



Integrated Management of Seed borne Nematode (*Aphelenchoides besseyi*) in T. Aman Rice (*Oryza sativa* L.)

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Abstract

Two experiments were conducted in the Nematology Laboratory of the Seed Pathology Centre and experimental field of the Department of Agronomy, Bangladesh Agricultural University (BAU), Mymensingh during 2004 to examine the influence of physical and chemical treatments on the seed borne nematode disease of rice. The integrated control with physical and chemical treatments against seed borne nematode (*Aphelenchoides besseyi*) causing white tip disease of rice varieties BR11 and Nizersial (T. Aman) rice revealed that farmer's saved seeds of both the varieties treated simultaneously with brine solution, hot water and Furadan 3G resulted in enhanced plant height, root length, panicle length, number of grains per panicle, total number of spikelet's per panicle, grain yield and lower association of seed borne nematode. Better responses were found with farmer's saved seeds treated with brine solution and hot water than field application of Furadan 3G, hot water treatment and treatment with brine solution having higher plant growth characters and yield of grains correspondingly with lower number of sterile and discolored spikelet's and seed borne nematode compared to the control.

Keywords: Seed borne nematode, T. aman rice, integrated management

1. Introduction

In Bangladesh, 82.9% of the total cultivated lands are used for rice covering an area of 11.53 million hectares and its total production is 33.91 million tons (BBS, 2012). However, the average yield of rice in our country is only 3.3 tons/ha, whereas those in Australia, Korea, and China are 9.7, 6.6 and 6.2 tons/ha, respectively (FAO, 2000). For this low yield of rice in Bangladesh, diseases caused by plant parasitic nematodes play a significant role (Fakir, 1989).

Rice crops suffer from more than 60 different diseases (Ou, 1985). In Bangladesh 43 diseases are known to occur on rice (Fakir, 2000). Among all these diseases, 27 are seed-borne. More than 150 species of plant parasitic nematodes have been associated with rice and rice soils. Estimates of annual yield losses due to plant parasitic nematodes of rice worldwide range from 10-20% (Hollis and Keoboornrueng, (1984).

Seeds carry several destructive pathogens that often take heavy toll causing severe diseases of crops raised from them (Suryanarayana, 1978). Rice seeds while growing, maturing and ripening in the standing plant in the field can be infected by seed-borne fungal bacterial and nematode pathogens. Among the plant parasitic nematodes, *Aphelenchoides besseyi* was first observed by Christic in 1984. It was found to be seed-borne in rice and may remain viable for 2-3 years on dry grains in quiescent state beneath the husk (Yoshii and Yamamoto, 1951). Among these seed-borne pathogens, *Aphelenchoides besseyi* causes white tip disease of rice. It is a major nematode pest of rice in Bangladesh.

The average yield losses range from 10-30% in fields, where all plants have been attacked. Maximum losses of 70% for the most susceptible cultivars and 20% for the most resistant cultivars have been reported. Atkins and Todd (1959) have estimated the loss in yield due to *Aphelenchoides besseyi* ranging from 17.4 to 54.17 % in varieties in Asahi, Early prolific, Magnolia, Sun bonnet, Fortuna, Tikhohu, Saikai 29 and Norin 18. Taylor (1969) has also reported reduction in yield up to 20-60% due to white tip disease. Proper management of the white tip disease is therefore of utmost importance to increase the production of rice. The present study was undertaken to:

- i) examine the effect of physical treatment with brine solution (20% NaCl) and hot water on seed-borne *Aphelenchoides besseyi*;
- ii) examine the effect of chemical treatment with Furadan-3 G on seed-borne *Aphelenchoides besseyi*; and
- iii) investigate the comparative effect of physical and chemical treatments either alone or in combination on the seed borne nematodes, its growth and yields of T. Aman rice.

2. Materials and Methods

2.1. Experimental sites and season

The experiments were conducted both in the Nematology laboratory of the Seed Pathology

Centre and experimental field of the Department of Agronomy, Bangladesh Agricultural University (BAU), Mymensingh, during 1st July to 10th December, 2004.

2.2. Seed Samples, designs and treatments

Seed samples of a high yielding variety BR11 and a local variety Nizersial were collected from individual farmers of village Boira under Sadar Thana of Mymensingh. For this, 100 seeds from each variety were taken randomly. By dehusking the seeds, different parts like empty glume, lemma, palea and grain were kept in small petridishes (2.5 cm) with (10-12 ml) distilled water. The petridishes were left for 24 hours at room temperature for the nematodes, if any, to come out into the water. After 24 hours, all the petridishes were checked under stereo- binocular microscope and the number of the released nematodes were counted with the help counting dish. Two hundred fifty grams (250) of farmers saved seeds of the two varieties were taken for hot water treatment at 55^o C for 10 minutes in hot water treating devices and were then taken for treating with brine solution (20% NaCl). Furadan 3G (carbofuran), 6g/plot @ 33kg/ha were applied at the time of final land preparation.

Six treatments were laid out in a Factorial Randomized Complete Block design used in this study to see their effect on different growth and yield parameters as well as number of seed borne nematode, *Aphelenchoides besseyi* in two Aman varieties of rice BR11 and Nizersial. The treatments are as follow:

- T₁= Farmers saved seed (control)
- T₂= Farmers saved seed soaked in brine solution (20% NaCl)
- T₃= Farmers saved seed treated with hot water at 55^o c for 10 minutes.
- T₄= Application of Furadan 3 G in the main field at the time of final land preparation
- T₅= Farmers saved seed+ brine solution (20% NaCl) + hot water treatment
- T₆= Farmers saved seed+ brine solution (20% NaCl) + hot water treatment + Furadan 3G

2.3. Agronomic management

Seed beds were prepared by ploughing land several times and the land was made even by adding additional water. Area of each seed bed was 1m² in the every location. Before broadcasting, seeds were soaked in water for 24 hours for sprouting; seeds were broadcasted in the seed bed @ 20-25 kg/500 m² for planting one hectare of land and 80 g seeds were sown in each seed bed. The 240 m² land was prepared by ploughing several times. The field was puddled by adding water and the land was made level with the help of ladder. The seedlings were transplanted in the field at 25 x 15 cm spacing. Each plot size was 5 m² (2.5 m x 2 m). A space of 1m land was kept free among the blocks. The land was irrigated several times as per requirement. Intercultural operations were done whenever necessary. For cultivation of Nizerzial (v1) and BR11 (v2) rice the land received fertilizers @ Urea 180 kg/ha, TSP 100 kg/ha, MOP 70kg/ha, Gypsum 60 kg/ha and Zinc sulphate 10 kg/ha.

The symptom in the leaf was not well marked till 15 days after sowing. So the data of the affected plants were taken from 15 days after sowing. The symptoms were visible in different leaves of an effected plant and became prominent gradually. When the symptoms were found at four leaf stage, the leaves were apparently smaller in size and typical symptom of white tip was observed at the tip of the leaves. Other related symptoms such as twisted flag leaves were visible. At the reproductive phase, the panicles at the time of emergence, revealed the nematode infection in the spikelets. After maturation of rice, it was harvested on 10 December, 2004 and weight of the grains was taken.

2.4. Data collection

Plant height from ground level to the tip of the largest leaf or panicle of hills was recorded from 10 randomly selected hills per plot. After uprooting the hills, the roots were washed out by water and then the root length was measured starting from ground level to the longest

available root apex. The total developed roots were measured from ten hills taken randomly per plot and the average root length was calculated. Length of panicle was measured from the base of the panicle to the apex of top most available spikelets. Fully developed grains panicle⁻¹ was counted from 10 randomly selected panicles. Number of sterile spikelets panicle⁻¹ was calculated from the difference between total spikelets and number of filled grains. Total number of spikelets panicle⁻¹ was counted covering both healthy and sterile spikelets from ten randomly selected hills. Number of discoloured spikelets were calculated from ten randomly selected panicles plot⁻¹ in each treatment and average was calculated. The grain yield was measured in gm per m² basis from each plot.

Population of nematode, *A. besseyi* was determined by modified Baermann funnel method. In this test, 10 g of seeds was used. Seeds were allowed to germinate over strainer for 5 days at 25^oC. Five to ten (5-10) ml of bottom sediment was examined for the presence of *A. besseyi*. The available nematodes were counted in number. Few nematodes released from seeds, leaves and spikelets were picked up from water suspension with the help of a fine pointed soft needle made of coconut leaf midrib and put into a drop of water on a slide looking through a stereo- binocular microscope. The live nematodes in the water drop over the glass slide were heated by the flame of a spirit lamp with upward and downward motion for few moments until the nematodes were killed and relaxed in straight or slightly curved way. Killed nematodes were fixed in TAF solution and kept in small vials with proper labeling for identification purpose. TAF solution was prepared by using Formalin (40% formaldehyde)-7ml, Tri ethanolamine- 2ml and Distilled water- 91 ml. Killed and fixed nematodes were placed in a drop of concentrated glycerin on a slide. Cover slip was placed on the drop after placing four small glass leads at four corners of the drop to give a protective support on the nematode specimens.

2.5. Statistical analysis

Analysis of variance was done following Factorial Randomized Complete Block Design (RCBD) with the help of computer package MS STAT- C software developed by Russel. The mean differences among the treatments were evaluated with the help of LSD test and DMRT.

3. Results and Discussion

3.1. Effect of treatments on growth and yield characters of BR 11 and Nizersial

plant height at maturity, length of root and length of panicle, number of grains/ panicle, number of sterile spikelets, total number of spikelets panicle⁻¹, number of discoloured spikelets panicle⁻¹, yield of grains and number of nematodes/10 g seeds are presented in Table 1. The influence of different treatments in relation to plant height was found to be statistically significant ($p=0.05$). The tallest plant (102.83 cm) were found in treatment T₆ (Farmers saved seed+ brine solution+ hot water treatment+ Furadan 3 G). The highest root length (23.39 cm) was observed in treatment T₆, while the lowest of 15.03 cm was observed in T₁ Treatment (Table 1). The longest panicles 23.84 cm were observed in treatment T₆.

T₆ gave the highest number of grains/ panicle (144.08), where as the lowest of 121.31 grains/panicle were observed in T₁ (Table 1). The lowest significant number of sterile (15.50) were recorded from the treatment T₆, while the highest number of sterile spikelets 20.11 was recorded in T₁. The highest total number of spikelets /panicle was noted in T₆ and the lowest number of 141.35 was in non-treated farmers saved seed (T₁). The highest number of discoloured spikelets panicle⁻¹ (7.86) was found in T₁, while the lowest number 3.46 resulted with treatment T₆ (Table 1). Two treatments T₆ and T₅ were found to have the highest and statistically similar grain yields of 438.56 and 420.19 g/m², respectively (Table 1). Non treated farmers

saved seeds (treatment T₁) were found to yield the highest number of 49.52 *Aphelenchoides besseyi* nematode/ 10g seeds. The lowest number of 3.89 and 2.72 nematodes/10 g seeds were recorded with the treatments T₅ and T₆, respectively (Table 1).

3.2. Growth and yield characters of two varieties T. Aman rice infected with *Aphelenchoides besseyi*

Responses of both the varieties with respect to plant height, root and panicle lengths were statistically insignificant (Table 2). Similarly, responses of the no. of grains, no. of sterile spikelets, total no. of spikelets and no. of discoloured spikelets panicle⁻¹, grain yields and no. of isolated nematode / 10gm of seeds were statistically insignificant (Table 2).

3.3. Interaction effects of treatments and varieties on various growth and yield characters of BR11 and Nizersial varieties of T. Aman rice infected with, *Aphelenchoides besseyi*

Interaction effects of treatments and varieties were found to be insignificant with respect to plant height, root and panicle length (Table 3). Interaction effects of the treatments and varieties with respect to the no. of grains, no. of sterile spikelets, Total no. of spikelets and no. of discoloured spikelets/panicle, yield of grains gm/m² and no. of isolated nematodes *Aphelenchoides besseyi* per 10 gm of seeds were found to be insignificant (Table 3).

Plant growth and yield characters with respect to plant height, root length, panicle length, number of grains, total number of spikelets and grain yields showed their positive response to treatments. Higher values of root length, plant height, panicle length, number of grains, total number of spikelets and yield of grains were low due to nematode activities with less physiological disturbances in the plant metabolism which allowed the plants to result in better growth and yields.

Table 1. Effect of treatments on growth and yield characters of BR 11 and Nizersial rice associated with *Aphelenchoides besseyi*

Treatments	Plant height at maturity (cm)	Length of root(cm)	Length of panicle (cm)	No. of grains panicle ⁻¹	No. of sterile spikelets panicle ⁻¹	Total no. of spikelets panicle ⁻¹	No. of discoloured spikelets panicle ⁻¹	Yield of grainy (g)/m ²	No. of nematodes 10g ⁻¹ seeds
T ₁ (Farmers saved seed)	92.25bc	15.03f	19.74f	121.31f	20.11a	141.35f	7.86a	290.47d	49.52a
T ₂ (Farmers saved seed soaked in brine solution)	97.65b	16.49e	20.71e	125.33e	18.47b	143.81e	7.25b	325.55c	15.28b
T ₃ (Farmers saved seed treated with hot water)	98.39abc	17.77d	21.99d	130.54d	17.76b	148.31d	6.30c	365.23b	9.89c
T ₄ (Application of Furadan 3G in the main field at the time of final land preparation)	99.67ab	20.45c	22.80c	136.31c	16.68c	153.00c	5.51d	358.63b	5.54d
T ₅ (Farmers saved seed +brine solution+ hot water treatment)	101.46ab	22.10b	23.25b	140.11b	16.25c	156.53b	4.58e	420.19a	3.89e
T ₆ (Farmers saved seed+ brine solution+ hot water treatment+ Furadan 3G)	102.83a	23.39a	23.84a	144.08a	15.50d	159.68a	3.46f	438.56a	2.72e
LSD (P=0.05)	6.12	0.91	0.27	2.40	0.72	2.61	0.41	24.68	1.22

Each Value is an average of three replications.

Values in the column having common letter(s) do not differ significantly at p=0.05 level by DMRT.

Table 2. Responses of two varieties on various Growth and yield character of BR11 and Nizersial as influenced by treatments

Variety	Plant height (cm)	Length of root (cm)	length of panicle (cm)	No. of grains/panicle	No. of sterile spikelets/panicle	Total no. of spikelets/panicle	No. of discoloured spikelets/panicle	No. of grains in gm/m ²	No. of nematode/10 gm of seeds
Variety-1(V1) (Nizersial)	103.73	19.17	22.39	151.36	17.58	152.02	5.74	384.14	15.32
Variety-2(V2) (BR11)	100.87	19.24	21.72	153.28	17.34	156.05	6.32	390.61	14.71
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS

Each value is an average of three replications; NS= Non significant

Table 3. Interaction effects of treatments and varieties on various growth and yield character of BR11 and Nizersial varieties of T. Aman rice associated with, *Aphelenchoides besseyi*

Interaction (Treatment*v ariety)	Plant height(cm)	Length of root(cm)	length of panicle (cm)	No. of grains panicle ⁻¹	No. of sterile spikelets/panicle	Total no. of spikelets panicle ⁻¹	No. of discoloured spikelets panicle ⁻¹	No. of grains in gm/m ²	No. of nematodes/10 gm of seeds
T1V1	100.03	15.21	20.15	145.15	20.38	145.18	7.84	297.27	50.00
T1V2	98.12	14.85	19.33	142.22	19.84	150.15	8.50	330.41	49.03
T2V1	102.58	16.30	21.14	150.05	18.30	149.23	7.34	344.32	17.01
T2V2	99.75	16.69	20.29	145.00	18.65	152.23	7.66	350.35	13.54
T3V1	103.27	18.00	22.17	151.13	17.86	152.51	6.41	398.31	10.58
T3V2	101.39	17.57	21.80	151.75	17.66	155.09	6.53	390.25	9.05
T4V1	104.10	20.36	23.11	153.26	16.86	155.37	5.28	410.38	6.08
T4V2	103.29	20.54	22.50	156.40	16.50	157.28	6.13	420.45	5.25
T5V1	105.35	22.00	23.50	155.29	16.50	157.52	4.37	422.42	4.77
T5V2	104.17	22.21	23.00	160.94	16.01	160.52	5.20	425.15	3.91
T6V1	107.04	23.16	24.26	124.75	15.60	158.31	3.25	432.17	3.43
T6V2	105.24	23.62	23.41	159.28	15.40	161.08	3.93	427.10	2.56
LSD p=0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS

Each value is an average of three replications; NS= Non significant

The combined treatments of farmers saved seeds with brine solution, hot water treatment and application of furadan 3G appeared to have superior effect on plant growth and yield characters. Farmers saved seeds with brine solution, hot water treatment was found to respond better in respect of plant growth and yield characters.

The treatments with furadan 3G appeared to have better effect on plant growth and yield characters. Furadan is a carbamate and systemic chemical, which inhibited the growth and activities of the nematode inside the plants. Consequently, plant metabolism was less disturbed and enhanced plant growth with less nematode incidence as also stated by Choi *et al.* (1989). Cho *et al.* (1987) reported that chemical control for the nematode *Aphelenchoides besseyi* was seed disinfection before seeding and carbofuran 3G treatment on the day before transplanting or seed disinfection+ carbofuran 3G water surface treatment at the early stage of injury also effective. Rahman and Taylor (1983) also reported similar (reduced) infestation of *Aphelenchoides besseyi* by the application of carbofuran at 3 kg ai/ ha incorporated in sowing.

Hot water treatment of seeds was found to exert good effect in respect of plant growth and yield characters. This result is supported by the studies made by Yoshi and Yamamoto (1951). Atkins and Todd (1959) reported that hot water treatment of the seed can be used to destroy *Aphelenchoides besseyi*. It was also found that *Aphelenchoides besseyi* was not recovered from rice seeds which received hot water treatment at 55 °C for 15 minutes but was observed in seeds subjected to hot water treatment at 50 °C. Tenente and Manso (1994) reported that there was no effect on seed germination or viability if seeds were planted quickly after treatments at low temperature 52 °C- 53 °C for 15 minutes for controlling *Aphelenchoides besseyi*.

Treatment with brine solution appeared to give good effect in respect plant characters. Sivakumar (1988) observed the effectiveness of pre soaking of rice seeds in water with 1% potassium chloride or 1% sodium chloride for 2 hours which gave 95 - 97% disinfestation of *Aphelenchoides besseyi*. Similar observation was also made in this experiment with brine solution (20% NaCl). Monirul (2000) reported that rice seeds with hot water and brine solution (20% NaCl) could minimize the white tip disease incidence with corresponding incidence of yield. All this reports support the results of the present study in relation to seed treatment with brine solution.

4. Conclusions

This research was carried out with an integrated control approach applying physical and chemical means against seed borne nematode, *Aphelenchoides besseyi*. Farmers saved seeds + brine solution + hot water treatment+ Furadan 3G gave the highest response in relation to plant height, root and panicle length, number of grains per panicle, total number of spikelets panicle⁻¹ and yield of grain per m². Farmers saved seeds + brine solution + hot water treatment had comparatively better response in plant growth and yield characters with corresponding lower number of sterile spikelets panicle⁻¹, no. of discoloured spikelets panicle⁻¹ and no. of nematode population. Furadan 3G had better effect on plant growth and yield characters with corresponding lower number of nematodes as well as no of sterile spikelets panicle⁻¹ and no. of discoloured spikelets panicle⁻¹. It is concluded that treatments of farmers saved seeds + brine solution (20% NaCl) + hot water treatment (55 °C for 10 minutes) + application of furadan 3G in the main field at the time of final land preparation would help greatly to minimize the loss of yield caused by seed borne nematode, *Aphelenchoides besseyi* in BR11 and Nizerial rice commonly cultivated in the country.

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