Effects of Neem-Based Formulations on Brown Planthopper *Nilaparvata lugens* (Ståhl) and Whitebacked Planthopper *Sogatella furcifera* (Horvath)

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Abstract

Toxicological and biochemical effects of azadirachtin and neem oil on both sexes of two planthopper species of rice* N. lugens *and* S. furcifera *were evaluated.

Results showed that with increasing concentrations of neem oil and azadirachtin higher mortality was observed to both sexes of the two planthopper species. Lesser mortality was observed in females than males which was attributed to size differences. Esterase activities were also found affected in treated insects although there existed variations in responses between the species tested.

Key Words: *N. lugens, S. furcifera,* azadirachtin, neem oil

Introduction

Damage inflicted by various insects is one major constraint in crop production affecting both the volume and quality of the produce. The use of synthetic and commercially produced insecticides provided a hasty solution. Pesticides usually cause several serious problems such as health hazards and environmental safety. Likewise, its high cost and high tendency to induce resistance to the target pest are major problems. These concerns prompted the need to replace the use of synthetic pesticides and look into other alternatives. Interests focused on the potentials of bioactive organic compounds from crude botanicals. Unlike synthetic pesticides which usually contain one single active ingredient, crude botanicals have various bioactive components which could act concertedly to yield greater effect. Extracts from the neem tree *Azadirachta indica* A. Juss for one have been used by farmers in Asia and Africa even before commercial insecticides were made available.

The neem tree grows in abundance in almost all dry forests of India and have been known to have various physiological and behavioral effects over a wide variety of insects. The ability of the Indian neem tree to retard the feeding activities of pests is well known. The antifeedant properties of the neem plant reduced pest damage due to the ability of the extracts to repel and disrupt growth and reproduction of a variety of insects. These effects have been directly associated to active compounds of neem extracts, mellantriol and the

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triterpenoids azadirachtin and salanin . These ingredients are nontoxic to man but can effectively repel insects (*Kubo and Nakanishi 1980*). Studies must be further conducted to gain full knowledge on its proper utilization and maximize its potential in pest management. In this study, the toxicity and effects on the esterase activities in two species of planthoppers were investigated.

Materials and Methods

Insects. Newly emerged adults of *N. lugens* and *S. furcifera* were treated topically with 0.2ul neem oil-acetone solutions using Burkhard microapplicator which delivered 25, 50, 75, 100 *ug* neem oil per adult. In the control group, adults were treated similarly with acetone only. The adults were then confined in TN1 rice seedlings for 24 h after which mortality of the adults was recorded. The remaining live insects were prepared for enzyme analysis. For purified azadirachtin tests, the same procedure applied but the concentration varied from 0, 1, 2.5, 5, 10, and 20 ppm respectively.

Acetylcholinesterase Assay. A typical run uses 40 ml pH 8 phosphate buffer, 200 ul and 100 ul substrate, and 100 ul dinitrobenzoic acid (DNTB). The standard consists of a solution of a buffer, substrate and DNTB. Transmittances were then read in a spectrophotometer 30, 60, and 90 min after preparation of samples. Transmittance readings were made at 412 nm. The absorbance was computed from transmittance readings using the formula: 2-log(T). Converted absorbance was computed for rate of production of acetylcholinesterase/min. The amount of ACTH produced per minute was computed using the standard curve as reference.

Nonspecific Esterase Assay. The substrate solutions were prepared by diluting a stock solution of alpha and beta napthylacetate in acetone (0.03M) in 0.04M phosphate buffer, pH 7. Diazoblue-sodium lauryl sulfate solution (DBLS) was also used to give the color reaction. Alpha napthol gives a strong blue color with maximum absorption at 600nm., while b-napthol gives a strong red color with a maximum absorption at 555nm. A typical run uses 25ul substrate, 25ul sample, 4 ml 0.04M phosphate buffer at pH 8 and 1 ml DBLS. Transmittance was read and absorbance was computed.

Results and Discussion

Toxicity. Results of the toxicity of various concentrations of neem oil to both sexes of planthopper species showed a generally upward trend in mortality (Table 1).

Table 1. Toxicity of various concentrations of neem oil on *N. lugens* and *S. furcifera*.

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	5.00 EF	1.75 F	3.30 D	0.00 D
25	16.67 CD	8.70 DF	7.83 CD	0.00 D
50	33.29 AB	11.93 CE	14.73 C	5.07 D
75	40.33 A	18.16 C	31.43 B	9.10 CD
100	40.70 A	27.19 B	49.03 A	27.79 B

In a column, treatment means having a common letter are not significantly different by DMRT $\alpha = 5\%$.

For the male brown planthopper, mortality was increasing as the concentration was increased. Low mortality was observed in the control group. However, the concentrations used were not sufficient to kill 50% of the test insects as the highest concentration killed only a maximum of 40.7%. As to the females, mortality was comparatively lower than males. The highest concentration killed only 27.9% (Table 1).

As to the whitebacked planthopper, a similar trend was obtained as to *N. lugens*. Males were observed to be more susceptible than females as shown by its higher mortality in increasing treatments (Table 1).

For the active ingredient in neem, azadirachtin was found to be toxic to the two insect species (Table 2). Comparing the effects on the two sexes in *N. lugens*, the results showed no significant differences as shown by ANOVA ($P \geq 0.05$). This indicates that the effect is not size dependent. Azadirachtin has the same effect regardless of sex.

Table 2. Toxicity of various concentrations of azadirachtin on *N. lugens*, and *S. furcifera*.

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	5.00 E	0.00 E	8.47 C	10.26 C
1.0	14.53 DE	13.13 DF	23.09 BC	36.11 B
2.5	39.30 C	28.77 CD	36.11 B	60.56 A
5.0	69.73 B	61.77 B	62.16 A	74.32 A
10.0	76.33 B	74.63 B	----	----
20.0	98.33 A	98.13 A	----	----

In a column, treatment means having a common letter are not significantly different by DMRT $\alpha = 5\%$.

For *S. furcifera*, azadirachtin effect was shown to be similar when compared to that of *N. lugens*. Females have higher mortality than males indicating that size is not a factor for the differences. Females are bigger than males and were expected to have lesser mortality than males but the reverse is true. Susceptibility to azadirachtin by females could be attributed to differences in physiology between the two sexes.

Acetylcholinesterase Analysis. The effect of various concentrations of neem oil on acetylcholinesterase activity of *N. lugens* and *S. furcifera* is presented in Table 3. The F-test conducted showed that the effect of neem oil on acetylcholinesterase activity on the two species was significant. Within species the activity showed differences between the two sexes. Males had higher activity than females even if females are generally larger than males. This is in contrast with results obtained with the effect of azadirachtin (Table 4). Within species, azadirachtin has affected acetylcholinesterase production more in *S. furcifera* than in *N. lugens*. Furthermore, the effect on *S. furcifera* is sex-dependent (Table 3). Females had higher acetylcholinesterase production than males although the trend is similarly decreasing. The differences in results on the effect of neem oil and azadirachtin could be attributed to the existence of other compounds present in neem oil other than azadirachtin which affected acetylcholinesterase production.

Table 3. Effect of various concentrations of neem oil on activity of acetylcholinesterase of *N. lugens* and *S. furcifera*

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.016 CD	0.011 DE	0.026 A	0.016 C
25	0.018 BC	0.010 E	0.026 A	0.015 CD
50	0.026 A	0.016 CD	0.024 A	0.014 CE
75	0.023 AB	0.015 CE	0.020 B	0.012 DE
100	0.026 A	0.022 AB	0.016 C	0.011 E

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

Table 4. Effect of various concentrations of azadirachtin on acetylcholinesterase activity of *N. lugens* and *S. furcifera*.

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.010 B	0.007 B	0.024 C	0.046 A
1.0	0.011 B	0.010 B	.0.024 C	0.042 A
2.5	0.010 B	0.007 B	0.019 C	0.036 AB
5.0	0.019 A	0.013 AB	0.011 D	0.027 BC
10.0	0.013 AB	0.019 B		

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

Nonspecific Esterase Activity. Alpha-esterase activity, *in vivo*, was insignificantly different across all levels of concentrations of neem oil in both *N. lugens* and *S. furcifera* (Table 5). No particular trend was observed across all levels of concentration for both species. Comparing the difference between sexes of *S. furcifera* revealed that alpha esterase activity was significantly higher in females than males while no significant differences were observed in *N. lugens*. The effect of neem oil on alpha-esterase activity of *S. furcifera* is sex-dependent while it is sex-independent in *N. lugens*. A contrasting result was observed however when a different substrate was used in esterase assay. The use of beta-napthyl acetate to elucidate b-esterase activity revealed significant differences between sexes not only between increasing treatments (Table 6). It was observed that females had higher amount of b-esterase activity than males.

Table 5. Effect of various concentrations of neem oil on alpha esterase activity of *N. lugens* and *S. furcifera*.

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.477 A	0.677 A	0.201 C	0.404 AB
25	0.501 A	0.593 A	0.232 BC	0.495 A
50	0.342 A	0.491 A	0.202 C	0.419 AB
75	0.499 A	0.513 A	0.222 C	0.423 AC
100	0.456 A	0.676 A	0.233 BC	0.367 AC

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

Table 6. Effect of various concentrations of neem oil on Beta esterase activity on *N. lugens* and *S. furcifera*.

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.323 AB	0.541 AB	0.192 B	0.379 AB
25	0.195 B	0.603 A	0.215 B	0.402 AB
50	0.179 B	0.546 AB	0.207 B	0.453 AB
75	0.550 AB	0.520 AB	0.172 B	0.390 AB
100	0.480 AB	0.671 A	0.376 AB	0.526 A

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

The effect of various concentrations of azadirachtin on alpha-esterase and beta-esterase activities of *N. lugens* and *S. furcifera* is presented in Table 7 and 8. Generally, females had higher amount of nonspecific esterase production than males. Within species however, higher amount was produced as the concentration of azadirachtin was increased but the difference was observed to be insignificant. An extreme case was observed with female *N. lugens* where the highest amount of azadirachtin used had significantly increased the productiion of *B-esterase*.

Table 7. Effect of various concentrations of azadirachtin on Alpha esterase activity of *N. lugens* and *S. furcifera*

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.347 B	0.683 AB	0.095 BC	0.342 A
1.0	0.369 B	0.292 B	0.058 C	0.228 AB
2.5	0.258 B	0.459 B	0.045 C	0.228 AB
5.0	0.413 B	0.449 B	0.045 C	0.320 A
10.0	0.552 B	1.136 A		

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

Table 8. Effect of various concentrations of azadirachtin on Beta esterase activity of *N. lugens* and *S. furcifera.*

	N. lugens		*S. furcifera*	
	Male	Female	Male	Female
Control	0.281 CD	0.798 B	0.149 B	0.425 A
1.0	0.189 D	0.489 BD	0.115 B	0.308 A
2.5	0.307 CD	0.589 BD	0.079 B	0.324 A
5.0	0.296 CD	0.632 BC	0.071 B	0.347 A
10.0	0.271 CD	1.508 A		

In a column, treatment means having a common letter are not significantly different by DMRT at $\alpha = 5\%$.

Esterases are involved in detoxification system by cleaving the ester linkages of a wide array of pesticide constituents. The significantly higher concentration of esterases observed in females would therefore render the females more resistant to the toxic constituents of the pesticide. It is highly probable that the significantly higher esterase activity in females is directly responsible for the relatively lower percentage in mortality.

Neem oil has antifeedant effects. These properties deter feeding by making plants unattractive and unpalatable to insects. Saxena et al (1980) demonstrated these antifeedant effects in a study conducted to show the potential of neem seed oil as an antifeedant for the control of *N. lugens*. The insects generally avoided the rice plants sprayed with the oil resulting to the retardation of growth and development and the reduced survival of the insect. This is further supported by a study done on newly emerged females of *N. lugens, S. furcifera*, and *N. virescens* which showed a significantly reduced food intake on susceptible TN1 rice plants sprayed with oil of neem (Saxena et al 1983). In a monitored feeding behavior of newly emerged females of *N. virescens* using electronic monitoring device on 21-day old seedlings that had been treated systemically by overnight root immersion of dipping the foliage in neem oil formulation showed wave formed patterns indicating that the duration of

phloem feeding was significantly reduced. This was accompanied by a corresponding significant increase in frequency in probing, salivation period and xylem feeding (Saxena and Boncodin 1988). Garcia and Rembold (1984) in their study of the effects of azadirachtin on ecdysis of *Rhodnius prolixus* showed that the feeding inhibition is an indirect effect due to an interference of azadirachtin to the endocrine system rather than the inhibitions of chemoreceptors.

The repellant effects of neem were also observed in many insects. A study conducted on *Sitophilus oryzae* showed pest repellency on neem-treated wheat. Likewise, studies on *Tribolium castaneum* and *S. zeamais* showed the same observations (Akou-Edi 1983).

Neem was also observed to disrupt or interfere with various physiological functions of various insects such as reduction of egg laying, hatching, growth disruption (Abdul 1984), and ecdysis disruption (Abdul Kareem et al 1987, Heyde at al 1983, Haasler 1983).

The results of this study confirm the above investigations. Effects on esterase activities are some of the biochemical explanation resulting from the physiological effects of neem oil.

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