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Elimination of white-tip nematode, *Aphelenchoides besseyi*, from rice seed

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Summary — Sixteen treatment combinations were tried in ten rice entries heavily infected with white-tip nematode for its elimination. The treatment schedule of soaking of the seed in 0.2 % solution of mancozeb and monocrotophos followed by vacuum fumigation (methyl bromide *ca* 32 g/m^3) for 2 h at 30 °C could eliminate the nematode in all the test entries. Even when the vacuum fumigation was substituted with atmospheric fumigation (aluminium phosphide *ca* 9.3 g/m^3), the treatment schedule was equally effective. Soaking in water followed by hot water treatment (52 °C for 30 min) and vacuum fumigation, though effective in elimination of white-tip nematode, resulted in complete loss of seed viability.

Résumé — ***Traitement des semences de riz pour l'élimination du nématode* Aphelenchoides besseyi, *agent du white-tip*** — Seize combinaisons de traitements sont testées sur dix entrées de riz gravement infestées en vue de l'élimination du nématode agent du white-tip. Le schéma de traitement comportant le trempage des semences dans une solution à 0,2 % de mancozeb et de monocrotophos suivi d'une fumigation sous vide (bromure de méthyle, env. 32 g/m^3) pendant 2 h à 30 °C élimine les nématodes chez toutes les entrées. Le remplacement de la fumigation sous vide par une fumigation à la pression atmosphérique (phosphore d'aluminium, env. 9,3 g/m^3) ne modifie pas le bon résultat du traitement. Le trempage des semences suivi d'un traitement à l'eau chaude (52 °C pendant 30 min) ou d'une fumigation sous vide, quoiqu'efficace pour éliminer les nématodes, produit une perte complète de la germinalité des semences.

Key-words : *Aphelenchoides*, rice, seed disease, treatment.

The white-tip nematode, *Aphelenchoides besseyi*, is a potential pest of rice in almost all rice growing areas of the world. Yield reductions due to this nematode were reported to be as high as 71 % in USSR (Tikhonova, 1966), 60 % in India (Muthukrishnan *et al.*, 1974) and Japan (Tamura & Kegasawa, 1959) and 54 % in USA (Atkins & Todd, 1959) on susceptible varieties. Seed is the main source of infection and the effective control of the nematode increased yields by 19-74 % (Komori *et al.*, 1963).

Hot water treatment at 52-55 °C for 10-30 min of presoaked (12-15 h) seed is being followed as a mandatory treatment for controlling this nematode in quarantine laboratories. However, after this treatment nematodes were not eliminated completely (Kononova & Vinnichuk, 1959; Nandakumar *et al.*, 1975). Further, the nematode has a wide host range including several economically important crops such as maize, strawberry and millets (Fortuner & Williams, 1975; Lal & Mathur, 1988). There are indications of the existence of two or more physiological strains of the nematode in different parts of the world (Fortuner & Williams, 1975). Hence, in spite of its wide distribution, the white-tip nematode carries quarantine significance. It is, therefore necessary to develop a treatment that completely eliminates the nematode without affecting the seed viability.

Materials and methods

One hundred rice entries from seed stocks of the Directorate of Rice Research, Hyderabad, India were screened for the presence of white-tip nematode. Ten entries (V1 to V10) with germination percentage ranging from 95 to 100 were selected for the present study with population ranging from 145 to 500 nematodes per 100 grains. Fifty grams of seed of each entry in a cloth bag was considered as one replication. Each treatment was replicated three times.

The seeds were dehusked manually and the kernels as well as the husks were placed in 3 cm diam. Petri plates and soaked in water. The nematode mortality was recorded after 12 h of soaking. One hundred seeds of each replication were placed on sterile moist blotter paper in a Petri plate and germination percentage was recorded after incubating for a week.

The following treatments were tested for their efficacy in eliminating the white-tip nematode from the seed :

T-1 : Dry heat treatment.
T-2 : Soaking in water followed by dry heat treatment.
T-2 : Soaking in 0.2 % V/V mancozeb solution.
T-4 : Soaking in 0.2 % V/V monocrotophos solution.
T-5 : Soaking in 0.2 % V/V buprofezin solution.
T-6 : Soaking in 0.2 % V/V carbosulfan solution.

T-7 : Soaking in 0.2 % V/V solution of mancozeb and monocrotophos followed by dry heat treatment.
T-8 : Soaking in water followed by hot water treatment (HWT).
T-9 : Vacuum fumigation (VF) for 4 h.
T-10 : Soaking in water followed by VF for 4 h.
T-11 : Soaking in water followed by HWT and VF for 4 h.
T-12 : VF for 4 h followed by soaking in water and HWT.
T-13 : Soaking in 0.2 % V/V solution of mancozeb and monocrotophos followed by VF for 2 h.
T-14 : Soaking in water followed by atmospheric fumigation (AF).
T-15 : Soaking in water followed by HWT and AF.
T-16 : Soaking in 0.2 % V/V solution of mancozeb and monocrotophos followed by AF.

Dry heat treatment was given in a seed dryer (Yorco Scientific Instruments Co., New Delhi, India) at a temperature of 60 ± 1 °C for a duration of 12 h. Seed soaking in chemicals or in water was for a period of 12 h at room temperature (26-28 °C).

Vacuum fumigation was done in one cubic meter chamber (G. Bea Ltd., Altham, U. K.) with methyl bromide *ca* 32 g/m^3 for 2 or 4 h at 30 °C as applicable to the treatment. Atmospheric fumigation was done in a metal chamber designed for the purpose with phosphene (aluminium phosphide *ca* 9.3 g/m^3) for 72 h at 26-28 °C. Hot water treatment was given at 52 ± 0.1 °C for 30 min in a hot water bath (Indian Equipment Corp., Hyderabad, India) provided with a thermostat and a circulating pump for maintaining uniform temperature. The percentage nematode mortality and seed germination were transformed to angular values and subjected to statistical analysis following completely randomised block design.

Results and discussion

Effects of treatments on seed viability

Presoaking in water for 12 h followed by hot water treatment and vacuum fumigation in succession (T-2) resulted in complete loss of seed viability in all the test entries. The adverse effect of fumigation on seed germination is also reported earlier (Todd & Atkins, 1959; Fortuner & Williams, 1975). When atmospheric fumigation succeeded presoaking and hot water treatment (T-15) the effect on seed viability varied with the cultivar (22.5 to 90 %), most susceptible being IET 12202 (V6). The percentage seed viability recorded ranged from 47.5 to 90, 40 to 90, 67.5 to 92.5 and 35 to 100 in the treatments T-1, T-2, T-12 and T-14 respectively. Rest of the treatments had no significant effect on seed viability at least in 50 % of the entries. The treatments T-13 and T-16 did not affect germination in any of the entries significantly.

Effects of treatments on nematode mortality

The effects of sixteen treatments on the nematode mortality is presented in Table 1. Soaking rice seed in buprofezin solution (T-5) had negligible effect on nematode mortality. The treatments T-11 (presoaking + HWT + VF), T-13 (soaking in mancozeb and monocrotophos followed by AF for 2 h) and T-16 (soaking in mancozeb and monocrotophos followed by AF) resulted in 100 % nematode mortality in all the test entries.

Presoaking of seed followed by AF alone (T-14) or HWT followed by AF (T-15) resulted in significant nematode mortality which was on par with 100 % in all the test entries except in V10.

The treatment T-8 (presoaking + HWT) brought about nematode mortality on par with 100 % in 50 % of the test entries (V4, V5, V7, V8, V10), while T-12 (V4, V5, V9), T-4 (V6, V7, V10), T-6 (V2, V8, V10), T-7 (V1, V6, V9) and T-9 (V1, V9, V10) in 30 % of the entries.

Dry heat treatment (T-1) could not eliminate the white-tip nematode in any of the test entries and hence is not suitable as quarantine treatment. Nematodes are known to be highly resistant to heat and chemicals in anhydrobiotic state. The ear cockle nematode *(Anguina tritici)* in anhydrobiotic state remains viable even at 300 °C (Bird, 1981). However, if the seed is soaked in water prior to dry heat treatment (T-3) the percentage nematode mortality generally increased and resulted in nematode mortality on par with 100 % in entries V8 and V9. Further, seed soaking in chemicals prior to dry heat treatment (T-7) increased nematode mortality in all the entries as compared to dry heat treatment alone. Seed soaking in mancozeb (T-3) had highly variable effect on nematode mortality ranging from 17 to 98 % in different entries. Yadav *et al.* (1985), observed that mancozeb at the same concentration (0.2 %) brought about 98.3 % *M. incognita* larval mortality. Seed soaking in monocrotophos (T-4) or carbosulfan (T-6) was very effective in bringing about nematode mortality and upto 100 % kill was observed in some of the entries. These chemicals were also reported to be effective against white-tip nematode by Prasad *et al.* (1986). The hot water treatment at 52 °C for 30 min after 12 h presoaking (T-8) though effective, white-tip nematode survived the treatment to an extent of 0.9 to 19 % in different entries. This supports the observations that the nematode incidence was recorded to an extent of 21 % on leaves (Sivakumar, 1988) and 30 % on ear heads (Muthukrishnan *et al.* 1974) even after receiving hot water treatment to seed at 52-55 °C for 10 min prior to sowing. Vacuum fumigation alone (T-9) resulted in nematode mortality ranging from 27.5 to 98.8 % depending on the test entry. Vacuum fumigation followed by soaking in water and HWT (T-12) was in general on par with T-8 suggesting that prior fumigation has no additional advantage in reducing the nematode population.

Table 1. Effect of sixteen treatments on white-tip nematode mortality in seed of ten entries of rice.

Treatments	V1 IET 12204	V2 IET 12188	V3 IET 12193	V4 IET 12190	V5 IET 12189	V6 IET 12202	V7 IET 12209	V8 IET 12354	V9 NP 125	V10 IET 12206
	Percentage nematode mortality									
T-1	62.5	36.9	29.3	48.4	51.9	80.0	73.8	78.8	87.0	76.4
T-2	86.3	94.1	79.3	82.7	91.9	70.6	100.0	80.0	92.0	90.0
T-3	40.0	17.0	32.3	49.2	80.7	84.3	33.2	91.3	98.0	33.5
T-4	67.5	93.4	37.8	70.4	93.8	100.0	97.7	80.3	67.0	100.0
T-5	0.0	0.0	2.5	0.0	3.0	2.5	3.5	0.0	0.0	2.5
T-6	92.5	99.0	58.7	95.3	77.5	73.6	57.9	98.7	93.5	100.0
T-7	100.0	88.7	77.3	96.2	85.2	98.2	88.3	85.0	95.5	80.9
T-8	96.3	95.7	93.5	97.6	98.4	81.0	98.0	99.1	89.5	96.5
T-9	98.8	94.6	86.8	90.7	27.5	85.5	96.4	78.8	94.5	93.9
T-10	96.2	94.1	93.3	88.8	75.5	82.5	96.0	67.5	93.0	87.5
T-11	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
T-12	71.3	89.6	89.5	97.5	98.4	91.0	93.5	86.5	97.5	92.5
T-13	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
T-14	90.0	95.0	96.4	98.5	98.5	100.0	98.6	100.0	90.0	95.3
T-15	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	86.0
T-16	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
C.D. at 0.05 *	7.2	10.0	13.1	9.2	10.9	7.3	9.5	15.0	14.2	7.5
C.V.	4.9	6.8	9.7	6.2	7.5	5.0	6.2	10.2	9.0	5.0

* Statistical analysis was done basing on the transformed angular values and C.D. and C.V. correspond to the angular values.

The treatments T-11, T-13 and T-16 brought about 100 % nematode mortality. However, T-11 resulted in complete loss of seed viability. Therefore T-13 and T-16 are the two treatments that we recommend for effective elimination of white-tip nematode without any significant effect on seed viability. Earlier, Merny *et al.* (1985) also achieved eradication of *Aphelenchoides besseyi* from seeds of *Panicum maximum* following a schedule : *i)* quick wetting of seeds under partial vacuum; *ii)* presoaking of seeds for 16 h; *iii)* thermal treatment at 55 °C for 20 min in hot water. However, the effective treatments (T-13 and T-16) in the present study did not involve any thermal treatment.

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