RESEARCH/INVESTIGACIÓN

APHELENCHOIDES BESSEYI MANAGEMENT: EVALUATION OF RICE VARIETY RESISTANCE AND CONTROL PRACTICES

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ABSTRACT

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Aphelenchoides besseyi is the causative agent of white tip disease. Studies were conducted to determine the prevalence of *A. besseyi* in Egyptian rice cultivation, evaluate the resistance of 10 rice genotypes, and evaluate the efficacy of nematicides for management. *A. besseyi* was found in 27.5% of 182 seed samples, with the rice variety 'Sakha 108' exhibiting the highest incidence. Resistance varied across genotypes, with 'GZ11190-3-8-2-3', 'GZ11190-3-13-1-1', and 'Giza 179' showing high resistance. An increase in the percentage of white tip symptoms was noticed in parallel with the density of *A. besseyi* over three consecutive seasons. A significant negative impact of *A. besseyi* infection was observed on rice growth parameters, grain yield, and quality traits in susceptible varieties, particularly on discolored grains. 'Sakha 108' and 'Sakha 109' had the highest grain yield reduction due to nematode damage. Plants treated with Vydate, Mocap, and Nemazone in the nursery, as well as those subjected to hot water treatment, exhibited no symptoms. These findings are valuable for future rice-breeding strategies, seed production, and management.

Key words: Aphelenchoides besseyi, management, *Oryza sativa,* rice, varietal assessment, yield reduction

RESUMEN

Hagag, E. S., A. M., Tahoon, y R. M. Elamawi. 2024. Manejo de *Aphelenchoides besseyi*: evaluación de la resistencia de variedades de arroz y prácticas de control. Nematropica 54:111-130.

Aphelenchoides besseyi es el agente causal de la enfermedad de la punta blanca. Se realizaron estudios para determinar la prevalencia de *A. besseyi* en el cultivo de arroz en Egipto, evaluar la resistencia de 10 genotipos de arroz y evaluar la eficacia de nematicidas para su manejo. Se encontró *Aphelenchoides besseyi* en el 27,5% de 182 muestras de semillas, siendo la variedad de arroz 'Sakha 108' la que presentó la mayor incidencia. La resistencia varió entre los genotipos, destacando 'GZ11190-3-8-2-3', 'GZ11190-3-13-1-1' y 'Giza 179' que mostraron una alta resistencia. Se observó un aumento en el porcentaje de síntomas de punta blanca en paralelo con la densidad de *A. besseyi* durante tres temporadas consecutivas. Se observó un impacto negativo significativo de la infección por *A. besseyi* en los parámetros de crecimiento del arroz, el peso de 100 granos, el rendimiento de grano y en características de calidad en variedades susceptibles, particularmente con granos descoloridos. 'Sakha 108' y 'Sakha 109' tuvieron la mayor reducción en el rendimiento de granos debido al daño de los nematodos. Vydate, Taglist y Nemazon resultaron eficaces para reducir los síntomas de nematodos cuando se aplicaron a las plantas en el vivero. Estos hallazgos son valiosos para futuras estrategias de mejoramiento del arroz, producción de semillas y manejo.

Palabras clave: Aphelenchoides besseyi, Arroz (*Oryza sativa*), evaluación varietal, reducción del rendimiento, manejo

INTRODUCTION

Rice (*Oryza sativa*) is one of the most significant crops produced globally. Egypt stands as the largest rice producer in the Middle East. In 2021, rice production in Egypt was approximately 500,000 ha, producing about 4.9 million tons (FAOSTAT, 2023) *Aphelenchoides besseyi* is a major nematode pathogen of rice, known as the causal agent of white tip disease. *A. besseyi* can infect rice in all environments where rice is grown. Infection and damage are generally more significant in lowland and deep-water systems than in upland environments (CABI, 2021). The parasitic activity of the nematode in rice primarily targets the meristematic tissues, causing characteristic symptoms such as chlorotic and necrotic tips on the youngest leaves. This parasitic activity may further distort the morphology of the flag leaves. Interference with normal growth and development leads to smaller panicles, reduced spikelet filling leading to a decline in yield, which can reach up to 60% under severe infections (Kanzaki *et al*., 2012). The wide distribution of *A. besseyi* in rice-growing regions across different continents, including Asia, tropical America, Africa, and Europe (Franklin and Siddiqi, 1972; Fortuner and Williams, 1975; Ou, 1985; Ozturk and Enneli, 1997), emphasizes its global importance as a seed-borne pathogen. Studies conducted in Egypt have identified the prevalence of *A. besseyi* in various rice cultivars (Abdel-Hadi *et al*., 2005; El-Sherif and Khalil, 2006).

In Egypt, *A. besseyi* was initially identified by Amin (2001). Korayem (2002) attributed to the introduction of *A. besseyi* into Egypt through imported dwarf rice varieties used in breeding programs. The disease is widespread across Egyptian governorates (Abdel-Hadi *et al*., 2005; El-Sherif and Khalil, 2006). The level of *A. besseyi*-infected grains in Egypt was comparatively moderate (95-120 nematodes/100 seed) compared to other countries (Korayem, 2002), resulting in an estimated grain yield loss of 1.7 to 2% in Egypt (Korayem, 2002). El-Shafey *et al*. (2010) found that the deformation in flag leaf anatomical structure led to reduced chlorophyll content, leaf area, and yield of susceptible rice cultivars. Subsequently, El-Shafey *et al*. (2014) noted significant yield reductions caused by *A. besseyi* in susceptible old rice cultivars like ‘Giza 171’ and ‘Reiho’, as well as in new cultivars like ‘Giza 177’, ‘Sakha 102’, and ‘Sakha 103’. The yield losses in the older cultivars reached 47% due to varying levels of infection.

Multiple strategies for controlling *A. besseyi* have been reported (El-Shafey *et al*., 2010; Pashi *et al*., 2017). Managing foliar plant-parasitic nematodes is challenging. Numerous approaches have been employed to control nematode dispersal, including resistant cultivars, cultural practices, plant extracts, compost, biofumigants, induced resistance, nano-biotechnology applications, and chemical control (El-Saadony *et al*., 2021). The aim of this study was to determine the incidence of *A. besseyi* in cultivated Egyptian rice fields, assess rice genotypes, including promising lines and local varieties, for resistance to *A. besseyi* in greenhouse and field experiments, and evaluate management approaches for this nematode.

MATERIALS AND METHODS

Survey of incidence of A. besseyi in Egyptian rice fields

Rice seed, 250 g, was randomly collected from different varieties and locations in Egypt during 2022 and 2023. *A. besseyi* was extracted from 100 seeds selected from each sample, with five replicates collected per field, according to El-Shafey (2007). The seeds were partially ground in a low-speed blender, soaked in water for 24 hr, and sieved through a fine plastic wire screen to discard crushed rice grains and isolate nematodes in a petri dish. The extracted nematodes were identified and counted using a microscope. The frequency of occurrence for each variety was estimated as a percentage using the following formula:

$$\text{Frequency of occurrence \%} = \frac{\text{Number of positive samples}}{\text{Number of collected samples from each variety}} \times 100$$

Evaluation of rice varieties for resistance to A. besseyi

Aphelenchoides besseyi were extracted from infected seeds of 'Giza 177' collected from fields in Kafr El-Sheikh province in northern Egypt. Nematodes were extracted according to methods of Abdel-Hadi *et al*. (2005) and Hooper (1990) and cultured on Potato Dextrose Agar supplemented with *Alternaria alternata* (Jamali *et al*., 2008). Extraction of the nematodes from *A. alternata* cultures involved washing with a 3% H_2O_2 solution in a beaker, allowing the suspension to settle for 10 min, and subsequently filtering the suspension through a 25 µm-pore sieve. Distilled water was used to eliminate fungal debris (Sun *et al*., 2009). The nematode suspension was subsequently used as inoculum in various experiments. The population of *A. besseyi* was identified with morphological and anatomical characteristics observed using a light microscope (OPTIKA, B-190/B-290 Series, Italy) equipped with a digital camera (LCMOS10000KPA). Measurements and morphometric data were recorded according to Sanwal (1961) and the values of a, b, c, and V% were calculated.

Reaction of varieties to the white tip nematode under greenhouse conditions

Ten rice genotypes, 'Sakha 108', 'Sakha 109', 'Sakha 106', 'Giza 179', 'Giza 178', 'Giza 177', 'Giza 183', 'GZ10101-5-1-1-1', 'GZ11190-3-8-2-3', and 'GZ11190-3-13-1-1' were obtained from the breeding program of Rice Research & Training Center (RRTC), Sakha, Kafr El-Sheikh Governorate, Egypt. Resistance screening in a greenhouse occurred at the Rice Pathology Department, Sakha Agricultural Research Station, Sakha, Kafr El-Sheikh. The seeds were soaked in water at 60ºC for 15 min to ensure the seeds were free of *A. besseyi* (Tülek and Çobunoglu, 2011). Two 30-day-old seedlings of each cultivar were planted in 30 cm-diam. plastic pots containing 5 kg of a steam-sterilized mixture of clay and sand soil (2:1 V/V). At 30 to 40 days after transplanting (DAT), plants were inoculated with a suspension of 500 *A. besseyi*/plant. Inoculum was delivered to the plants using a pipette and nematodes were placed in the cavity between the leaf sheath and culm. Control plants (uninfected or healthy plants) received an equivalent aqueous suspension without nematodes. All plants were kept at 25±5°C in a greenhouse. The genotypes were replicated six times and the experiment was conducted twice in a randomized complete block design.

White-tip nematode symptoms were observed throughout the experiment for inoculated and control plants. Some plant characters were recorded, including: flag leaf length (cm), length (cm) and weight (g) of panicle, and weight of 100 grains (g) at approximately 70 to 80 DAT. The number of nematodes in 100 ripe grains was determined as the final population (Pf) density. Plant resistance to *A. besseyi* was assessed based on symptoms and Pf values using a disease index scale described by Popova *et al*. (1994), with some modifications. The resistance was rated as follows: 0 = no white tip, no small grains and erect panicles (SGPs), no nematodes; 1 = white tip absent, SGPs absent; Pf = 1-50 nematodes per 100 grains; 3 = either white tip or SGPs present; Pf >50 nematodes per 100 grains; 5 = white tip present, SGPs present; Pf >50 nematodes per 100 grains. The average index of infection for each cultivar was calculated as: $P = \Sigma (B \times n)/N$ where n = sum of the number of plants, B = index of infection, and N = total number of infected plants. The 10 tested varieties were classified into five different categories based on the average index of infection; 0: immune, 0.1-1.0: highly resistant, 1.1-3.0: moderately resistant, 3.1-4.0: moderately susceptible, and 4.1-5.0: highly susceptible.

Nematode density and the average index of infection data were statistically analyzed using a one-way analysis of variance (ANOVA). Growth parameter data were analyzed using a two-way ANOVA. Treatment means were separated using Duncan's multiple range test (DMRT) according to Duncan (1955), with all analyses conducted at a significance level of $P \leq 0.05$. All statistical analyses were performed using CoStat statistical analysis software (www.cohortsoftware.com).

Reaction of varieties to A. besseyi *under field conditions*

The field experiments were conducted at the Rice Research & Training Center (RRTC), Sakha, Kafr El-Sheikh Governorate, Egypt (31°5′17″N, 30°56′44″E). Prior to seeding, the seedbed was plowed, dry-grounded, irrigated, then wet-leveled.

Seeds of the 10 varieties evaluated in the greenhouse experiment were directly sown in nursery beds in mid-May. One month later, two seedlings/hill were transplanted manually in plots measuring 3 × 3.5 m^2 at 20 × 20 cm with a buffer area of 0.5 m × 0.5 m between the plots. Weed management was done manually. Nitrogen fertilizer in the form of urea (46% N) was applied twice for a total of 165 kg N ha^{-1}. The first split equaled two-thirds of the total N amount and was applied and incorporated into soil just before the first irrigation, while the second split equaled one-third and was top-dressed 30 days after application of the first split. Other culture practices were undertaken as recommended. There were two field experiments conducted.

In Experiment 1, during 2021, 2022, and 2023, natural field resistance of various rice varieties to white tip disease as well as the transmission rate of *A. besseyi* were evaluated. The study was arranged in a randomized complete block design with three replicates. Visually infected seeds from each variety were selected from the breeding program plots at RRTC farm. The seeds were obtained from infected panicles from the previous season and replanted each season. In addition, highly susceptible and infected 'Sakha 108' was planted in the area between the blocks and rows to further encourage nematode infection. The percentage of plants with symptoms of white tip was recorded after 70 to 80 DAT. Rice plants were harvested at the end of each growing season and number of *A. besseyi*/100 seeds was determined following the method described above.

Experiment 2 was conducted in two separate trails during 2022 and 2023. In 2022, the trial was arranged to determine the percentage of plants of the 10 varieties with symptoms of white tip disease and number of *A. besseyi*/100 grains in a completely randomized block design with three replications. Seeds treated with water at 60ºC for 15 min from each variety were used to ensure that seeds were free of nematodes. To encourage nematode infection, infected seeds of 'Sakha 108' were planted in areas between rows. Additionally, 5,000 *A. besseyi*/m^2 were added to plants at 20 DAT. The percentage of plants with symptoms of white tip disease was determined at 70 to 80 DAT. At harvest, 250 g of grain was collected to determine the number of *A. besseyi*/100 seeds as described above. In the sconed trial, the experiment was arranged to determine grain yield, discoloration, and grain quality of the 10 varieties in a split plot design with three replications. The main plots were the different varieties, and the subplots were infected and noninfected (control) plants with *A. besseyi*. Seeds were treated with hot water at 60ºC for 15 min to ensure seeds were free of nematodes. To encourage nematode infection, infected seeds of 'Sakha 108' were planted in areas between rows of each variety. Additionally, 5,000 *A. besseyi*/m^2 were added to plants at 20 DAT. Nematode-free seeds (noninfected) were used as control (Tülek and Çobanoğlu, 2011). At harvest approximately, 100 DAT, grain yield/m^2 was determined for each plot. Samples of rice grains (250 g) were taken from each variety to analyze grain discoloration percentage and grain quality. Reduction percentage for selected characters was calculated as:

$$\text{Reduction \%} = \frac{\text{Uninfected (Control)} - \text{infected}}{\text{Uninfected}} \times 100$$

The grain quality characters were calculated as percentage of hulling, milling, and head rice according to Cruz and Khush (2000) in addition to weight of 100 hulling grain and coloring grain percentage. Cleaned rough rice, 150 g, at 14% moisture content was dehulled using an Experimental Huller Machine (Satake, Japan). The brown rice was separated and weighed and then the hulling percentage was calculated as:

$$\text{Hulling \%} = \frac{\text{Weight of brown rice (g)}}{\text{Weight of rough rice (g)}} \times 100$$

The brown rice was milled using MC GILL Rice Miller No. 2. (S.K. Appliances, India). The total milled rice (whole milled grains + broken milled grains) was weighed, and the milled rice percentage was calculated as:

$$\text{Milling \%} = \frac{\text{Weight of milled rice}}{\text{Weight of rough rice}} \times 100$$

Whole milled grains were separated from the total milled rice using a rice sizing device SKU: 61-220-50 (Seedburo, Des Plaines, IL). The percentage of head rice (whole grains) was calculated as:

$$\text{Head rice (whole grains) \%} = \frac{\text{Weight of head rice (g)}}{\text{Milled rice weight (g)}} \times 10$$

Evaluation of treatments for the management of A. besseyi in the field

In 2022 and 2023 treatments for the management of *A. besseyi* were evaluated in a randomized complete block design. The treatments were: T1) hot water seed treatment prior to planting (60 ºC for 15 min.), T2) nursery treatment with Potassium silicate 2% (El-Gomhoria Chemical Company, Egypt), T3) foliar treatment at 30 DAT with Potassium silicate 2%, T4) nursery treatment with Taglis@ 90% w/w (*Tagetes* sp. 80% and Algae sea 10%; Shoura Chemicals, Egypt), T5) foliar treatment at 30 DAT with Taglis@ 90% w/w, T6) nursery treatment with Vydate 24% (Oxamyl; Corteva Agriscience, USA) applied at 3L/fed., T7) foliar treatment at 30 DAT with Vydate 24%, T8) nursery treatment with Mocap 10% GR (Ethoprophos; AMVAC Chemical Corporation, USA) applied at 30 kg/fed., T9) foliar treatment at 30 DAT with Mocap 10% GR, T10) nursery treatment with Nemazone@ 10% GR (Fosthiazate; Hebei Veyong Bio-chemical Co., Ltd., China) applied at 12.5 kg/fed., T11) foliar treatment at 30 DAT with Nemazone 10% GR, T12) nursery treatment with Kongest (3% Abamectin and 12% Imidacloprid; Qingdao Audis Bio.Tech Co., Ltd, China) applied at 20 cm/100L, T13) foliar treatment at 30 DAT with Kongest, and T14) untreated control (infected seeds).

Nematode infected rice of 'Sakha 108' was obtained from the seed production program at RRTC. Prior to planting the seedbed was plowed, dry-grounded, irrigated, then wet-leveled. The seedbed was divided into 14 4 m^2 plots for each treatment with a requisite buffer area 0.5 m × 0.5 m between the plots. Infected 'Sakha 108' rice seeds were directly sown in seedbeds plots. For nursery treatments, approximately 500 g of soil granules were mixed with 50 ml of fresh water and nursery treatment then uniformly spread three days after sowing. All plots received N in the form of urea (46.5% N) 20 days after sowing. Weed management was done manually. After 30 days, seedlings were uprooted from the seedbed and transplanted into plots measuring 3.5 x 3 m. The transplanting operation was done manually at a planting density of 20 x 20 cm and two seedlings per hill. At 30 DAT, the foliar treatments were applied. The nitrogen fertilizer and culture practice were done as described above. The percentage of plants with white tip nematode symptoms was recorded at 70 to 80 DAT. The population density of *A. besseyi* was determined in the laboratory by extracting nematodes from collected grains as described above.

In all field experiments, ANOVA was performed and treatment means were separated using Duncan's multiple range test (Duncan, 1955). All analyses were conducted at a significance value of $P \leq 0.05$. All statistical analyses were performed in CoStat statistical analysis software.

RESULTS

Morphological identification of A. besseyi

The morphological characteristics of *A. besseyi* in the present study were compared with published reports (Christie, 1942; Amin, 2002; Fortuner, 1970; El-Shafeay, 2007). The measurements of the *A. besseyi* used in the experiments closely aligned with previous characterizations of the nematode. Comprehensive details and visual representations can be found in Table 1.

Incidence of A. besseyi *in rice samples from Egyptian fields*

A total of 182 samples representing nine different cultivars were collected from seven governorates of Egypt (Kafr El-Sheikh, Beheira, Sharquia, Dakahlia, Gharbiya, Damietta, and Port Said) in 2022 and 2023. Among the nine cultivated varieties examined, six were infested with *A. besseyi* (Table 2). *A. besseyi* was detected in 27.5% of the samples. The cultivar 'Sakha 108' exhibited the highest incidence of infestation, while 'Sakha Super 300' had the highest density of *A. besseyi*.

Reaction of rice varieties to A. besseyi under greenhouse conditions

White tip disease symptoms were observed on tillers of the rice varieties. Leaf tips, 3 to 5 cm, whitened and then died off and eventually became shredded. The panicles appeared shorter and often

Table 1. Morphometric comparison between an Egyptian population of *Aphelenchoides besseyi* and other *A. besseyi* populations (Christie,1942; Yokoo, 1948; Allen, 1952; Fortuner, 1970; Amin, 2002; El-Shafey, 2007; and present study, 2024).

	Aphelenchoides besseyi						
Character	Christi, 1942	Yokoo, 1948	Allen, 1952	Fortuner, 1970	Amin, 2002	El-Shafey, 2007	Present study, 2023
Female							
Length (µm)	660-750	740-840	620-880	570-840	540-770	520-880	580-810
Width (µm)	17-22	-	-	-	15-18	15-20	15.7-22.20
Spear length (µm)	-				8.5-13	-	12.94-14.98
A	32-42	39-53	38-58	39-53	36-51.2	34.7-44	32.22-46.12
B	10.2-11.4	9.2-13.1	9-12	9.2-13.1	9.7-12.7	8.7-13.1	10.15-13.71
b′	-	4.06-5.77	-	4.06-5.77	4.7-6.3	3.7-5.9	4.56-6.57
C	17-21	13.8-20.4	15-20	13.8-20.4	15.4-20.1	14.9-21.5	14.29-20.50
Tail length (µm)	36-42	-	-	-	30-45	35-41	38.19-44.61
V%	68-70	68-73.6	66.72	68.7-73.6	69.2-74.6	67.3-73.9	67.45-76.81
Male							
Length (µm)	540-640	530-610	440-720	530-610	520-660	480-740	467-691
Width (µm)	14-17	-	-	-	15-18	14-19	12.85-16.94
Spear length (µm)	-				9-12	-	12.65-14.84
A	36-39	40.7-46.9	36-47	40.7-46.9	33-41.6	34-39	30.12-44.66
B	8.6-8.8	8.87-10.70	9-11	8.8-10.7	8.98-10.6	8.6-10.1	8.94-9.37
b′	-	3.57-4.91	-	3.57-4.91	3.8-5.1	3.4-4.93	4.25-6.16
C	15-17	16-20	14-19	16-20	15.5-22	14-18	14.76-19.15
Tail length (µm)	34-37	28-52	-	-	30-39	35-41	32.41-41.20
Spicules length (µm)	-		-	11.4-18.21	15-18	-	15.98-19.19

Table 2. Incidence of *Aphelenchoides besseyi* in Egyptian rice fields in 2022 and 2023.

Variety	Rice type	Number of collected samples	Number of samples infested with *A. besseyi*	Mean number of *A. besseyi* in infested samples/100 seeds (range)	Occurrence % of *A. besseyi*
Sakha 101	Japonica	22	6	20.1 (10.1 - 29.2)	27.3
Sakha 103	Japonica	2	0	0	0
Sakha 104	Japonica	18	6	13.4 (2.1 - 27.6)	33.3
Sakha 106	Japonica	2	0	0	0
Sakha 108	Japonica	40	22	18.9 (2.6 - 65.0)	55.0
Giza 177	Japonica	18	4	48.6 (10.8 - 86.4)	22.2
Giza 178	Indica Japonica	22	2	13.8 (12.5 - 15.1)	9.1
Giza 179	Indica Japonica	6	0	0	0
Super 300	Japonica	52	10	62.9 (2.7 - 180)	19.2
Total		182	50		

atrophied at the tips. Moreover, the fertile flowers sometimes produced malformed grains (Figs. 1 and 2). The rice varieties varied in the number of *A. besseyi* recovered. 'Sakha 108' and 'Giza 177' had the highest nematode densities per 100 grains. Based on the average index of infection, five varieties were highly susceptible ‘Sakha 108’, ‘Sakha 109’, ‘Sakha 106’, ‘Giza 177’, and ‘GZ10101-5-1-1-1’, whereas, ‘Giza 179’, ‘GZ11190-3-8-3-2, and ‘GZ11190-3-13-1-1’, were highly resistant (Table 3). Two varieties, ‘Giza 178’ and ‘Giza 183’, were moderately resistant.

Figure 1. White tip disease symptoms caused by *Aphelenchoides besseyi* on rice: A = leaves, B = flag leaf, C = grains, and D = panicles (Right: uninfected, left: infected).

This detailed evaluation underscores the variation in susceptibility and resistance among rice varieties to *A. bessyi*, highlighting the importance of selecting resistant varieties to manage the disease effectively. Susceptible varieties had lower values in the parameters length of flag leaf and panicle, weight of panicle, and weight of 100 grains compared to control plants (Table 4). Among these, ‘Sakha 108’ had the greatest reduction of flag leaf length (32.8%) while ‘Sakha 109’ exhibited the highest reduction of panicle weight and weight of 100 grains (29.6 and 33.1%, respectively). ‘Giza 177’ had the highest reduction of panicle length with decreases of 24.9 %.

Figure 2. Development of white tip disease symptoms caused by *Aphelenchoides besseyi* on rice leaves.

Table 3. Host status of rice varieties to *Aphelenchoides besseyi* based on the average index of infection under greenhouse conditions.

Variety	Number of nematodes/100 seeds (average)	Symptom's reaction[w]	Average index of infection[x]	Resistance category[y]
Sakha 108	202.1 a[z]	WT&SPGs	4.5 a	HS
Sakha 109	164.3 b	WT&SPGs	4.5 a	HS
Sakha 106	162.3 b	WT&SPGs	4.5 a	HS
Giza 179	49.0 d	-	1.0 c	HR
Giza 178	89.0 c	SPGs	1.5 c	MR
Giza 177	168.5 b	WT&SPGs	4.2 a	HS
GZ10101-5-1-1-1	143.3 b	WT&SPGs	4.5 a	HS
Giza 183	51.2 d	WT&SPGs	2.5 b	MR
GZ11190-3-8-2-3	13.0 e	-	1.0 c	HR
GZ11190-3-13-1-1	10.6 e	-	1.0 c	HR

[w]WT = white tip; SPGs = small panicles and grains; - = no symptoms.
[x]Average index of infection; 0: immune, 0.1-1.0: highly resistant, 1.1-3.0: moderately resistant, 3.1- 4.0: moderately susceptible, 4.1-5.0: highly susceptible
[y]HR, highly resistant; MR, moderately resistant; HS, highly susceptible.
[z]In the same column, Means followed by a common letter(s) are not significantly different at the 5% level by Duncan's Multiple Range Tests (DMRT).

Transmission rate of white tip nematode disease across various rice varieties under natural field conditions over three consecutive seasons

There was an increase in the percentage of plants with white tip disease symptoms in susceptible varieties over three consecutive seasons (Fig. 3A). Specifically, in 2023 'Sakha 109' had the highest value for nematode density (273 *A. besseyi*/100 seeds) (Fig. 3B). Conversely, 'Giza 179', 'Giza 178', 'GZ11190-3-8-2-3', and 'GZ11190-3-13-1-1' did not display white tip disease symptoms across seasons and had the lowest density of *A. bessyi*. Only weak symptoms appeared on 'Giza 183' across all years. These findings showed the consistent susceptibility of certain rice varieties to *A. besseyi*, while highlighting the resistance of others, which is crucial for guiding future breeding and cultivation practices aimed at minimizing the impact of this pest on rice production.

Reaction of varieties to A. besseyi under field conditions

In the infection trials, the rice varieties 'Giza 179', 'Giza 178', 'Giza 183', 'GZ11190-3-8', and 'GZ11190-3-13' had no panicles with white tip disease symptoms (Fig. 4A).'Sakha 109' had the highest percentage of plants with white tip disease symptoms in both years (53 and 55%, respectively). 'Sakha 109' had the highest *A. besseyi* population densities with 337 and 357 nematodes /100 seeds in both seasons, respectively (Fig. 4B).

For grain yield, *A. besseyi* infection significantly reduced grain yield in susceptible varieties in the infected plots compared with the control (Table 5 and Fig. 5). Among the infected plants, 'Sakha 109' had the highest percentage in grain yield reduction with decreases of 23.9 and 31.3%, in 2022 and 2023, respectively. Additionally, 'Sakha 108' matured later than other varieties as a result of nematode infection (data not shown). The quality attributes of rice grains displayed notable negative impacts due to *A. besseyi* infection in the infected susceptible varieties in comparison to the uninfected plants during 2022 and 2023 (Table 5 and Fig. 6). The highest reduction in hulling percentage was 15.4% in 'Sakha 108' during 2022 while it was 14.7% in 2023 with 'Sakha 109'. The highest reduction in milling percentage was 16.6% in 'Sakha 109' during 2022 while it was 14.9% during 2023 in 'Sakha 108'. Also, 'Sakha 108' had the most prominent decreases in head rice percentage, 58.3 to 45.0%, with a reduction of 22.9% in 2022, while 'GZ10101-5-1-1-1' had 55.0 to 42.0% with reduction 23.6 % in 2023. Symptoms of infection by *A. besseyi* expressed as transverse and

Table 4. The influence of *Aphelenchoides besseyi* infection on rice varieties characteristics under greenhouse conditions.

Varity	Treatment[y]	Flag leaf length (cm)	Reduction %	Panicle length (cm)	Reduction %	Panicle weight (g)	Reduction %	Weight of 100 grains (g)	Reduction %
Sakha 108	I	12.3 i[z]	32.8	20.0 d-g	18.9	2.0 d-g	14.2	2.0 f-i	13.2
	H	18.3 g	-	24.7 bc	-	2.3 abc	-	2.6 bc	-
Sakha 109	I	15.0 h	25.0	17.0 hij	12.1	1.8 ghi	29.6	1.8 i	33.1
	H	20.0 fg	-	19.3 e-h	-	2.5 ab	-	2.7 b	-
Sakha 106	I	21.0 f	16.0	18.0 ghi	18.2	1.7 hi	19.8	2.2 c-i	15.6
	H	25.0 e	-	22.0 cde	-	2.1 c-f	-	2.6 bcd	-
Giza 179	I	25.0 de	3.8	15.0 jk	6.2	1.9 f-i	12.9	2.3 b-h	12.6
	H	26.0 cde	-	16.0 ijk	-	1.9 e-h	-	2.6 bc	-
Giza 178	I	26.0 cde	7.1	17.0 hij	5.6	2.1 c-f	4.6	1.9 ghi	14.9
	H	28.0 bc	-	18.0 ghi	-	2.2 cde	-	2.3 b-i	-
Giza 177	I	15.3 h	19.5	14.0 k	24.9	1.9 f-i	28.5	2.4 b-g	32.1
	H	19.0 fg	-	18.7 f-i	-	2.6 a	-	3.5 a	-
GZ10101-5-1-1-1	I	18.0 g	10.0	19.7 d-h	24.4	1.8 f-i	16.7	2.2 b-i	15.5
	H	20.0 fg	-	26.0 ab	-	2.2 cde	-	2.6 b	-
Giza183	I	24.3 e	6.5	22.3 cd	-9.8	1.6 i	18.5	2.4 b-f	2.0
	H	26.0 cde	-	20.3 d-g	-	2.0 d-g	-	2.5 b-e	-
GZ11190-3-8-2-3	I	28.7 ab	7.7	21.0 def	5.9	2.0 d-g	11.1	2.0 e-i	5.1
	H	31.00 a	-	22.3 cd	-	2.3 bcd	-	2.1 d-i	-
GZ11190-3-13-1-1	I	27.7 bcd	4.8	25.3 ab	9.5	1.8 f-i	5.2	1.8 hi	3.2
	H	29.0 ab	-	28.0 a	-	1.9 e-h	-	1.9 ghi	-

[x] I= Infected plants, H= Control plants (uninfected).

[z] In the same column, means followed by a common letter(s) are not significantly different at the 5% level by DMRT.

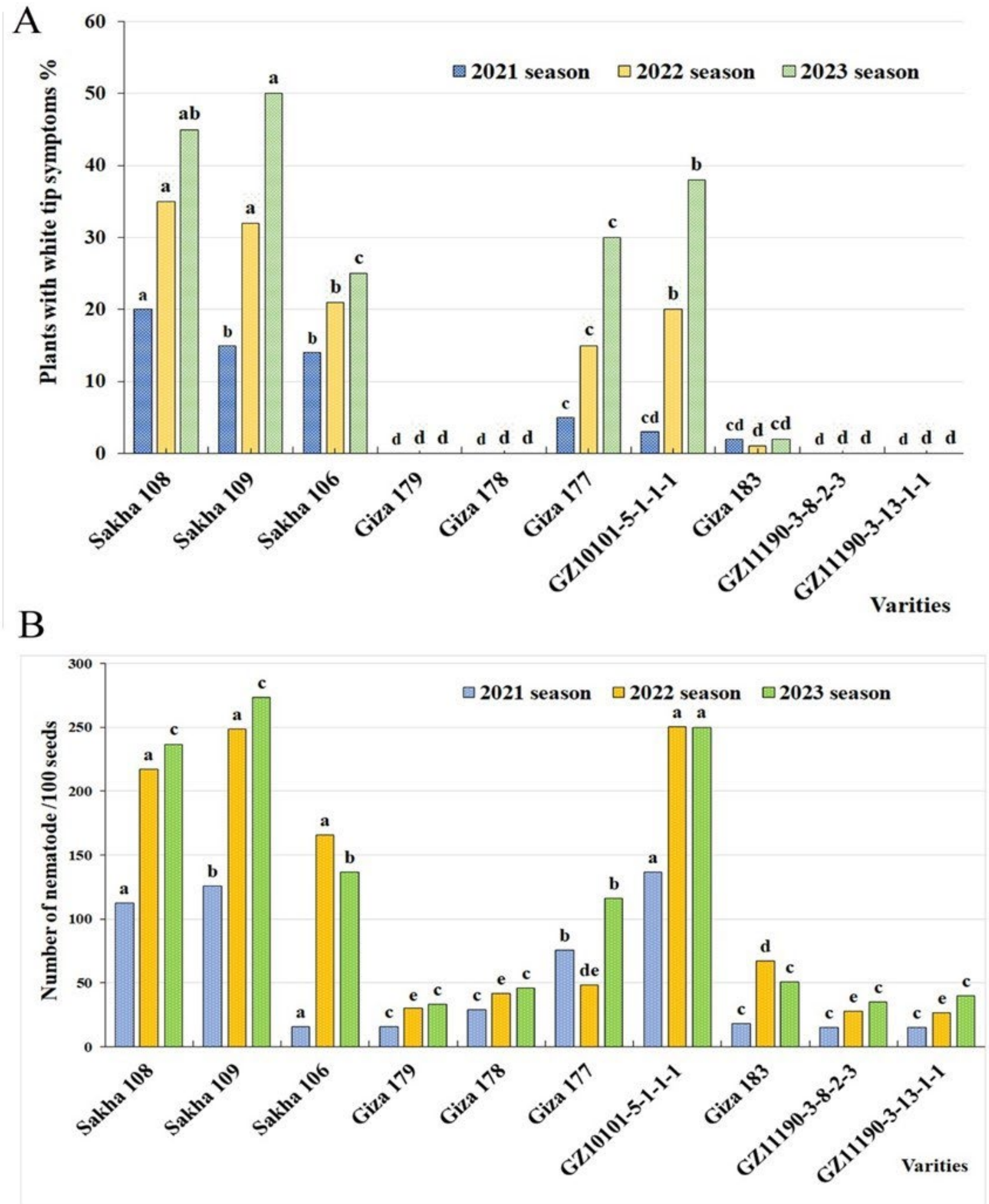

Figure 3. Transmission rate of *Aphelenchoides besseyi* across various rice varieties under natural field conditions over three consecutive seasons, 2021 to 2023. A: Plants with white tip disease symptoms %, B: Density of *A. besseyi* /100 seeds.

longitudinal cracks in rice grains (Fig. 7A). 'Sakha 108' had the highest percentage increase in discolored-grains reaching 94.5 and 93.9%, during 2022 and 2023, respectively, followed by 'Sakha 109' and 'Sakha 106' (Fig. 7B).

Efficacy of control methods on white tip disease and A. besseyi under field conditions

Application of all nematicides and bio-compounds reduced both the incidence of plants exhibiting white tip disease symptoms and the density of *A. besseyi* per 100 seeds. Treatments proved more effective when applied in the nursery stage rather than when applied 30 DAT (Table 6). Plants treated with Vydate, Mocap, and Nemazone in the nursery, as well as those subjected to hot water treatment, exhibited no symptoms in both years. There was a considerable reduction in *A. besseyi* per 100 seeds with Vydate 24% SL being

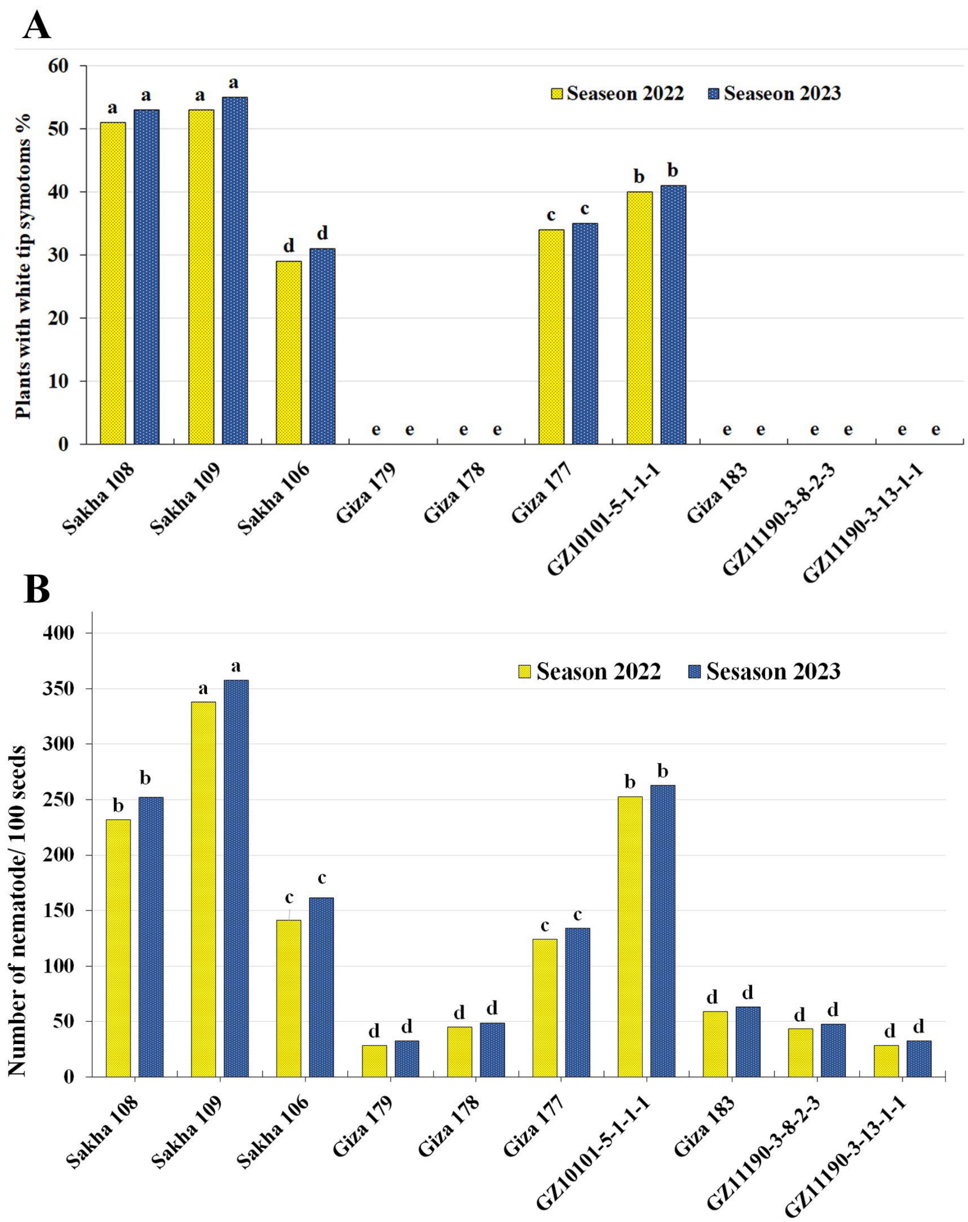

Figure 4. Reaction of rice varieties to *Aphelenchoides besseyi* during 2022 to 2023 under field conditions. A: Plants with white tip disease symptoms %, B: Number of *A. besseyi* /100 seeds.

very effective whether applied in the nursery or 30 DAT, with reductions of 98.8% and 93.3%, respectively, in 2022. This reduction was comparable to the efficacy of hot water treatment, which resulted in 98.1% and 92.3% reduction, respectively, in 2023. Mocap 10% GR, Taglis, and Nemazone 10% GR were more efficient when applied in the nursery, with similar efficacy to Vydate.

DISCUSSION

Aphelenchoides besseyi is a seed-borne nematode that causes white tip disease. Rice seed infested with *A. besseyi* allows for the widespread dispersion of this nematode. The detection of *A. besseyi* in seeds poses a challenge since not all visibly symptomatic seeds are necessarily infested by the nematode. Previous surveys in Egypt (Abdel-Hadi *et al*., 2005), revealed the widespread occurrences of white tip disease across most rice

Table 5. The impact of *Aphelenchoides besseyi* on grain yield and grain quality of rice cultivars under field conditions in 2022 and 2023.

Variety	Treatment[y]	Grain yield (kg/m^2)		Hulling %		Milling %		Head rice (HR)%	
		2022	2023	2022	2023	2022	2023	2022	2023
	I	0.7 gh[z]	0.7 j	69.7 ij	70.7 g	56.3 hi	57.0 fg	45.0 e	46.0 g
Sakha 108	H	0.9 ef	0.9 fg	82.3 abc	81.0 b	67.3 c	67.0 c	58.3 a	56.0 bc
	I	0.7 h	0.7 j	71.0 hij	72.3 fg	52.0 j	52.7 h	44.0 e	45.0 g
Sakha 109	H	0.9 ef	1.0 f	82.7 ab	84.7 a	62.3 fg	61.7 ef	55.3 abc	56.3 b
	I	0.8 fgh	0.8 hi	73.0 ghi	76.0 cde	54.3 i	56.0 g	54.7 abc	56.3 b
Sakha 106	H	1.0 ef	1.0 f	77.0 d-g	74.3 ef	57.7 h	57.0 g	55.0 abc	56.4 bc
	I	1.3 d	1.2 d	75.0 fgh	77.0 cde	74.7 b	76.3 ab	52.7 cd	54.7 bcd
Giza 179	H	1.3 bcd	1.2 d	78.0 def	77.3 cde	77.7 a	77.0 a	52.3 cd	53.3 def
	I	1.4 a-d	1.4 abc	74.0 fgh	75.7 dec	76.0 ab	76.7 ab	53.3 bcd	55.0 bcd
Giza 178	H	1.5 abc	1.4 ab	75.3 efg	74.3 ef	77.3 a	77.0 a	54.3 a-d	55.0 bcd
	I	0.7 h	0.7 ij	77.0 d-g	77.0 cde	64.0 ef	64.7 c-f	42.7 e	43.7 fgh
Giza 177	H	0.9 fg	1.0 f	84.0 a	83.3 ab	74.3 b	73.7 b	55.0 abc	56.0 bc
	I	0.9 ef	0.8 gh	75.0 e-h	74.7 def	60.7 g	61.3 f	42.3 e	42.0 h
GZ10101-5-1-1-1	H	1.1 e	1.1 e	80.3 a-d	82.0 ab	68.0 c	67.0 c	54.0 a-d	55.0 bcd
	I	1.4a-d	1.5 a	67.0 j	67.0 h	64.7 de	65.7 cd	51.0 cd	52.0 ef
Giza183	H	1.5 a	1.5 a	68.3 j	66.7 h	67.3 c	66.7 cd	52.7 cd	53.7 cde
	I	1.3 cd	1.3 cd	72.7 ghi	77.0 cde	62.3 fg	63.3 def	50.0 d	51.0 f
GZ11190-3-8-2-3	H	1.4 a-d	1.3 bc	73.7 ghi	77.3 cd	60.3 g	64.3 c-f	52.3 cd	53.0 def
	I	1.4 abc	1.4 ab	78.3 cde	77.0 cde	65.0 de	64.7 cde	57.7 ab	60.3 a
GZ11190-3-13-1-1	H	1. 5 ab	1.5 a	78.7 b-e	77.7 c	66.3 cd	65.3 bc	57.3 ab	59.3 a

[y]H= Control plants (uninfected), I = infected plants.

[z]In the same column, means followed by a common letter(s) are not significantly different at the LSD 5% level by Duncan's multiple range test.

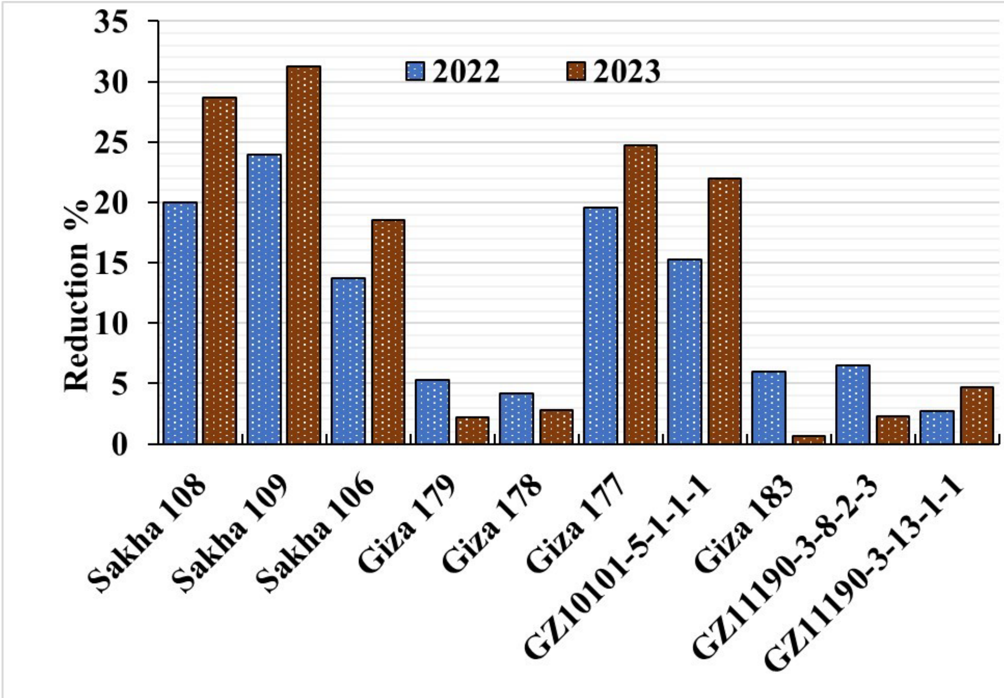

Figure 5. Effect of *Aphelenchoides besseyi* on grain yield reduction during 2022 and 2023.

cultivation areas. The observed *A. besseyi* densities in infected grains across Egypt were moderate (ranging from 95 to 120 nematodes/100 seed) when compared to other countries (Youssef, 2014). Our findings indicated the presence of *A. besseyi* in 28% of the 182 samples examined. Notably, 'Sakha 108' displayed the highest incidence, with 55% prevalence among 40 samples, and 'Sakha Super 300' exhibited the highest density of *A. besseyi* (180 nematodes/100 seeds). During our survey, 'Sakha 108' and 'Sakha Super 300' are considered the most widely grown varieties of rice. The severity of *A. besseyi* infestation was categorized based on the number of nematodes/100 seeds (Shahabi *et al*., 2016). Accordingly, within the infested rice seed samples, approximately 84% had a low population density (<50 nematodes/seed), 8% had moderate population densities (50-100 nematodes/seed), and the remaining 8% had a high population density (>100 nematodes/seed). Comparable surveys conducted in various rice-growing regions have demonstrated varying degrees of *A. besseyi* infestation. In Turkey, infestation rates of *A. besseyi* were 16% in 2007 and increased to 43% in 2008 (Tülek and Çobanoğlu, 2010). In Louisiana, the incidence of *A. besseyi* was reported as 7% in 1984 (McGawley *et al*., 1984), while a survey conducted in 2016 reported an increased percentage of *A. besseyi* incidence, 12% (Godoy *et al*., 2019). Jamali *et al*. (2006) suggested that the differences in *A. besseyi* densities within rice seed samples obtained from diverse regions might be attributed to climatic variations in the respective rice environments.

In this study, 10 rice varieties were evaluated for their resistance to *A. besseyi* in both greenhouse and field experiments. Greenhouse results indicated that assessing resistance using both white tip disease symptoms or SPGs and *A. besseyi* population densities together was more effective. Our results categorized three rice varieties as highly resistant, two as moderately resistant, and five as highly susceptible. The trends observed in resistance to *A. besseyi* under field conditions aligned with the greenhouse findings. The presence or absence of white tip disease symptoms and *A. besseyi* by the end of the season consistently served as significant indicators of resistance (Cook, 1974). Furthermore, the absence of white tip symptoms on the flag leaf of the resistant cultivars was in line with previous studies (Tülek, 2016).

Figure 6. Effect of *Aphelenchoides besseyi* on discolored grains on rice seed endosperm on susceptible varieties (Left: infected; right: uninfected).

In our experiments, infection with *A. besseyi* led to a significant reduction in growth parameters and weight of 100 grains of susceptible varieties compared with the control plants in the greenhouse experiment. These reductions were consistent with findings reported by McGawley *et al*. (1984), Tsay *et al*. (1998), and El-Shafey (2007). The consequences of *A. besseyi* infection includes whitened and shredded leaf tips, crinkled or distorted leaves, abnormal leaf greening, distorted floral parts, and empty grains and rice panicles with fewer filled grains and lower 1,000-grain weight (Waele, 2002; El-Saadony *et al*., 2021).

Under field conditions, our findings revealed an increase in the percentage of plants with white tip disease symptoms with increasing densities of *A. besseyi* in susceptible varieties cross three consecutive seasons. These results may have been due to infestation level of white tip disease increasing each year where the seeds were obtained from infected panicles from the previous season and replanted each season. These findings are in accordance with El-Sherif and Khalil (2006).

Our findings revealed a significant negative impact of *A. besseyi* infection on the quality traits of rice grains in susceptible varieties. There was a reduction in the percentage of hulling, milling, and head rice. Furthermore, there was a substantial increase in the percentage of discolored grains in the susceptible varieties, reaching more than 93% in both season in ‘Sakha 108’, which matures late. Similar results have been reported by EL-Shafey *et al*. (2014) and Asadi *et al*. (2015). Infection by *A. besseyi* led to the production of smaller and malformed seeds attributed to nematode feeding on the grain embryo (Shukla *et al*., 2003). Overall, *A. besseyi* infection not only caused yield loss but also compromised the rice quality by causing black blots on husked rice. Additionally, the smaller kernels from infected plants resulted in reduced agronomic yield, as reported by Asadi *et al*. (2015). However, yield loss was more severe in the panicle with white tip symptoms. Also, *A. besseyi* population densities do not always cause plants symptoms but can lead to yield loss (Feng *et al*., 2014).

The present results under field conditions supported that, in the susceptible varieties, yield reduction ranged of 13.7-23.9% in 2022 and 18.6-31.3% in 2023. ’Sakha 109’ had the highest number of *A. besseyi*/100 seeds in both seasons (337 and 357 nematodes /100 seeds, respectively). ‘Sakha 109’ had the highest *A. besseyi* population densities and percentage of plants with white tip disease symptoms in 2022 and 2023, respectively, resulting in the highest percentage in yield loss of 23.9 and 31.3%. Tülek (2016) reported that cultivars considered resistant, supporting only low reproduction of *A. besseyi*, still sustained yield losses from 8 to 19%. Yield losses caused by *A. besseyi* varied among cultivars and geographic areas. Tülek *et al*. (2015) reported that yield losses ranged from 8 to 52% on 39 cultivars in Turkey.

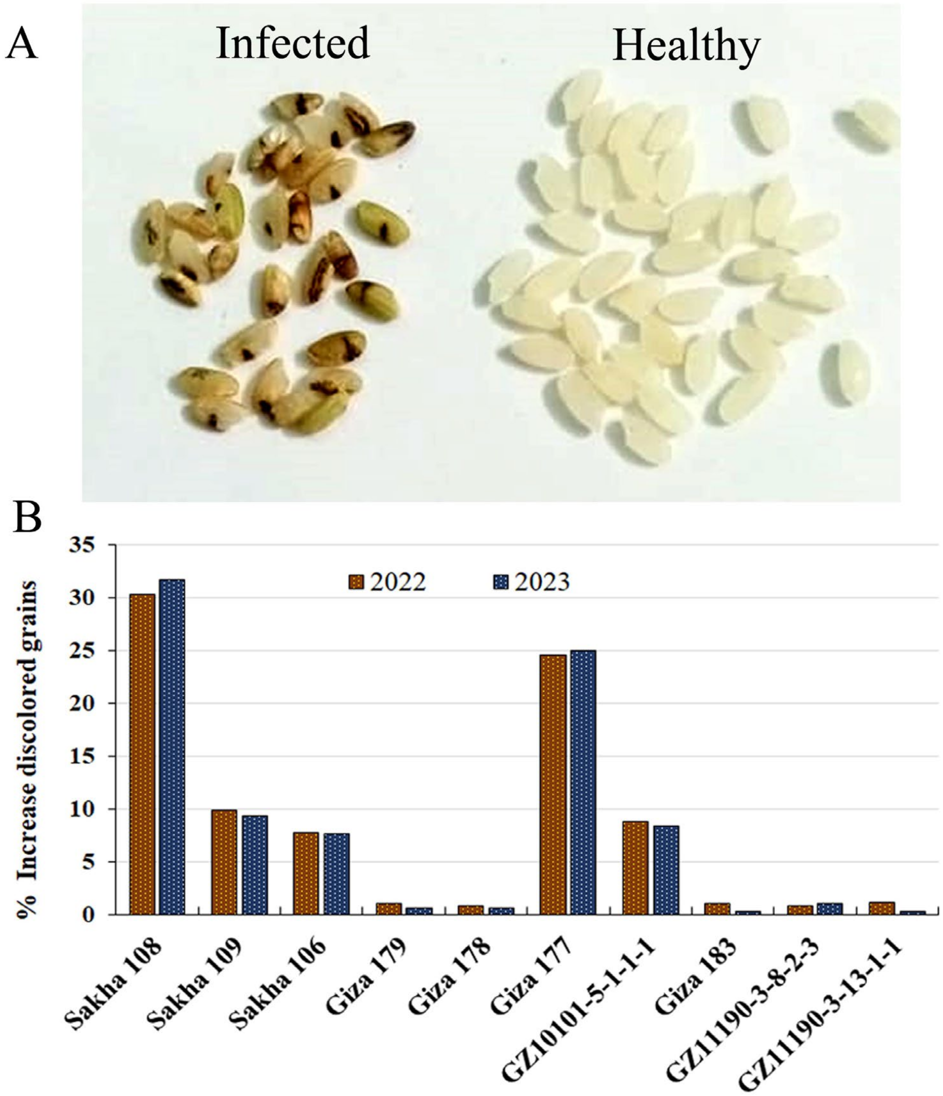

Figure 7. Effect of *Aphelenchoides besseyi* on, A: Transverse and longitudinal cracks of rice grains, B: Percentage of increased discolored grains

Previously, the estimation of grain yield loss in Egypt was 1.7 to 2% (Korayam, 2002) and in 2005 approximately 39% (El-Sherif *et al*., 2006). *A. besseyi* caused a yield reduction in susceptible cultivars, with losses reaching 47% (El-Shafey *et al*., 2014). The question is why this nematode has become more widespread in Egypt despite reduced severity. Rice growers in Egypt usually use a part of the harvested rice seeds for the following season. Thus, the infestation level of white tip disease increases each year. Also, Luc *et al*. (1990) stated that within a locality incidence and severity of the disease can change from year to year and are strongly influenced by cultural practices and local rice types. However, using insecticides either during seed storage or seed treatment and resistant cultivars may limit the spread and development of *A. besseyi* (Godoy *et al*., 2019).

In the present study, all control treatments were effective for the management of rice white tip disease with a reduction of the percentage of plants with white tip disease symptoms and number of *A. besseyi*/100 seeds. The treatments were efficient when applied early rather than after white tip disease symptoms appeared. Hot water treatment of seeds and nematicides as nursery applications were the most effective in management *A. besseyi*. These results are in accordance with the findings of Atkins and Todd (1959), who stated that hot water

Table 6. Efficacy of control treatments applied in the nursery (N) or 30 days after transplanting (DAT) on *Aphelenchoides besseyi* in 2022 and 2023.

Treatment	Plants w/white tip symptoms %			No. of nematodes/100 seed		
	2022	2023	Reduction	2022	2023	Reduction
Hot water (Seed treatment)	0.0 h[x]	0.0 h	100.0	0.0 f	1.6 g	98.4
Potassium silicate 2% (N)[y]	11.3 f	12.3 f	82.5	18.3 e	17.3 e	83.4
Potassium silicate 2% (DAT)[z]	36.4 b	37.0 b	21.7	82.0 b	87.2 b	16.2
Taglis 90% (N)	2.3 gh	3.0 gh	94.3	6.0 ef	7.3 fg	93.0
Taglis 90% (DAT)	31.3 c	32.0 cd	66.9	34.6 d	34.7 d	66.6
Vydate 24% (N)	0.0 h	0.0 h	98.8	1.3 f	2.0 g	98.1
Vydate 24% (DAT)	17.3 d	18.0 e	93.3	7.0 ef	8.0f g	92.3
Mocap (N)	0.0 h	0.0 h	98.1	2.0 f	3.3 g	96.8
Mocap (DAT)	27.3 d	28.0 d	85.1	15.6 e	14.8 ef	85.8
Nemazone (N)	0.0 h	0.0 h	93.0	7.3 ef	8.3 fg	92.2
Nemazone (DAT)	28.3 d	29.0 d	61.8	40.0 cd	37.6 d	63.8
Kongest (N)	4.0 g	5.0 g	90.4	10.0 ef	9.3 fg	91.1
Kongest (DAT)	33.3 c	34.3 bc	51.0	51.3 c	52.0 c	50.0
Untreated control	45.0 a	45.7 a	-	104.7 a	104.0 a	-

[x]In the same column, means followed by a common letter(s) are not significantly different at the 5% level by Duncan's multiple range test.
[y](N) = Nursery treatment
[z](DAT)= 30 Dayes After Transplanting

treatment can be used to eradicate rice white tip disease. Also, Cho *et al*. (1987) reported that effective chemical control for *A. besseyi* was seed disinfection before seedling. *A. besseyi* control with pre-harvest nematicide treatments was only partially effective (Aleksandrova, 1981). In our experiment, applications of Vydate, whether applied at the nursery or after symptoms appeared, and nursery applications of Mocap, Taglist, Nemazon, and Kongest were not significantly different from hot water treatment in both seasons. The efficiency of these nematicides ranged from 90.4 to 98.8% and 91.1 to 98.1% reduction in nematode population during 2022 and 2023, respectively. El-Shafey (2007) reported that nursery application is the proper time for the application of nematicides, and the application of Vydate and Mocap at the nursery resulted in the lowest severity of *A. besseyi* infection. These compounds are systemic nematicides that inhibit feeding and temporarily inactivate, repel, or kill nematodes in the plant rhizosphere (Al-Azzeh and Abu-Gharbieh, 2004).

Tagiles (composed of 80% *Tagetes* sp. and 10% seaweed algae) resulted in a substantial decrease in *A. besseyi* population densities when applied in the nursery. This effect can be attributed to the generation of various toxic bioactive compounds. Alpha-terthienyl is considered the primary allelopathic compound responsible for nematode suppression (Marahatta *et al*., 2012; Adam *et al*., 2023). Kongest, a formulation containing 3% abamectin and 12% imidacloprid, significantly reduced *A. besseyi* population densities when applied in the nursery. Abamectin, a member of the avermectin group produced by *Streptomyces avermitilis*, has insecticidal, acaricidal, and nematicidal properties. Its mechanism involves altering the electrical conductivity of neuronal cells, causing hyperpolarization by activating glutamate-gated chloride channels, ultimately leading to paralysis and death (Bloomquist, 1993; Jayakumar, 2009; El-Marzoky *et al*., 2022). Meanwhile, imidacloprid, a chloronicotinyl systemic insecticide, acts on nicotinic acetylcholine receptors (nAChRs), facilitating sodium (Na^+) entry and potassium (K^+) exit while disrupting nerve communication between neurons, resulting in rapid insect death (Nugnes *et al*., 2023).

Silicon accumulation strengthens plants and forms a protective cuticle-Si layer, reducing disease incidence (Sun *et al*., 2021). The utilization of potassium silicate in our study resulted in a 82.5 and 83.4% reduction in *A. besseyi*/100 seeds in 2022 and 2023, respectively. Silicon-based compounds, recognized for their eco-friendly nature, have shown promise in controlling plant-parasitic nematodes. Yu *et al*. (2022) reported their effectiveness in suppressing nematode reproduction and reducing galling, and disease severity caused by nematodes. Furthermore, calcium silicate inclusion increased lignin thioglycolic acid derivatives in coffee, reducing galls and eggs of *Meloidogyne exigua*. The application of calcium silicate increased the secondary lignin metabolism in roots, reducing root galls and eggs across various *Meloidogyne* species in beans, tomatoes, and coffee (Silva *et al*., 2010).

In conclusion, the rice varieties 'Giza 179', 'GZ11190-3-8-2-3', and 'GZ11190-3-13-1-1' were highly resistant to *A. besseyi,* which is crucial for guiding future breeding. These varieties can be a promising source of resistance for the management of rice white tip disease in Egypt. Also, hot water treatment, bio-nematicides (Tagiles and Kongest), and potassium silicate have the potential to be used for nematode control in the nursery, and could be used as safe nematicide alternatives. The three chemical nematicides evaluated here can be used for nematode control after white tip disease symptoms appear.

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