Neem as Seed Treatment for Rice Before Sowing: Effects on Two Homopterous Insects and Seedling Vigor

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ABSTRACT Compared with treated seedlings, fewer first-instar *Nephotettix virescens* (Distant) nymphs reached the adult stage on 'TN1' rice seedlings raised from seeds treated before sowing with ≥2.5% neem kernel extract or with 2% neem cake. Likewise, fewer *Nilaparvata lugens* (Stål) nymphs became adults in treatments with neem extracts. Neem cake treatments were not effective. Neem treatments affected neither seed germination nor root length, shoot length, or chlorophyll content of rice cultivars 'IR36' and 'IR42.' Seedlings from seeds treated with neem were more vigorous, and had higher root and shoot growth indexes and dry weights than those that germinated from the control.

KEY WORDS Insecta, neem, *Nephotettix virescens, Nilaparvata lugens*

THE HOMOPTERANS *Nephotettix virescens* (Distant) (Cicadellidae) and *Nilaparvata lugens* (Stål) (Delphacidae) attack rice plants from the seedling stage onward and damage the crop by excessive sucking of photosynthates and by vectoring viruses (Ling 1972, 1977; Hibino 1983). If not managed properly from the early growth stage of the crop, these pests can inflict serious losses. Because chemical control of these insects with synthetic insecticides is expensive and harmful to natural enemies, less expensive and effective but selective pest control materials are needed. Certain plant derivatives that can reduce pest damage below the economic injury level are being considered as potential alternatives for synthetic insecticides (Saxena 1983). Plant derivatives may not necessarily be toxic but they may affect the pest by altering its behavior and physiology. This makes them ideal for use in integrated pest management.

Among the wide range of plants, neem (*Azadirachta indica* A. Juss [Meliaceae]) derivatives have shown great potential in controlling insect pests. They affect more than 195 species of insects including the hemipterous pests of rice (Jacobson 1986, Saxena 1989). *Nephotettix virescens* is highly sensitive to neem treatments and its feeding is disturbed on plants treated with neem (Heyde et al. 1984; Saxena & Khan 1985, 1986; Saxena et al. 1987). The insect's survival and its transmission of tungro viruses were reduced on rice plants treated with neem oil (Mariappan & Saxena 1983, 1984). Likewise, application of neem oil or cake to rice plants decreased feeding and retarded the growth and development of *Nilaparvata lugens* (Saxena et al. 1981, 1984). Neem oil was also highly effective in reducing *Nilaparvata lugens* survival and in suppressing transmission of grassy and ragged stunt viruses of rice (Saxena & Khan 1985).

Appropriate formulations, proper timing, and correct method of application are important considerations in the use of neem for controlling rice insect pests. Rice seed treatments before storage, during storage, and before-sowing stages have been developed to improve rice seed viability and seedling vigor (Krishnasamy & Seshu 1986). Insecticides and fungicides (Reddy et al. 1975; Prakash & Kauraw 1983) or plant materials (Israel & Veda Moorthy 1953, Prakash et al. 1982) have been mixed with rice seed before storage and at storage to prevent damage by insect pests and seed-borne diseases without impairing germination and seedling vigor.

Seed treatment with systemic insecticides that are harmless or beneficial to seedling quality may be useful in controlling the insects that attack the plant at the seedling stage (Banerjee & Bera 1969). Therefore, we evaluated rice seed treatments before the sowing stage with neem seed kernel extract or neem cake for leafhopper or planthopper control at the seedling stage. We also measured effects of neem treatment on seed germination and seedling vigor.

Materials and Methods

Neem seeds and cake from the 1987 crop were obtained from Tamil Nadu Agricultural University, Coimbatore, India. The neem seeds were dehulled and the kernels were crushed with a mortar and pestle. A specified quantity of kernel meal was loosely bundled in a clean cheesecloth and soaked

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for 12 h in 50% of the volume of water required to make a known concentration of the solution. After 12 h, the soaked kernel meal was squeezed while water was added to make extract concentrations to 2.5, 5, and 10% in water (wt/vol).

Healthy seeds of insect-susceptible rice cultivar 'TN1' and common cultivars 'IR36' and 'IR42' (lots of 50 g each) were soaked separately in plastic trays (10 by 15 cm) that contained kernel extract solutions. After 24 h, the seeds were drained off the kernel extract, covered with filter paper, and incubated at room temperature for 48 h. Seed lots soaked in water for 24 h in another set were dressed separately with 1 or 2% finely powdered neem cake (wt/wt) before incubation. In the control, seeds were soaked in water and incubated normally.

Insect Growth and Development. Stock cultures of *Nephotettix virescens* and *Nilaparvata lugens* were maintained on 30- to 40-d-old potted plants of susceptible 'TN1' in cages in the insectary. To determine insect growth and development, 7-d-old 'TN1' seedlings raised from treated and control seeds were placed singly in test tubes (1.5 by 15 cm) containing about 2 ml water. Infestation was at a rate of one first-instar *Nephotettix virescens* or *Nilaparvata lugens* nymph per seedling. Insect survival was recorded daily until all nymphs became adults or died. Growth was measured by the number of nymphs that became adults and the time taken to reach the adult stage. The growth index of each insect species on treated and control seedlings was calculated as the ratio of percentage of nymphs developing into adults to the mean development period in days (Saxena et al. 1974). The lower the growth index, the more effective was the treatment. Each treatment included 10 replications and each replication used 10 seedlings and 10 nymphs.

Seed Viability and Seedling Vigor. Treated and control seeds of 'IR36' and 'IR42' in lots of 25 each were germinated separately using the ragdoll method (International Seed Testing Association 1985). Seeds in a row (25 each per replication) were evenly distributed on a rag; four rows representing four replications were made. Rags with seeds were rolled separately, moistened, and kept in a germinator at 25 ± 1°C and 97% RH. Fourteen days later, the rags were unrolled and the percentage of normal seedlings that emerged was recorded.

The effect of neem treatment on seedling vigor was determined by noting the seedling growth rate. 'IR36' or 'IR42' seeds, arranged in single rows on moist germination papers spread on glass plates (20 by 20 cm), were covered with 5-cm-wide moist germination paper strips and secured with rubber bands. The glass plates with seed, held at an angle of 60° in a Plexiglas tray containing water 2 cm deep, were placed inside a germinator at 25°C and 97% RH. Seedling growth was recorded by daily measurements of the root and shoot growth of the germinating seed. Each treatment included 25 seeds and was replicated four times. Seedling growth rate was expressed as root or shoot growth index and was computed as follows:

$$\text{Root/shoot growth index} = \frac{B - A}{B} \times 100$$

A = number of seedlings whose root or shoot length reached 4 or 2 cm, respectively, on the fifth day of germination; and B = number of seedlings whose root or shoot length reached 4 or 2 cm, respectively, on the seventh day of germination. Root and shoot lengths of 10 randomly selected normal seedlings from each treatment were measured 14 d after germination. The same seedlings were dried at 85°C for 16 h and weighed.

To determine the chlorophyll content, five 14-d-old seedlings were randomly selected from each treatment including the control and processed by the method of Yoshida et al. (1972). Two grams of freshly cut leaf tissue of treated or control seedlings was crushed thoroughly with a mortar and pestle and completely homogenized in acetone. The supernatant was filtered through Whatman #42 filter paper and made up to 100 ml in a volumetric flash. A 5-ml aliquot of this solution was transferred to a 50-ml volumetric flask and made up to volume with acetone. The absorbance of the tissue extract was measured at 652 nm using a spectrophotometer (Bausch & Lomb, New York) and computed as follows:

$$\text{Total chlorophyll } (\mu g/g) = \frac{D652}{34.5}$$

D652 is the absorbance at 652 nm. At 652 nm, chlorophyll *a* and *b* intersect, and 34.5 is the specific absorption coefficient for both pigments at this wavelength.

All data were analyzed with Duncan's (1951) multiple range test ($P = 0.05$).

Results

Insect Growth and Development. Treatment with 2% neem cake dressing before seed incubation significantly reduced the percentage of *Nephotettix virescens* nymphs that became adults on 'TN1' seedlings (Table 1). Although the development period was not affected, the reduced adult emergence on seedlings that emerged from seed treated with neem cake significantly lowered the growth index on control seedlings. Seed dressing with neem cake did not affect the growth and development of *Nilaparvata lugens*. However, seed treatment with 5 and 10% neem seed kernel extract significantly reduced the growth and development and the growth index of both species.

Seed Viability and Seedling Vigor. The germination rate of 'IR36' and 'IR42' seeds, shoot length and root length, and chlorophyll content of seedlings were unaffected by neem treatments (Table 2). In addition, significantly higher shoot and root growth indexes indicated that soaking seed in neem extract increased seedling vigor. Neem cake treat-

Table 1. Development of *Nephotettix virescens* and *Nilaparvata lugens* nymphs on 'TN1' rice seedlings raised from seeds treated with neem kernel extract of neem cake before sowing

Treatment, % neem	*Nephotettix virescens*			*Nilaparvata lugens*		
	% Nymphs becoming adults	Development period, days	Growth index	% Nymphs becoming adults	Development period, days	Growth index
Neem seed kernel extract						
2.5	45b	22.7a	2.0b	83a	14.0a	5.9a
5	40b	21.5ab	1.8b	44b	14.1a	3.1b
10	7c	22.6a	0.3c	22c	14.1a	1.6c
Neem cake						
1	82a	20.5b	4.0a	89a	14.9a	6.0a
2	52b	21.6ab	2.4b	89a	14.0a	6.3a
0 (control)[a]	90a	22.2a	4.0a	90a	14.0a	6.4a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1951] multiple range test). Average of 10 replications, 10 first instars per replication.

[a] Control was common for the treatments.

ment also enhanced seedling vigor but not as much as did neem extract. The dry weight of 'IR36' and 'IR42' seedlings germinating from untreated seed was significantly less than the dry weight of seedlings germinating from seed treated with neem.

Discussion

Vigorous and elite rice seedlings are required to obtain good plant stand and crop establishment (Seshu et al. 1988). Consequently, rice seeds must not only be genetically pure but also free from pests and diseases and have high germinability and vigor. Treating seeds with systemic insecticides may be useful in controlling insects that attack the rice plant at the seedling stage (Krishnasamy & Seshu 1986). Without affecting seed viability, many insecticides are effective in this respect (Bang & Floyd 1962, Gloria et al. 1975). However, treatment of seeds before sowing with certain systemic insecticides such as carbofuran was reported to be phytotoxic and decrease seed germination (Venugopal & Litsinger 1980). Insecticide treatments also render unused, treated seed unfit for human or animal consumption. In contrast, neem treatments are safe to man and animals (Jacobson 1981, Qadri et al. 1984). Seed treatment with neem derivatives would also be more economical to rice farmers.

The seed cake of the "pinnai" (*Calophyllum inophyllum* L.) plant is effective against storage

Table 2. Germination of 'IR36' and 'IR42' rice seeds and seedling vigor when seeds were treated with neem kernel extract or neem cake before sowing

Treatment, % neem	% Germination	Growth index[a]		Length, mm		Dry wt, mg/10 seedlings	Chlorophyll content, µg/g[b]
		Root	Shoot	Root	Shoot		
'IR36'							
Extract							
2.5	95a	78a	66a	250a	105a	91a	51a
5	95a	80a	71a	249a	104a	92a	50a
10	94a	82a	70a	251a	106a	90a	52a
Cake							
1	94a	61b	60b	249a	106a	90a	50a
2	96a	55c	64b	250a	106a	89ab	51a
0 (control)	94a	35d	44c	250a	105a	85b	49a
'IR42'							
Extract							
2.5	96a	44ab	47b	218a	80a	83ab	106a
5	96a	40b	48b	220a	81a	85a	106a
10	95a	49a	52a	220a	80a	84ab	105a
Cake							
1	95a	25c	22c	220a	80a	84ab	105a
2	95a	25c	26c	221a	81a	82abc	106a
0 (control)	94a	16d	17d	219a	81a	78c	104a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1951] multiple range test). Average of four replications, 25 seedlings per replication.

[a] Root or shoot growth index was computed on the number of seedlings whose root or shoot length reached 4 or 2 cm, respectively, on the fifth/seventh day of germination.

[b] On fresh weight basis.

pests but adversely affects the viability of rice seeds (Prakash et al. 1982). In contrast, neem seed derivatives applied before seed was sown not only affected the development of rice leafhoppers and planthoppers systemically, but also increased seedling vigor. The systemic action of neem treatments against insects is well known (Gill & Lewis 1971, Heyde et al. 1984, Saxena et al. 1984). In addition, a significant increase in dry weight of seedlings obtained from neem-treated seeds, without concomitant increase in seedling root and shoot length, indicated that neem treatments produced robust seedlings. The possible role of active principals of neem, such as azadirachtin, in promoting the vigor of rice seedlings remains to be investigated.

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