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Determining yield losses in rice cultivars resulting from rice white tip nematode *Aphelenchoides besseyi* in field condition

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Abstract

This study was carried out to determine the effects of rice white-tip nematode *Aphelenchoides besseyi* on yield and yield components in the experimental fields of the Thrace Agricultural Research Institute, Turkey in 2012. Four rice cultivars commonly grown in Turkey were used in trial. The experiment was conducted as split plot in a completely randomized block design with four replications with treatments (naturally infected seeds, artificially infected plots, 5000 *A. besseyi* m^{-2} and non-infected) as a main plots, cultivars (Halilbey, Gala, Tunca and Edirne) as a sub-plots were evaluated. In nematode-contaminated plots where it was seen that decreases of yield and weight of 1000 seeds found statistically important at the level 0.01 ($P < 0.01$). Compared to the control treatment, in naturally infected treatment cultivars Halilbey, Edirne, Gala and Tunca exhibited 26.7%, 15.8%, 15.6% and 8.1% yield decrease, respectively. In the artificially infected treatment cv. Halilbey, Edirne, Gala and Tunca exhibited 29.6%, 14%, 13.5% and 5.1% yield decrease, respectively.

Keywords: Rice, white-tip nematode, *Aphelenchoides besseyi*, yield

Rice white tip nematode *Aphelenchoides besseyi* Christie is widely found in rice growing areas across the world and causes extensive losses of rice in economic aspect. Because *A. besseyi* is transmitted through its seeds and a wide scale of infection can occur. Its economic importance varied by locations, regions and countries. Annual variations in density and severity of the damage by the pest are affected by cultural practices and sowing local varieties. Damage to susceptible plants often changes according to rate of infection in sown seeds and number of *A. bessseyi* in the infected seeds.

Affected plant tillers the tip of the leaves whiten for a distance of 3 to 5 cm and then die off and shred. The panicles are shorter and often atrophied at the tips. The fertile flowers sometimes produce misshapen grains with a low and delayed germination potential (Tamura & Kegasawa, 1959). Fukano (1962) reported that the nematode multiplied in rice florets much faster than on seedlings where only somatic organs of the host were available for the parasitization. Accumulation of nematodes around the basal portion of a floret or a grain suggest that fleshy tissues such as ovary, stamens, lodicules and embryo are probably the major sites of feeding (Huang & Huang, 1972).

In Turkey, *A. besseyi* was first reported in 1995 in the Ipsala and Gönen districts of Edirne and Balıkesir provinces (Özturk & Enneli, 1997). Various studies found that the pest spreading across other rice growing regions was gradually seen by 7.80-43% in Balıkesir, Çanakkale, Çankırı, Çorum, Edirne, Tekirdağ and Kırklareli (Tülek & Çobanoğlu, 2010).

Turkey ranks 54th by 98966 ha growing areas and 37th by 860000 ton annual production of paddy rice, (FAO, 2013). Almost all rice varieties grown in Turkey are included in Japonica type and therefore varieties used in the study also belonging to Japonica type. Preliminary studies showed reactions of the registered varieties to *A. besseyi*. Resistant to *A. besseyi* varieties Asahi, Rinaldo Bersani and Bluebonnet were used as controls and observed that Halilbey variety susceptible. Edirne and Gala varieties were moderately susceptible whereas, Tunca variety moderately resistant. The present study aimed to test the four varieties widely grown in Turkey under field conditions to finally determine yield losses accounted for by rice white tip nematode.

Materials and methods

The trial on yield loss was established in the experiment area of Thrace Agricultural Research Institute, Turkey in 2012. Varieties Edirne, Gala, Halilbey and Tunca extensively grown in Turkey were tested. Infected and nematode-free seeds of varieties used in the experiment were grown in the year before. The experiment was established as split plot in a completely randomized block design in four replications with treatments (naturally infected seeds, artificially infected plots, 5000 *A. besseyi* m^{-2} and non-infected) as a main plots. Cultivars Halilbey, Gala, Tunca and Edirne as sub-plots were evaluated. Drilling technique of direct seeding was used. Seeds were sown dry by driller and sowing norm calculated by the plot sizes of 10.5 m^2 and 500 seeds per square meter. The seeds were sown with 6 intervals of 20 cm in 5-6 cm depth. Intermittent irrigation was performed until seedlings appeared and continous irrigation by the harvest, with plants submerged in 15 cm water. All the testing materials were planted in the second half of May. The related treatments were as below.

Treatment 1: *A. besseyi*-infected seeds of every variety produced in the year before were used and nematode analyses made on 100 seeds of each variety in 4 replications. The obtained values constitute initial population. The rate of reproduction was calculated by the formula "Multiplication rate of rice white tip nematode = final population (Pf) / inital population (Pi)".

Treatment 2: Nematode-free seeds were used for this purpose and they submerged in hot water as a control prior to the sowing. Therefore, seeds were presoaked for 3-5 hrs in cold water followed by their submergence at 55-60 °C for 15 minutes (Tülek & Çobanoğlu, 2011). The study was conducted using electric Benmari equipment with 12 l water.

Treatment 3: Nematode-free seeds were used to perform artificial inoculation after the seedling germination. Inoculum density was calculated as 5000 *A. besseyi* individuals per square meter. The required nematodes were obtained from two sources. The first was carrot disk method to reproduce *A. besseyi* individuals. The tested nematode *A. besseyi* was initially isolated from infested seeds and reared on carrot disk in laboratory (Tülek *et al*., 2009). The second source used was infected Halilbey seeds produced the year before. *A. besseyi* individuals were sprayed on to water-filled rice plots by atomizer 10 days after the seedling germination.

All other cultural practices were conducted as in conventional rice farming. For the weed control in the plots *Penoxulam* 25.2 g l^{-1} (1000 cc ha^{-1}) and *Bentazon* 480 g l^{-1} (2000 cc ha^{-1}) were applied before the rice tillering. Following harvest which was done at the end of September when 80% of kernels were straw yellow, nematode analysised and counts made on 100 seeds taken from every plot and mixed as bulks in 4 replications. After the harvest, variance analysis was also made concerning nematode counts and yields.

Laboratory analysis methods: Baermann funnel method was used to obtain rice white tip nematode both before the sowing and after the harvest (Hooper, 1986). Seeds were separated from hulls (lemma and palea). *A. besseyi* individuals were found localized inside hulls and on the cargo by 97% and 3%, respectively (Tülek & Çobanoğlu, 2011a). Therefore, only hulls were used for analysis. Samples were kept in water in conic flask for 48 hrs, bottom-sedimented nematodes were put into tubes in volumes of 20 ml. The nematodes in two 1 ml aliquants of water suspension from each extract were counted in counting dishes, using a stereomicroscope and the average of the two counts was calculated and referred to 100 paddy seeds. After the harvesting, 100 gram paddy rice was taken from each sample and determined milled rice yield (%).

Results

In the first treatment in which naturally infected seeds were used and initial population (Pi) was determined, nematode analysis was performed on seeds of every rice variety prior to sowing. Following the harvest, final populations (Pf) were established to determine multiplication rates in every rice variety. Results are shown in Table1.

Finally, yield loss caused by rice white tip nematode and values of control plots were obtained using variance analysis (Table 2).

Table 1. Means of multiplication rate related to *Aphelenchoides besseyi* in naturally infected plots (1st treatment).

Rice cultivars	*A. besseyi*/100 grains (Pi)	*A. besseyi*/100 grains (Pf)	Multiplication rate (Pf/Pi)
Edirne	95	461.6	4.85
Halilbey	82	689.1	8.40
Tunca	28	152.8	5.45
Gala	164	328.7	2.00

*Pi = Initial population, Pf = Final population.

Table 2. Decrease (%) in paddy yield (t ha^{-1}) 1000 kernel weight (g), milled rice yield (%) nematode density (number/100 seed) caused by *Aphelenchoides besseyi*.

Components	Non-infected (Control)	Artificially infected	Naturally infected	Decrease in naturally infected plots (%)	Decrease in artificially infected plots (%)	F-value
Yield (t ha^{-1})	5.793 a	4.869 b	4.820b	16.8	15.9	54.4111***
1000 Kernel weight (g)	35.3 a	29.9 c	31.2 b	11.6	15.2	207.7983***
Milled rice yield (%)	61 a	60.5 a	59.6 a	2.3	0.8	1.3610**
A. besseyi/100 grains	0 c	185.2 b	408.1 a	-	-	88.7032***

Means followed by different letters in each line are significantly different at P= 0.05.
** P <0.05; *** P <0.01

It was found that the results were statistically significant at the level of 0.01 considering the weight of 100 kernel and number of nematode per 100 seeds and yield between nematode-free and nematode-infected blocks. The difference between the treatments was found statistically insignicant in terms of milled rice yield. Comparison with controls showed that seeds naturally infected with *A. besseyi* had differences of 16.8% and 11.6% in yield and

1000 kernel weight, respectively. Artificial inoculation showed that there were reduction of 15.2% in yield and 1000 kernel weight. Based on variety, comparison of treatments with controls indicated that highest and lowest yield loss 29.6 and 5.1% in Halilbey and Tunca varieties for artificial inoculation, respectively. Assessment on 1000 kernel weight showed that highest and lowest yield loss were 18.6 and 1.8% in artificial inoculation for Halilbey and naturally infected Tunca, respectively (Table 3).

Table 3. Decrease in yield and 1000 kernel weight of rice varieties caused by white tip nematodes *Aphelenchoides besseyi*.

Cultivars	Decrease in naturally infected plots (%)		Decrease in artificially infected plots (%)	
	Yield	1000 kernel weight	Yield	1000 kernel weight
Halilbey	26.8	16.5	29.6	18.6
Edirne	15.8	10.4	14.1	16.0
Gala	15.6	18.6	13.5	20.8
Tunca	8.1	1.8	5.1	5.4

Post-harvest evaluation was made in terms of the density of nematodes. The analysis of variance showed that the difference between treatment x variety interaction was significant at the 0.01 level (P <0.01) (Table 4).

Table 4. Nematode *Aphelenchoides besseyi* density per 100 seed related to treatment x cultivar interaction.

Cultivars	Nematode density (number/100 seed)	
	Naturally infected plots	Artificially infected plots
Halilbey	689 a	315 c
Edirne	462 b	164 d
Gala	328 c	148 d
Tunca	152 d	113 d

[*]In each line, means followed by different letters are significantly different according to LSD tests at P= 0.05.

Discussion

There are numerous reports on yield losses caused by *A. besseyi*. Yield losses were reported in susceptible rice varieties of 17.5, 49 and 6.6% in the USA in different years in 1950's (Atkins & Todd, 1959) and 10-30% in Japan (Yamada & Shiomi, 1950). Losses of yield in rice varieties in China was reported 45% and more when infection over 50% (Tsay *et al*., 1998). Yield losses vary with cultivar, year, temperature, cultural practices and other factors. In infested fields, the average losses range from 10 to 30%. In fields where all plants have been attacked, maximum losses up to 70% for the most susceptible cultivars and 20% for the most resistant cultivars have been reported (Prot, 1992).

Fukano (1962) reported that not considerable loss with 30 and fewer live nematodes in 100 seeds. Nematode analysed after the harvest showed that number of nematodes was significantly higher than Fukano reported.

Tülek & Çobanoğlu (2011b) observed yield loss of 57.9% caused by *A. besseyi* to cv. Halilbey when 324 nematode *A. besseyi*/panicle were found during the flowering stage and an average of 233.4 (120.5-423.4)/100 paddy seeds recorded at harvest. It is clear from nematode number in 100 seeds that some 233.4 *A. besseyi* caused a loss of yielding of 57.9% in cv. Halilbey whereas, values from naturally infected plots showed that an average of 689 *A. besseyi* led to yield loss of 29.6%, which suggested that yield loss caused by rice white tip nematode in a variety and nematode density on harvest were affected by climatic conditions (humidity, temperature and rainfall), nematode density of sown seeds and percentage of infected plants (percentage of plants with evidence of white tip in flag leaves).

Tülek & Çobanoğlu (2010) conducted study using susceptible cv. Halilbey showed that panicles with white tip symptoms and panicle without white tip symptoms found rice white tip nematode giving 741.6 and 10.9 *A. besseyi*/100 seeds, respectively. In the same study, panicle with rice white tip symptoms showed the number of fertile kernels per panicle decreased by 46.1%. Sterile kernel numbers were increased 88.1% due to *A. besseyi* infestation. Also, the weight of 1000 kernels decreased by 30.4% and there was a significant ($P = 0.01$) negative correlation between the numbers of *A. besseyi* specimens per 100 seeds and the weight of 1000 grains ($r = -0.6418$, significant at $P = 0.01$) in the infected seed samples. The effects of *A. besseyi* on the susceptible cv. Halilbey indicated that panicles with white tip symptoms were significantly shorter (27.1%) and lighter (60.7%) than those without white tip symptoms.

It was recorded that comparison of tolerant plants with controls showed a decrease less than 15% in seed weight while susceptible varieties 30-70% losses when number of nematode was relatively more (Popova *et al*., 1980). Considering this fact that Tunca variety was tolerant in the study.

It has been concluded from previous studies made in Turkey that rice white tip nematode accounted for yield losses ranging from 5.4% to 57.9% in different Japonica varieties in different years.

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