EFFECTS OF WHITE TIP NEMATODE ON PHYSICAL PROPERTIES OF RICE GRAINS

Zohre Asadi[1], Salar Jamali[1]*, Samad Sabouri[2], and Fatemeh Habibi[2]

[1]Plant Protection Department, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran. [2]Rice Institute Research of Iran, Rasht, Iran. * Corresponding author: jamali@guilan.ac.ir; jamali_s2002@yahoo.com

ABSTRACT

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Physical properties of agricultural crops are important in the design of more efficient milling, planting and harvesting machines, and also for reducing losses and improving safety in post-harvest handling. In this study, some physical properties of paddy rice, including grain length, width, thickness, geometric diameter, arithmetic diameter and sphericity of the grain, and the percentage of broken rice, healthy rice and milling yield were determined at five levels of *Aphelenchoides besseyi* in 2012 and 2013. Seedlings of *Oryzae sativa* cv. Hashemi, moderately susceptible to white tip nematode, were transplanted to pots and nematodes inoculated at the three- to five-leaf stage. The results showed that moisture content, grain length, thickness, arithmetic diameter, geometric diameter, and sphericity decreased with increasing inoculation level from 0 to 700 nematodes. The percentage of rice in the head, milling yield, and 1,000 grain weight were also decreased ($P<0.05$) by the nematode. Broken grain and unfilled grain were increased significantly in 2012 and 2013, respectively, by *A. besseyi*, but no effect was seen for grain width. Based on these results, white tip nematode could be a significant preharvest factor in causing high variability in physical properties as well as in reducing the milling quality of rice.

Key words: *Aphelenchoides besseyi*, crop loss, *Oryzae sativa*, physical quality.

RESUMEN

Asadi, Z., S. Jamali, S. Sabouri, y F. Habibi. 2015. Efectos del nematodo causante de la punta blanca sobre las propiedades físicas del grano de arroz. Nematropica 45:51-58.

Las propiedades físicas de los cultivos agrícolas son importantes en el diseño de maquinaria como molinos, cultivadoras y cosechadoras más eficientes, así como para reducir las pérdidas y mejorar la seguridad en el manejo post-cosecha. En este estudio, se determinan varias propiedades físicas del arroz con cascara, como longitud, anchura, grosor, diámetro geométrico, diámetro aritmético y esfericidad del grano. El porcentaje de arroz partido, arroz sano y rendimiento en la molienda se determinaron a cinco niveles de *Aphelenchoides besseyi* en 2012 y 2013. Plántulas de *Oryzae sativa* cv. Hashemi, moderadamente susceptibles al nemátodo de la punta blanca, se trasplantaron a macetas que se inocularon con nematodos en el estadio de tres a cinco hojas. Los resultados muestran que el contenido en humedad, longitud, grosor, diámetro aritmético, diámetro geométrico y esfericidad del grano se redujeron con niveles crecientes de inóculo de 0 a 700 nematodos. El porcentaje de arroz entero, rendimiento de la molienda y peso de 1,000 granos de arroz también se redujeron ($P<0.05$) por el nematodo. Los granos rotos y vacíos se incrementaron significativamente en 2012 y 2013, respectivamente, por *A. besseyi*, pero no se observaron efectos sobre la anchura del grano. Basados en estos resultados, el nematodo de la punta blanca podría ser un factor precosecha importante causante de una alta variabilidad en las propiedades físicas, así como en la reducción de la calidad de la molienda del arroz.

Palabras clave: Aphelenchoides besseyi, pérdidas de cultivo, *Oryzae sativa*, calidad fisisca.

INTRODUCTION

Rice is the world's single most important food crop and a primary food source for more than a third of the world's population (Khush, 1997). Global paddy rice production increased at an average rate of 16.5 million tons per year during the last 10 yr, reaching about 718.3 million tons in 2011 (FAO, 2011). Rice white tip nematode (*Aphelenchoides besseyi* Christie, 1942) is one of the most important nematode pests of rice production throughout the world, including in Iran (Bridge *et al.*, 2005; Jamali*et al.*, 2006). This nematode was reported first in Iran from the Khomam and Lahijan regions in 1971 (Kheiri, 1971). A study of the white tip nematode in Turkey (Tulek and Cobanoglu, 2010), showed a significant negative correlation between the population densities of *A. besseyi* in infected seed samples and the weight of 1,000 grains in 2008. The effect of *A. besseyi* on the susceptible rice cultivar Halilbey showed that panicles with white tip symptoms were significantly shorter (27.1%) and lighter (60.7%) than those without white tip symptoms. Losses in yield and yield components of the cultivar Giza 171 due to white tip nematode infection were reported under Egyptian conditions (El-Shafey *et al.*, 2012).

A knowledge of the physical and mechanical properties of agricultural products, including rice grain is of fundamental importance for proper storage and handling, including for design and manufacture of equipment for optimum post-harvest processing (Corrêa *et al.*, 2007). Fukano (1962) determined an economic damage threshold density (300 live nematodes per 100 seeds), which provides a useful basis for damage prediction (Bridge *et al.*, 2005).

A study on resistance of rice cultivars to white tip disease caused by *A. besseyi* showed that Hashemi rice cultivar was moderately susceptible to the nematode (Jamali and Mousanejad, 2011). Hashemi cv. is the most widely grown and important cultivar in Iran due to its high physical qualities, but the physical traits of *O. sativa* cv. Hashemi rice grain infected with *A. besseyi* have not been fully investigated. The objective of this study was to determine the effect of white tip nematode on physical properties of Hashemi rice.

MATERIALS AND METHODS

Nematodes were extracted from paddy samples collected from Guilan Province using the Mathur and Lal method (Mathur and Lal, 1989). In this method, seeds were soaked in a glass petri dish for 16 hr, and then the shells of seeds were isolated with aid of a dissecting microscope. Anabiotic nematodes in the shell of seeds were released. Nematodes extracted from each sample were cultured on *Alternaria alternata* grown on potato dextrose agar (PDA) plates (Jamali *et al.*, 2006; 2008). The nematodes were harvested from culture plates after 4 wk of incubation at 28°C and relative humidity of 95% by rinsing with water. The number of nematodes in the collected water suspension were determined. Seedling rice was inoculated in leaf pods at booting stages using the plastic tube method on July 29 (Jamali *et al.*, 2006). Five inoculum levels were used including; 0 (distilled water), 100, 300, 500 and 700 nematodes per plant. The experiment was conducted in a greenhouse at the temperature of 28°C to 30°C and 85% to 90% relative humidity.

Soil used for the experiments consisted of 19% sand, 21% silt, and 60% clay). The soil pH was 6.6, and the EC was 0.98 $dS.m^{-1}$. The soil for greenhouse pots was sterilized by metham sodium at 145 ml/m^2. Following sterilization, soil was aerated for 5 wk to allow escape of residual pesticide. To ensure freedom from nematodes, *O. sativa* cv. 'Hashemi' seeds were soaked in water at 55°C for 15 min prior to planting (Gergon and Prot, 1993). The seedlings were transplanted to pots (20-cm diameter × 25-cm depth) at the three to five leaf stages with five plants in each pot on May 22.

Seeds of the rice were harvested at the end of growing season (September 5). The moisture content of rice after harvest was determined, and then the samples were stored in a refrigerator (4°C).The samples were removed from the refrigerator and kept overnight at room temperature (23°C ± 2°C) before the milling experiment. The moisture content of each sample was measured prior to milling using a digital moisture meter (Model GMK-303 RS, G-won Hitech Co., Ltd: Woolim E-biz Center, 170-5, Guro-dong, Guro-gu, Seoul 152-769 Korea) and expressed as a percent on a dry basis. The moisture content of all treatments was approximately 8% and was suitable for milling. Before hulling samples, the length (L), width (W), and thickness (T) of 100 randomly collected grains per replicate were measured using a digital caliper with 0.01-mm accuracy. The average diameter of the grain was calculated from the arithmetic mean and geometric mean of the three axial dimensions. The arithmetic mean diameter (Da - Equation [1]), geometric mean diameter (Dg - Equation [2]) and sphericity (φ - Equation [3]) of the grains were calculated by using the following relationships (Mohsenin, 1986):

$$D_a = \frac{L + W + T}{3} \quad [1]$$

$$D_g = (L.W.T)^{0.333} \quad [2]$$

$$\varphi = \left(\frac{D_g}{L}\right) \times 100 \quad [3]$$

All paddy seeds were hulled with a laboratory scale rubber roll type test husker (Model THU-35A, Satake Engineering Co., Tokyo, Japan). The clearance between two rubber rolls was set to 0.65 mm. This setting allowed producing brown rice with minimum breakage. Weight of brown rice was measured and recorded for each treatment. The brown rice was subsequently milled using a laboratory polisher (Model TM 05, Satake Engineering Co. Ltd., Japan) for whitening of the kernels. The weight of broken rice and head rice was determined after the whitening of the brown rice and used to calculate the head rice percentage and percent of broken rice. The head rice percentage [4], broken rice [5] and milling yield [6] were calculated by using the following relationships:

$$\%\,Head\ rice = \frac{Wt\ of\ whole\ grains}{Wt\ of\ paddy\ samples} \times 100 \qquad [4]$$

$$\%\,Broken = \frac{Wt\ of\ broken\ grains}{Wt\ of\ paddy\ samples} \times 100 \qquad [5]$$

$$\%\,\text{Milling yield} = \frac{Wt\ of\ white\ rice}{Wt\ of\ whole\ grains} \times 100 \qquad [6]$$

Nematode population density was measured at the end of growing season. Nematodes were extracted from rice seeds of each treatment by Mathur and Lal (1989) method mentioned above, and the extracted nematodes were counted.

The experiments were completely randomized with four replicates and conducted in two years (2012 and 2013). The results obtained were subjected to analysis of variance using SAS, and means were compared using Tukey's test. An interaction was found between years and the percentage of broken grains and milling yield. Differences among means were considered significant at $P \leq 0.05$.

RESULTS

The mean number of *A. besseyi* that were extracted from each pot at the end of the experiment was 0 (control), 35.4 (treatment 1), 252.2 (treatment 2), 408.5 (treatment 3), and 540.6 (treatment 4) in 2012. In 2013 the mean number of nematodes that were extracted at the end of the experiment was 0 (control), 45.5 (treatment 1), 240.9 (treatment 2), 442.3 (treatment 3), and 562.5 (treatment 4).

Decreased moisture content was seen in both 2012 and 2013 (Fig. 1). The highest moisture content was in the control samples with 23.3%, and 23.5% in 2012 and 2013, respectively. Nematodes at all the infestation levels reduced moisture content compared with the untreated control in both years, with decreasing moisture content at increasing nematode inoculation levels.

In 2012, inoculation with 500 or 700 nematodes per plant resulted in decreased grain length (Fig.

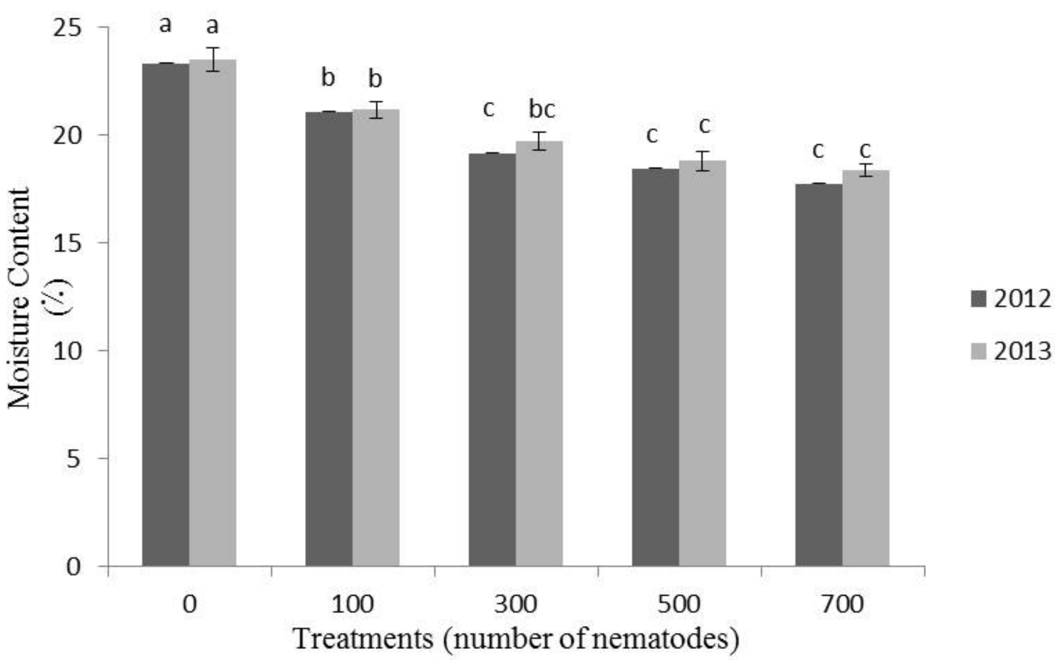

Fig. 1. Effect of *Aphelenchoides besseyi* initial population density (treatments) on moisture content of rice. Means with the same letter within a year are not significantly different according to Tukey's test (P<0.05).

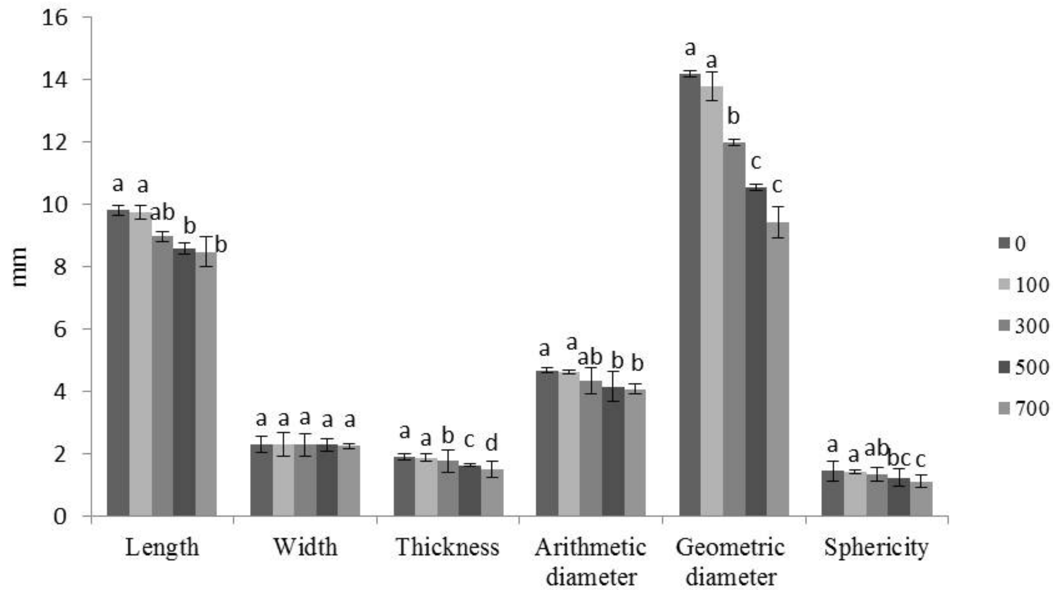

Fig. 2. Effect of *Aphelenchoides besseyi* initial population density (0, 100, 300, 500, or 700 nematodes per pot) on rice grain dimension (length, width, thickness), arithmetic diameter, geometric diameter, and sphericity in 2012. Means with the same letter within a response variable are not significantly different according to Tukey's test (P<0.05).

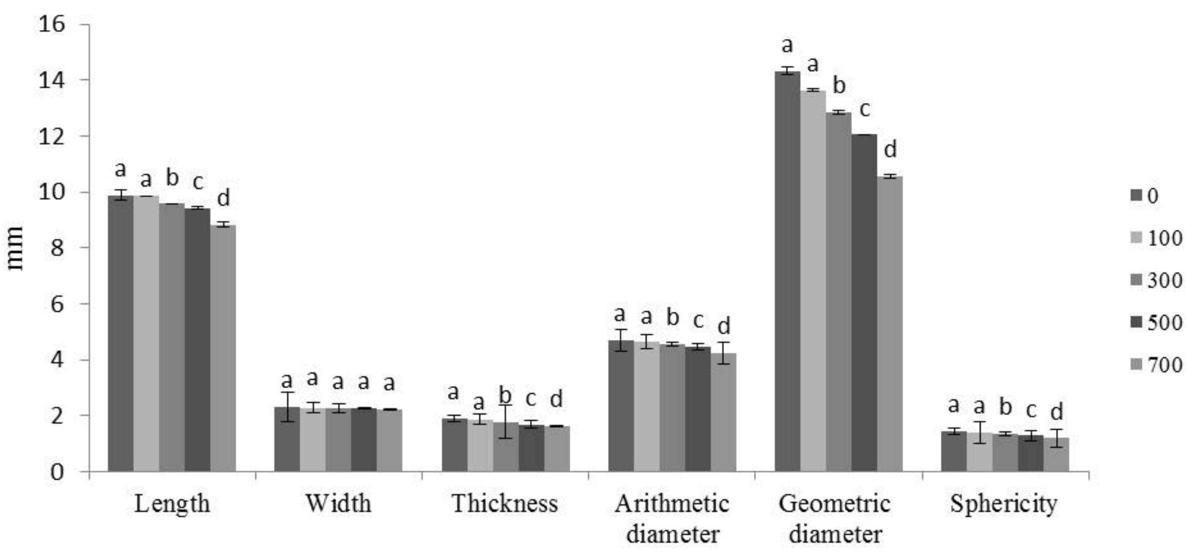

Fig. 3. Effect of *Aphelenchoides besseyi* initial population density (0, 100, 300, 500, or 700 nematodes per pot) on rice grain main dimension (length, width, thickness), arithmetic diameter, geometric diameter, and sphericity in 2013. Means with the same letter within a response variable are not significantly different according to Tukey's test (P<0.05).

2). Nematodes did not affect grain width at any infestation level, but nematodes at levels of 300 per plant or higher lowered grain thickness. Arithmetic diameter and sphericity were lowered at both 500 and 700 nematodes per plant while rates of 300 or greater lowered geometric diameter. The same patterns of results were observed in 2013 (Fig. 3).

The weight of 1,000 kernels was decreased at inoculation levels of 500 or 700 nematodes per plant in 2012, and at 300, 500, and 700 nematodes per plant in 2013 (Fig. 4). Inoculation with 100 nematodes did not affect weight of 1,000 grains either year. Increasing nematode inoculation densities above 100 nematodes increased the weight of unfilled grain both

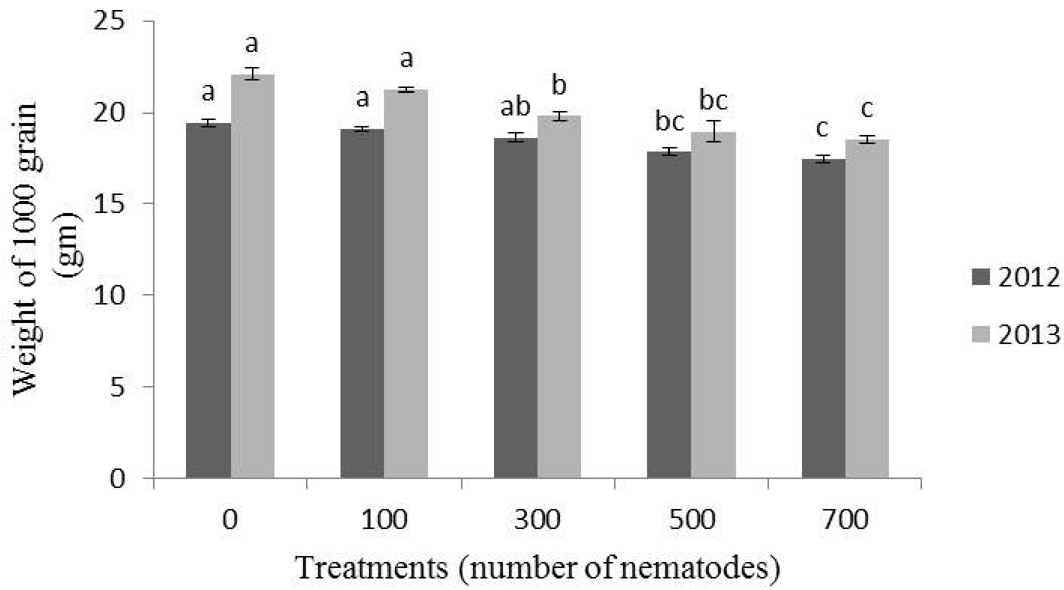

Fig. 4. Effect of initial population density of *Aphelenchoides besseyi* (treatments) on the weight of 1,000 seeds. Means with the same letter within a year are not significantly different according to Tukey's test (P<0.05).

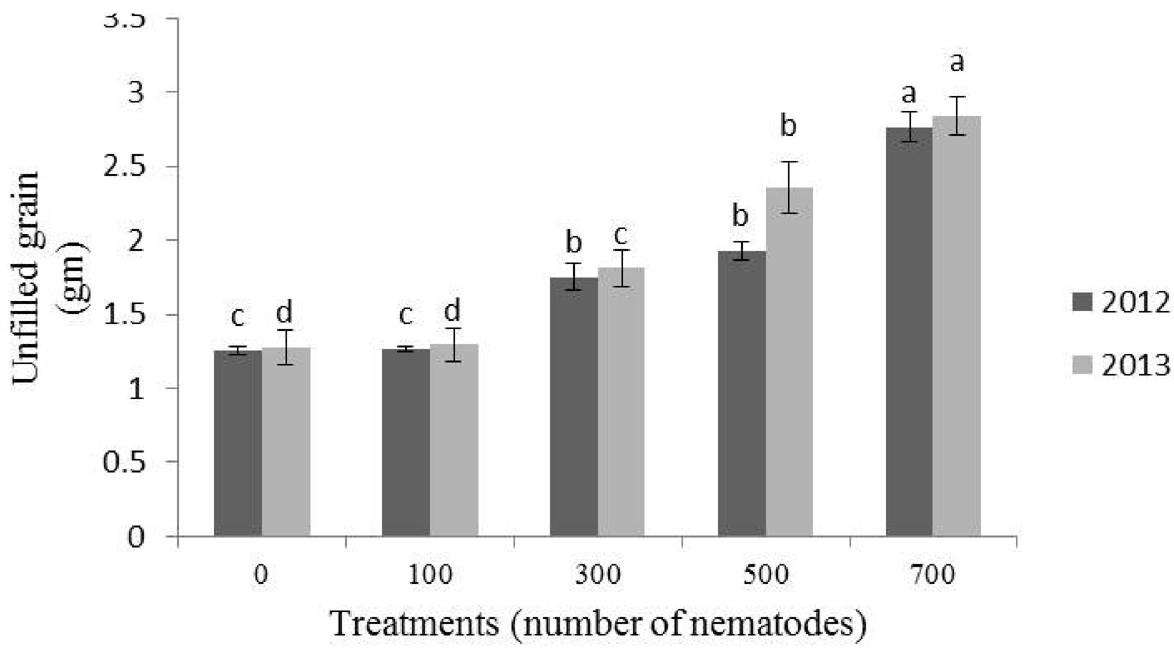

Fig. 5. Effect of initial population density of *Aphelenchoides besseyi* (treatments) on weight of unfilled grain. Means with the same letter within a year are not significantly different according to Tukey's test (P<0.05).

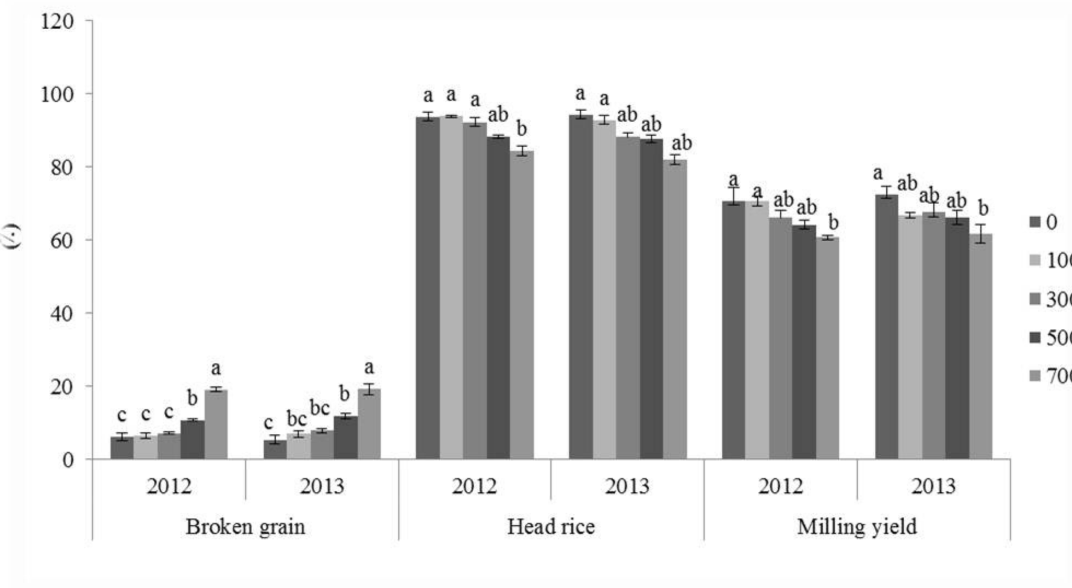

Fig. 6. Effect of *Aphelenchoides besseyi* initial population density (0, 100, 300, 500, or 700 nematodes per pot) on, broken grain and head rice percentage and milling yield. Means with the same letter within a year and response variable are not significantly different according to Tukey's test (P<0.05).

years (Fig. 5). The interaction between years (2012, 2013) and length, thickness, D_a, D_g, weight of 1,000 kernels and weights of unfilled grain was significant (P<0.05).

All inoculation levels that were tested increased the percentage of broken rice that was seen (Fig. 6). The percentage of head rice was lowered by 700 nematodes per plant in 2012, but no significant differences were seen in the percentage of head rice in 2013 at any inoculation level. Milling yield was lower at the highest inoculation level in both years.

The interaction between years (2012, 2013) on moisture content, width, sphericity, milling yield, percentage of head rice and broken grain was not significant, while interaction effects on length, thickness, D_a, D_g, weight of 1,000 kernels, and weights of unfilled grain were significant (P<0.05).

DISCUSSION

Increasing inoculation levels of *A. besseyi* reduced the length, thickness, arithmetic diameter, geometric diameter, sphericity and decreased the width of rice. Candole *et al.* (2000) reported that rough rice from blast-infected panicles was drier by 7 to 10 percentage points and 10% thinner than rough rice from blast-free panicles. Decreasing moisture content of grain also decreased geometric diameter, arithmetic diameter, and sphericity significantly (Reddy and Chakravertty, 2004; Al-Mahasneh and Rababah, 2007; Kashaninejad and Rezaghah, 2007; Askari*et al.*, 2010).

White tip nematode infection resulted in small and deformed seeds due to nematode feeding on the embryo of grain (Shukla *et al.*, 2003). The generally smaller kernels from infected plants would likely, in turn, result in lower agronomic yields. The present results indicate that infection by the white tip nematode negatively affected grain weight. Similar results have been reported by El-Shafey *et al.* (2012) where a reduction in grain yield due to white tip up to 47% in both 2003 and 2004 seasons was seen. White tip nematode infection also increased straw yield, the number of unfilled grains per panicle, the number of unproductive tillers per square meter, and lowered plant height, flag leaf area, 1,000-grain weight, the number of tillers and panicles per square meter, and hulling and milling percentage in their study. Yield loss is due to the incidence of pathogenicity of the nematode, population density, host sensitivity and tolerance as well as environmental factors (Trudgill and Phillips, 1998).

Inoculation with the nematode increased the weight of unfilled grains. Feeding on the embryo of the seed disrupts the seed filling process by causing some flowers to be sterile. Nematodes feed ectoparasitically on ovary, stamens, lodicules, and embryo of the grain (Bridge *et al.*, 2005). Infected clusters are small, deformed, and their maturation is delayed. Some flowers are sterile, and the numbers of unfilled grains increase. Similar effects have been seen with some fungal pathogens of rice (Candole *et al.*, 2000). The results of our study indicated that increasing inoculation levels generally lowered head rice percentage, milling yield, and increased the broken grains. The objective of rice milling systems is to remove the husk and the bran layers from paddy rice to produce whole white rice kernels that are sufficiently milled, free of impurities, and contain a minimum number of broken kernels. *Aphelenchoides besseyi* could contribute to this loss if the infection level is sufficiently high. Cell division in the endosperm decreases at lower moisture contents, reducing the material capacity storage grain resistance to the milling process (Mushtaq *et al.*, 2008). According to these results, it seems that white tip disease was effective on resistance of rice during the conversion process. The reduction in moisture content could be the main cause of increasing broken grains after milling.

In conclusion, *A. besseyi* is a seed borne nematode and can survive in stored seeds for several years under dry conditions. The quality of rice grains can be affected by the nematode. Our results demonstrated the negative effect of white tip nematode on the physical properties of rice grain, including drier and thinner, unfilled grains. *Aphelenchoides besseyi* could be a significant pre-harvest factor affecting the physical properties of rice. The preceding conclusions were based on a limited number of rice genotypes and locations. Further studies with more rice cultivars are warranted.

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