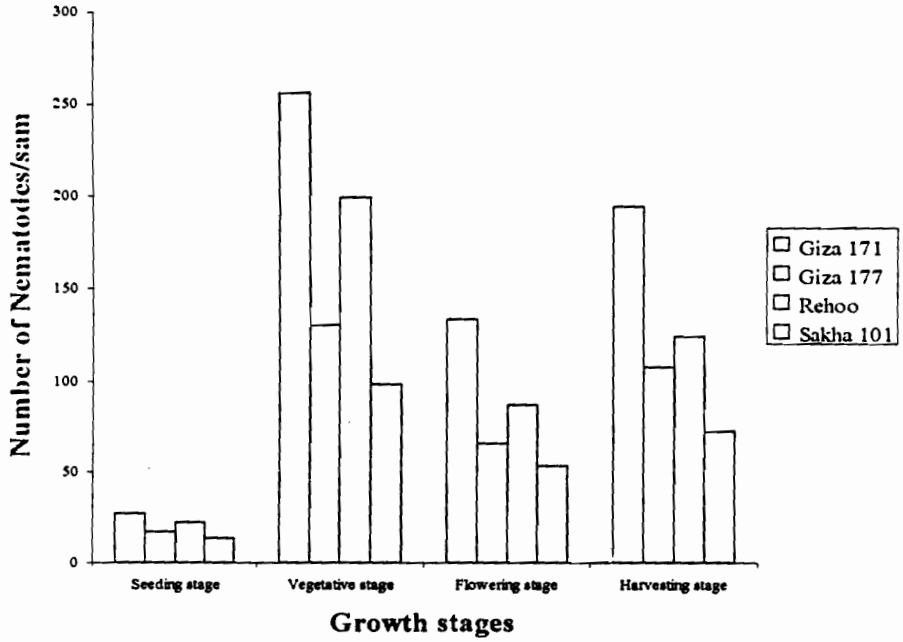

Fig. (1): Population changes of white-tip nematode *Aphelenchoides besseyi* on different stages of four rice cultivars grown in pots under outdoor conditions

Data in Table (2) documented the numbers of *A. besseyi* recovered from soaking 100 rice grains (seeds) of each rice cultivar tested i.e. Giza 171 & 177, Rehoo and Sakha 101 for 48 or 96 hrs at 25 ± 2°C, respectively. It is obvious that the population density of *A. besseyi* resulted by soaking rice grains for 96 hrs was significantly greater than that of 48 hrs for all rice cultivars examined. Among the rice grains cultivars tested, Giza 171 gave the highest numbers of *A. besseyi* per 100 rice grains soaked for 48 or 96 hrs, followed by Rehoo with values of 124.6 or 157.0 and 126.0 or 168.0 individuals, respectively. On the other hand, rice grains of Giza 177 that were soaked either for 48 or 96 hrs ranked the third after rice cv. Rehoo in the number of *A. besseyi*/100 grains, with values of 99.7 or 130.3 individuals. Whereas grains of rice cv. Sakha 101 revealed the least average number of *A. besseyi* recovered after soaking either 48 or 96 hrs in tap water with values of 77.6 or 107.0 individuals, respectively. It can also be noticed that grains of rice cvs. Rehoo and Giza 171 achieved the highest level of *A. besseyi* infestation with values of 35.20%, and 36.75%, respectively. However, rice grains cv. Giza 177 showed the moderate level of nematode infestation (28.75%), whereas the least value was accomplished by rice cv. Sakha 101 (23.10%), respectively.

Table 2: Numbers of white tip nematode, *A. besseyi* recovered from two sets of one handred rice grains/cultivar each; one set after 48 hrs, and the other set after 96 hrs of incubation in tap water at 25 ± 2°C.

Rice Cultivar	Population density of *A. besseyi* at harvesting time after		% infestation
	*48 h	*96 h	
Giza 171	124.6a	157.0a	35.20 %
Giza 177	99.7c	130.3b	28.75 %
Rehoo	126.0b	168.0a	36.75 %
Sakha 101	77.6d	107.0c	23.10 %
**Average	106.9B	140.6A	

Each figure represented the average number of *A. besseyi* in four replications.
* Means in each column followed by the same small letter didn't differ at P < 0.05.
**Means in each column followed by the same big letter did not differ at P < 0.05.

Data in Table (3) represented the rate of *A. besseyi* build-up on four rice cvs i.e. Giza 171 & 177, Rehoo and Sakha 101 grown in pots under outdoor conditions. It is evident that rice cv. Giza 177 accomplished the highest value of nematode build-up with value of 10.61, followed by rice cv. Rehoo (9.8) and Sakha 101 (9.09), whereas the least value was recorded by rice cv. Giza 171 which was amounted to 8.93.

Table 3: Rate of whit-tip nematode, *A. besseyi* build-up on rice plant plus grains of certain rice cultivars grown in pots under outdoor conditions.

Rice cultivars	Number of *A. besseyi* in one gram of plant or grains incubated in water for 48 hrs.		No. of nemas. per one g. of plant plus grains	***Rate of build-up
	*Plant tissue	**Grains		
Giza 171	194.3^{A}	63.10^{A}	128.7	4.80
Giza 177	101.2^{C}	38.95^{C}	70.1	4.07
Rehoo	136.6^{B}	49.80^{B}	93.7	4.16
Sakha 101	74.8^{D}	30.66^{D}	52.7	3.93

* Each figure represented the average number of *A. besseyi* in rice plant tissue of three replicates (growth stages).

** Each figure represented the average number of *A. besseyi* in rice grains of four replicates.

Means in each column followed by the same big letter did not differ at $P < 0.05$.

$$***\text{Rate of build-up} = \frac{\text{Final population } (P_f)}{\text{Inital population } (P_1)}$$

where the (P_f) = Giza 171 = 26.8 nemas./g. rice grains at sowing.
Giza 177 = 17.2 nemas./g. rice grains at sowing.
Rehoo = 22.5 nemas/g. rice grains at sowing.
Sakha 101 = 13.4 nemas/g. rice grains at sowing.

DISCUSSION

Examination of on gram of the rice plant samples at different growth stages i.e. vegetative, flowering and harvesting for each rice cultivar significantly revealed that the population changes of *A. besseyi* obviously depend upon the rice cultivar as well as the growth stage. Apparently, the nematode population density increased during the vegetative stage, then sharply decreased during the flowering stage and tended to increase once again at the harvesting stage but not exceeded that of the vegetative period. This phenomenon may be related to the situation of water flooding, high atmosphere humidity and free moisture on the leaf surface during flood that play an important role in spread and multiplication of the nematode during the vegetative period (June. – July) as well as near the end of rice growing season (mid-August – mid-September). This speculation agreed with the findings of Rahman and Miah (1989) who found that deepwater rice plants were less affected by *A. besseyi* during period before flooding and number of nematodes per infested plant was also low which could be due to the slow rate of nematode development and multiplication under pre-flood conditions.

Apparently, the population density of *A. besseyi* obtained by soaking 100 rice grains in tap water for 96 hrs was significantly greater than that of 48 hrs for all rice cultivars tested. Among the rice cultivars examined Giza 171 as well as Rehoo showed the highest nematode population densities per 100 rice grains soaked for 48 or 96 hrs in water with values of 124.6 or 157.0 and 126.0 or 168.0 individuals, respectively. However, grains of rice cvs. Giza 177 and Sakha 101 ranked the second to them with values of 99.7 or 130.3 and 77.6 or 107.0 individuals, respectively. These results agreed with the findings of Khalil and El-Sherif (2003) who reported that grains of Rehoo

cultivar gave the highest numbers of *A. besseyi* whereas, Sakha 101 showed the least with values of 57.0 or 76.4 and 33.8 or 43.6 individuals when rice grains were soaked in water for 48 or 96 hrs, respectively.

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تغير تعداد نيماتودا أوراق القمة البيضاء *Aphelenchoides besseyi* على أصناف الأرز المنزرعة تحت ظروف خارج الأبنية.

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أسفر فحص تعداد سلوك تعداد نيماتودا القمة البيضاء *Aphelenchoides besseyi* وكذا معدل تكاثرها على أربعة أصناف أرز هى جيزة ١٧١، ١٧٧، ريهو وسخا ١٠١ منزرعة فى أصص قطر ٤٠ سم خلال موسم زراعة الأرز ٢٠٠٥ تحت الظروف الخارجية (شبه حقلية) عن أن تعدادها مختلف معنويا على حسب صنف الأرز ومرحلة النمو. وبصفة عامة زاد تعداد النيماتودا بدرجة ملحوظة فى مرحلة النمو الخضرى (التفريع) وانخفض بدرجة واضحة فى مرحلة التزهير، ثم زاد مرة ثانية فى مرحلة الحصاد ولكن لم يتفوق فى ذلك على مرحلة النمو الأولى. وكان الصنف جيزة ١٧١ هو الذى احتوى على أعلى عدد كلى من النيماتودا فى المراحل الثلاثة تحت الدراسة بمعدل ١٩٤,٣ فرد فى جرام واحد من عينة النبات التى فحصت ومعدل تكاثرها عليه كان ٤,٨، بينما أعطى الصنف سخا ١٠١ أقل قيمة فى كثافة أعداد النيماتودا وبمعدل تكاثر ٣,٩٣ أما بخصوص تعداد النيماتودا الناتج من نقع ١٠٠ حبة فى ماء لمدة ٤٨ أو ٩٦ ساعة كان الصنف ريهو هو الذى احتوى على أعلى أعداد النيماتودا بمعدل (١٢٦ أو ١٦٨ فرد) يليه فى ذلك صنف جيزة ١٧١ (١٢٤,٦ أو ١٥٧ فرد)، كذلك الصنف جيزة ١٧٧ (٩٩,٧ أو ١٣٠,٣ فرد)، بينما كان الصنف سخا ١٠١ يحتوى على أقل أعداد نيماتودا بمعدل ٧٧,٦ أو ١٠٧ فرد على التوالى. وفى نفس الوقت أعطى الصنف ريهو وكذلك جيزة ١٧١ أعلى مستوى بنسبة إصابة فى الحبوب بمعدل ٣٦,٧٥% و٣٥,٢٠% على التوالى، بينما أعطى الصنف سخا ١٠١ أقل مستوى إصابة (٢٣,١%).