J. Agric. Sci. Mansoura Univ., 29 (4): 2127 - 2136, 2004

EFFECT OF STORAGE, HOT AIR-DRYING, HOT WATER AND NEMATICIDE SEED SOAKING TREATMENTS ON WHITE TIP NEMATODE, *Aphelenchoides besseyi* SURVIVAL IN RICE SEEDS.

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ABSTRACT

Aphelenchoides besseyi has been detected in rice seeds collected from five Egyptian governorates (Behaira, Dakahlia, Gharbia, Kafr El-Shaikh and Sharkia). Data indicated that the number of nematodes is reduced as seed age increased, in which it can survive for up to three years. Several warming treatments have been evaluated to disinfect rice seeds contaminated with the white tip nematode, *A. besseyi*. Hot water at 50 °C for 15 minutes and hot air-drying treatment at 70 °C for 24 hours achieved good and satisfied results in controlling the rice nematode without affecting seed sprouting. Also, the efficacy of disinfestation by soaking seeds in different concentration levels of Cadusafos (Rugby 20%) or Oxamyl (Vydate 24%) for 24 hours was evaluated. Cadusafos was more effective than Oxamyl in increasing the percentage of nematode mortality. However, the nematicidal effect increased as concentration increased. Cadusafos at 200 and 400 ppm and Oxamyl at 400 ppm showed maximum nematode mortality without damage on seed sprouting.

Keywords: *Aphelenchoides besseyi*, Cadusafos, control, hot air-drying, hot water treatment, nematicide, Oxamyl, seed borne nematode, storage.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crops worldwide, providing the primary source of calories for 40% of world's population (De Datta, 1981). Rice is 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). *Aphelenchoides besseyi*, Christie is a seed-borne nematode causing the "white tip" disease in rice. It has been recorded in most rice growing countries (Giudici and Villa, 1997; Villa and Giudici, 1998; Hoshino and Togashi, 1999, Giudici and Villa, 2003 and Giudici, *et al.*, 2003). Recently, it was recorded in Egyptian rice fields and seeds (Amin, 2001 and Koraim, 2002). The average yield loss ranges between 10 and 30% depending on the growing variety, and nematode population density (Prot, 1992).

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 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 and Hoshino 2001). Rice seeds are therefore, the main source of the nematode

infestation. Besides, field wastes, related weeds, irrigation water may be involved.

Egyptian farmers usually use a part of the harvested rice seeds for the following season. Thus, the infestation level of white tip disease tends to increase from year to year. In Japan, management of *A. besseyi* usually involves soaking of rice seeds in aqueous emulsion or solution of nematicides for 24 hours (Hoshino and Togashi, 2000). After soaking, the seeds are air-dried for a few days until they are soaked again in water for sprouting. However, no study has been carried out in Egypt on the nematode management. The air drying and chemical seed treatments are the most effective means which allow good control (Hoshino and Togashi, 2000). Giudici *et al.*, (2003) reported that the Italian foundation seed of main rice varieties have been treated yearly with hot water (55-61 °C for 10-15 minutes) to control the diffusion of the nematode. The present work aimed to study the effect of storage time on survival of the nematode in rice and control of infested seeds by hot air-drying, hot water and nematicide seed soaking treatments.

MATERIALS AND METHODS

Rice seed storage: Seeds of rice cultivars were collected in September and October 1998, 2001, 2002 and 2003 from five Governorates of Northern Egypt (Behaira, Dakhahlia, Gharbia, Kafr El-Sheikh and Sharkia). After sampling, the seeds were stored in dark in a warehouse till January 2004, resembling the normal circumstances of rice seed storage.

Nematode extraction: Nematode mortality within dry seeds was determined using mass extraction method described by Hoshino and Togashi (2002). Individual seeds were bisected crosswise with small scissors and then placed into 9 cm diam Petri-dish contained 10 ml distilled water at 25 ±2 °C for 24 hours. Then, alive and dead nematodes were counted using a Hawksely counting slide. Nematodes that do not move when prodded with a needle were considered dead.

Rice seed cultivar: All seeds used for the experiments were Egyptian cultivar, Giza 177, collected in September, 2003.

Rice seeds soaking in hot water: Muslin bundles containing 100 rice seeds were considered as experimental replicates. The bundles were inserted in 500 ml beakers filled with 300 ml hot water at ten various temperature degrees (25-75 °C) for 15 min. Eight replicates were used for each temperature degree treatment. After treating, seeds of 4 replicates of each treatment were spread in 10 ml distilled water in 9 cm diam Petri-dishes and incubated at 25 ±2 °C for 72 hours. Untreated 8 replicates were incubated at laboratory temperature (control) for 72 hours without hot water treating. Percentages of the nematode survival were determined. The other remaining

four replicates of each treatment as well as those of the control were tested for sprouting within 72 hours.

Effect of hot-air drying on survival of *Aphelenchoides besseyi* in rice seed:

One hundred of rice seeds per Petri-dish (8 replicates/treatment) were treated for nematode survival (4 replicates) and seed germination (4 replicates) with hot air drying at seven various temperature degrees (40 °C to 100 °C) for 24 hours. Then, the seeds were crosswise-bisected and incubated in 10 ml-distilled water in 9 cm diam Petri dishes at 25 °C for 24 hours. Untreated 8 replicates were incubated at 25 ±2 °C for 24 hours. Then, the percentages of nematode survival were determined. Treated and untreated seeds (second group) were tested for sprouting within 72 hours.

Rice seeds soaking in some nematicides:

One hundred of split rice seeds per replicate (4 replicates/treatment and 4 replicates were left without seed splitting for germination) were soaked in 9 cm diam Petri-dish containing 10 ml of an aqueous emulsion (25,50,100,200 and 400 ppm) of Cadusafos (Rugby 20% E.C.) and Oxamyl (Vydate 24% E.C.) for 24 hours at 25 ±2 °C, Then, the supernatant was poured through a 325 mesh sieve and the nematodes retained on the sieve were collected and counted. Nematodes that did not move when prodded with a needle were considered dead. Treated un-split seeds were washed three times with water and then spread on 9 cm diam Petri-dishes and incubated at 25 ±2 °C for germination.

Statistical analyses: Contingency tables made by number of surviving and dead nematodes were used for comparison of mortality among treatments. Duncan's multiple range test (DMRT) was calculated to compare the total number of survived nematodes between treatments. Additionally, corrected mortality was calculated as described by Abbott (1925) to exclude the initial mortality in samples, i.e. ((% survival for untreated control) – (% survival after treatment)/ (% survival for untreated control)) X 100. While % survival is obtained by subtracted % mortality from 100.

RESULTS

a. Effect of rice seeds storage on survival of *A. besseyi*:

Data on the effect of storage of rice seeds on survival of *A besseyi* are illustrated in Fig. (1). The total population (alive + dead)of recovered nematodes from the stored seeds was higher in 1998 season followed by 2002 season as they were 165 and 178 nematodes/100 rice seeds, respectively. Many alive nematodes emerged from split rice seeds in spite of being stored more than three years. However, percentage of nematode mortality was greatly increased as the seed storage period increase. No alive nematodes were recovered from those seeds of 1998, while considerable counts of alive nematode were released from seeds of 2002 and 2003.

b. Effect of hot water soaking of rice seeds on survival of *A. besseyi*:

Mortality of *A besseyi* was greater in hot water soaking seeds at 50 °C and up at which it reached to 100%. The other lower hot water treatments exhibited lower percentages of mortality which positively correlated with the temperature of hot water. The seed sprouting was not affected with hot water-soaking seed till 70 °C (Table 1 and Fig. 2).

c. Effect of hot air drying of rice seeds on survival of *A. besseyi*:

Mortality of *A besseyi* was greater when rice seeds were exposed to hot air at 70 °C for 24 hours. It achieved more than 80% nematode mortality. Higher hot air temperatures (80 -100 °C) caused 100 nematode mortality. The seed sprouting was not affected by hot air treatment at (40 to 80 °C) compared with those of untreated one. However, the higher treatments completely prevented seed germination (Table 2 & Fig. 3).

d. Effect of nematicidal of rice seeds soaking on survival of *A. besseyi*:

Mortality of *A. besseyi* within rice seeds was greater in the nematicidal soaking treatments at all concentration level than in the untreated control. Cadusafos soaking treatment showed better performance than that of Oxamyl soaking one at all concentrations. In general, mortality of the nematicidal treatments was increased as nematicide concentration increased (Table 3). Cadusafos at 200 or 400 ppm and Oxamyl at 400 ppm gave 100% nematode mortality. Namely, all Cadusafos concentrations gave significant reduction in the number of survived nematode whereas Oxamyl at 100, 200 and 400 ppm recorded significant reduction in survived nematode (Table 3 and Fig 4). Variable percentages of mortality were obtained with the nematicide concentrations. Most nematicide concentrations did not affect rice seed sprouting (Table 3).

DISCUSSION

Since *A. besseyi* is considered as a serious rice seed-borne parasitic nematode in Egypt (Amin, 2001), it seems that the distribution in all paddy fields becomes epidemics (Korayem, 2002). It was detected in most different rice cultivar seeds collected from five Egyptian governorates. At the maximum infestation level, the greatest yield loss was recorded in dried seeded crop in the last 6 years (unpublished data). Fucano (1962) reported that the densities of approximately 30 live nematodes per 100 seeds were correlated to a maximum 5% loss in yield for susceptible varieties. Yamaguchi (1977) determined an economic damage threshold density of 300 live nematodes per 100 seeds. *A. besseyi* can survive up to three years in dormant state. Although, storage period can affect nematode survival in rice seeds, a number of nematodes can survive for up to three years (Prot, 1992). However good seed storage conditions can probably prolong nematode survival.

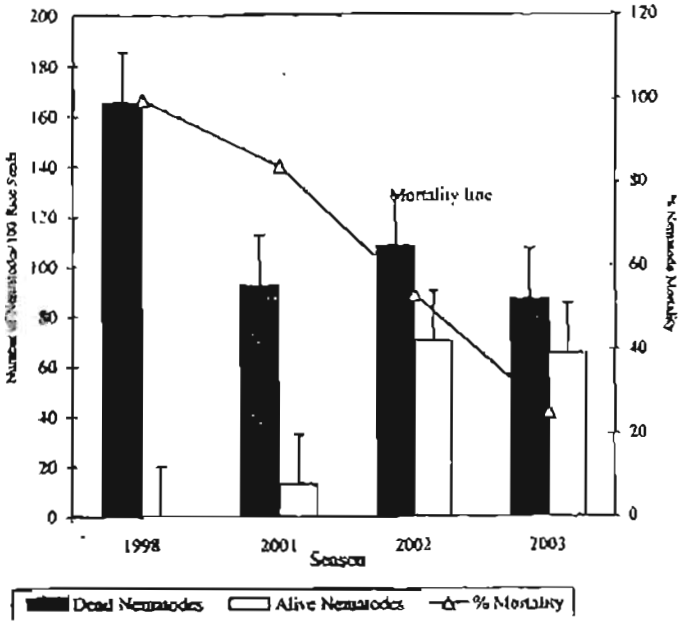

Fig. (1): **Effect of storage period on survival of *Aphelenchoides besseyi* in rice seeds.**

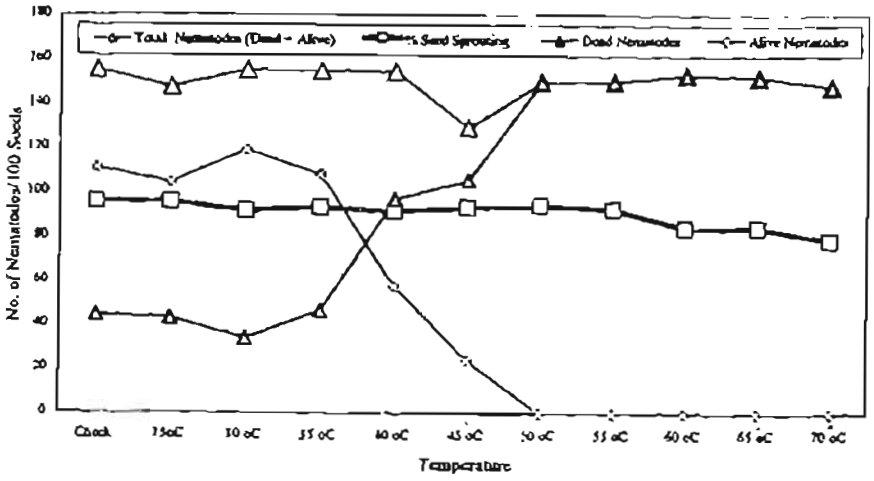

Fig. (2): **Effect of hot water treatment on survival of *Aphelenchoides besseyi* and rice seeds sprouting.**

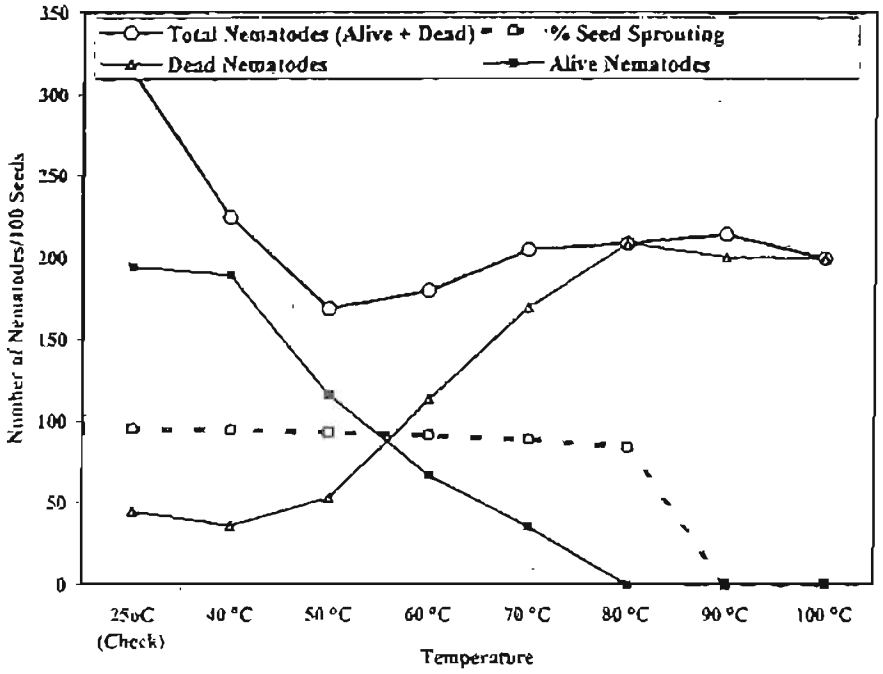

Fig. (3): Effect of hot air treatment on survival of *Aphelenchoides besseyi* and rice seeds sprouting.

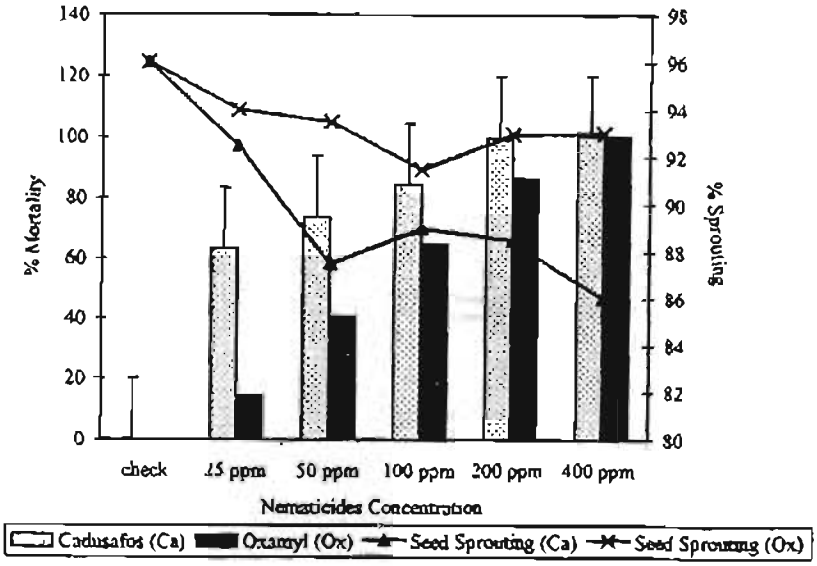

Fig. (4): Effect of different concentrations of Cadusafos (Ca) and Oxamyl (Ox) on *Aphelenchoides besseyi* mortality and rice seeds sprouting.

Table 1: Effect of hot water seed soaking on survival of *A.besseyi* and seeds sprouting.

Temperature	No of survived nematodes*	No of dead nematodes	Total of emerged nematodes	Mortality (%)	Survival (%)	Corrected Mortality (%)	% seed sprouting
25 °C	104.3 b	43.0	147.3	29.2	70.8	9.1	94.0
30°C(Check)	118.8 a	33.8	152.6	22.1	77.9	0.0	95.5
35 °C	108.0 ab	46.5	154.5	30.1	69.9	10.3	95.5
40 °C	57.6 c	97.0	154.5	62.8	37.2	52.2	91.5
45 °C	23.8 d	105.5	129.3	81.6	18.4	76.4	93.0
50 °C	0.0 e	150	150.0	100	0.0	100	94.0
55 °C	0.0 e	150	150.0	100	0.0	100	92.5
60 °C	0.0 e	153	153.0	100	0.0	100	86.5
65 °C	0.0 e	152	152.0	100	0.0	100	84.0
70 °C	0.0 e	148	148.0	100	0.0	100	78.5
Tap water	110.8 ab	44.3	155.1	28.6	71.4	8.3	94.0

*Means followed by the same letter(s) within a column in each block are not significantly different (P≤ 0.05) according to Duncan's multiple range test.

Table 2: Effect of hot air-drying on survival of *A. besseyi* and seed sprouting.

Temperature	No of survived nematodes*	No of dead nematodes	Total of emerged nematodes	Mortality (%)	Survival (%)	Corrected Mortality (%)	% seed sprout-ting
40 °C	188.8 a	35.8	224.6	15.9	84.1	4.3	94
50 °C	115.8 b	52.8	168.6	31.3	68.7	21.8	93
60 °C	66.5 c	112.8	179.3	62.9	37.1	57.8	91
70 °C	35.3 d	169.3	204.6	82.7	17.3	80.3	88.5
80 °C	0.0 e	208.0	208.0	100	0.0	100	84
90 °C	0.0 e	200	200	100	0.0	100	0
100 °C	0.0 e	199.0	199.0	100	0.0	100	0
Check	194.3 a	44.5	238.8	13.1	86.9	0.0	95

*Means followed by the same letter(s) within a column in each block are not significantly different (P≤ 0.05) according to Duncan's multiple range test.

Under stress of hot water treatment during seed soaking, *A. besseyi* are killed by high concentration of CO_2 and low oxygen content followed by respiration stress causing high nematode mortality (Hoshino and Togashi, 2000). Also, freshly hydrated nematodes are physiologically unprepared to survive under stress of either hot water soaking or hot air drying because adults and juveniles of *A. besseyi*-infested seeds are, in most cases, in a dormant state (Nandakumar, *et al.*, 1975) and can survive in seeds up to three years. Also, soaking rice seeds in hot water is at 50 °C for 78-88 hours sufficiently rapid for causing 50% of nematode mortality (Tamura and Kegasawa, 1957). More than 90% of dormant nematodes can revive 3 hours after absorbing water (Chiyonisho and Nakazawa, 1988). Thus, the fatal effect of either hot water seed soaking or hot air drying on *A. besseyi*-infested seeds may be attributed to the above mentioned reasons. Conclusively, worming treatment of rice seeds could be the best control method for rice seeds nematode. A treatment for 10-15 min at 55-61 °C can destroy the nematodes without affecting germination of the seeds (Giudici *et al.*, 2003). For these reasons in EU, the Council Directive No. 2000/29 states that rice

seeds can be imported in the Community only after they underwent an effective treatment, i.e. hot water – air drying. Besides, the nematicidal treatments gave satisfied results, that they can be used for influential effects on the nematode survival in rice seeds. The direct contact of the nematicides on sensitive and unprepared rice nematodes has good action (Hoshino and Togashi, 2000). They also, suggested that a combination of water soaking plus air-drying to seeds followed by a nematicidal application to seedlings before transplanting may be a good control for *A. besseyi* in rice.

Table 3: Effect of nematicidal seed soaking on survival of *A. besseyi* and seeds sprouting.

Nematicide	Concentration (ppm)	No of survived nematodes	No of dead nematode	Total of emerged nematodes	Mortality (%)	Survival (%)	Corrected Mortality (%)	% seed sprouting
Cadusafos (Rugby)	25	60.8 c	154.4	214.3	71.7	28.3	63.5	92.5
	50	44.3 c	158.0	198.3	79.7	20.3	73.8	87.5
	100	23.8 c	172.5	196.3	87.8	12.2	84.3	89.0
	200	0.0 c	180.3	180.3	100.0	0.0	100.0	88.5
	400	0.0 c	192.8	192.8	100.0	0.0	100.0	93.0
Oxamyl (Vydate)	25	135.0 ab	68.5	203.5	33.7	66.3	14.5	94.0
	50	73.8 bc	86.5	160.3	54.0	46.0	40.6	93.5
	100	47.3 c	125.3	172.6	72.6	27.4	64.6	91.5
	200	22.3 c	190.3	212.6	89.5	10.5	86.5	93.0
	400	0.0 c	204.5	204.5	100.0	0.0	100.0	86.0
Check	0.0	187.8 a	54.5	242.3	22.5	77.5	0.0	96.0

*Means followed by the same letter(s) within a column are not significantly different (P≤ 0.05) according to Duncan's multiple range test.

REFERENCES

Amin, A. W. (2001). First record of *Aphelenchoides besseyi* Christie, 1942 (Nematoda: Aphelenchoididae) in Egypt causing white tip leaf disease on rice. Opusc. Zool., Budapest, XXXIII : 3-6.

Anonymous (2003). Central Administration for Agriculture Economics and Statistics, Ministry of Agriculture, Egypt.

Chiyonishio, T. and H. Nakazawa. (1988). Basic studies on the control of rice white-tip nematode by seed disinfestation. I. The technique of the nematode disinfestation. Bulletin of the tollori prefecture Agricultural experiment station. 24:1-37.

De Datta, S. K. (1981). Principles and practices of rice production. Ed:Willy and Sons. New York.

Fucano, H. (1962). Ecological studies on white-tip disease the rice plant caused by *Aphelenchoides besseyi* Christie and its control. Bulletin of the Fukuoka Agricultural Experiment Station. 18:1-108. (English summary).

Giudici, M.L. and Villa, B. (2003). Different white tip symptoms in Italian rice varieties. Proceedings of the third international temperate rice conference, 10-13 March, Punta del'Este, Uruguay.

Giudici, M.L. and Villa, B. (1997). Sintomi di White tip su riso ed estrazione del nematode. L'Informatore Agrario. LIII (29): 63-66.

Giudici, M.L.,Villa, B., A. M. Callegarin and L. Tamborini (2003). White tip disease in Italian rice. Proceedings of the third international temperate rice conference, 10-13 March, Punta del'Este, Uruguay.

Hollis, J. P. and S. Keoboonrueng (1984). Nematode parasites of rice Pp. 95-146 in W.R.Nickle, ed. Plant and insect nematodes. New York:Maecel Dekker.

Hoshino, S. and K. Togashi (2002). Mass extraction method for determining *Aphelenchoides besseyi* density in *Oryza sativa* seeds. Japan J. Nematol., 32(2):25-30.

Hoshino, S. and K. Togashi (2000). Effect of water-soaking and air-drying on survival of *Aphelenchoides besseyi* in *Oryza sativa* seeds. J. Nematol., 32(3):303-308.

Hoshino, S. and K. Togashi (1999). A simple method for determining *Aphelenchoides besseyi* infection level of *Oryza sativa* seeds. Supplement to the Journal Nematol., 31(4s):641-643.

Korayem, A. M. (2002). Detection of *Aphelenchoides besseyi* Christie on rice plants in Northern Nile-Delta, Egypt. Egypt.J.Phytopathol.,30(1): 93-97.

Nandakumar, C., J. S. Prassad, Y. S. Rao, and J. Rao. (1975). Investigation on the white-tip nematode (*Aphelenchoides besseyi* Christie, 1942) of rice (Oryza sativa). Indian J. Nematol., 5:62-69.

Prot, J. C. (1992). White Tip. In " Compendium of rice disease" Ed. by R.K. Webster and P. S Gunnel, A.P. S. Press: pp 46-47.

Tamura, I. And K. Kegasawa (1957). Studies on the ecology of the rice nematode, *Aphelenchoides besseyi* Christie. On the swimming away water temperature. Jap. J. Ecology. 7: 111-114.

Togashi, K. and S. Hoshino (2001). Distribution pattern and mortality of white tip nematode, *Aphelenchoides besseyi* (Nematoda: Aphelenchoididae) , among rice seeds. Nematology, 2(1):17-24.

Villa, B. and Giudici, M.L. (1998). Estrazione di *Aphelenchoides besseyi* dai semi di riso. Sementi elette, XLIV (1): 17-22.

Yamaguchi, T. (1977). New method of rice seed disinfection in Japan. Rev. Plant Protec. Res., 10:49-59.

J. Agric. Sci. Mansoura Univ., 29 (4), April, 2004

تأثير فترة التخزين ، المعاملة بالتجفيف الساخن ، غمر الحبوب فى الماء الساخن أو بمبيدات النيماتودا على حيوية نيماتودا حبوب و أوراق الأرز phelenchAoides besseyi داخل حبوب الأرز

أمين وفدى أمين و منى السيد الشلبى
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اكتشفت نيماتودا حبوب و أوراق الأرز *Aphelenchoides besseyi* فى عينات الحبوب التى جمعت من خمس محافظات شمال دلتا النيل بمصر. وهى البحيرة و الدقهلية و الغربية و كفر الشيخ و الشرقية و ذلك خلال أعوام ١٩٩٨-٢٠٠٠-٢٠٠١-٢٠٠٢-٢٠٠٣) تم حفظ العينات فى ظلام على درجة حرارة المخزن لحين بدأ التجارب و الفحص فى يناير ٢٠٠٤. أشارت النتائج أن مستوى الأعداد الحية للنيماتودا و حيويتها داخل الحبوب تقل بزيادة مدة تخزين الحبوب رغم قدرة بعض الأفراد البقاء لمدة ثلاث أعوام.

تم تقييم استخدام المعاملة بالحرارة للقضاء على النيماتودا داخل حبوب الأرز صنف جيزة ١٧٧ و مكافحتها . حيث أعطى استخدام الحرارة بالتجفيف الساخن عند حرارة ٧٠ م لمدة ٢٤ ساعة أو الغمر فى الماء الساخن عند حرارة ٥٠ م لمدة ١٥ دقيقة نتائج مقنعة فى خفض أعداد نيماتودا الأرز إلى أقل مستوى لها داخل الحبوب بدون التأثير على إنبات الحبوب. و عند غمر حبوب الأرز صنف جيزة ١٧٧ فى تركيزات مختلفة من مبيد كادوسافوس (راجبى ٢٠%) و مبيد أوكساميل (فايديت ٢٤%) لمدة ٢٤ ساعة أوضحت النتائج أن الغمر فى تركيزات كادوسافوس كان أكثر تأثيرا من الاوكساميل مع كل التركيزات المختبرة فى زيادة نسبة الموت لأفراد النيماتودا بين قشرة حبوب الأرز. و لوحظ زيادة نسبة الموت بزيادة تركيز المبيد. و أعطى الغمر فى مبيد الكادوسافوس عند استخدام تركيز ٢٠٠ و ٤٠٠ جزء فى المليون و تركيز ٤٠٠ جزء فى المليون لمبيد اوكساميل أعلى نسبة موت (١٠٠%) لأفراد نيماتودا أوراق و حبوب الأرز داخل الحبوب بدون التأثير على حيوية و نسبة إنبات الحبوب. يتضح من ذلك أن المعاملة الحرارية أو الغمر فى مبيدات النيماتودا يؤديان الى الحصول على حبوب غير حاضنة لنيماتودا الأرز. و بهذا يمكن زراعة حبوب غير مصابة فتقل الإصابة عام بعد آخر.