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Resistance and Resurgence Studies of Neem Oil 50% EC Against Rice Brown Planthopper, *Nilaparvata lugens* (Stål)

N.M. Mozaddedul Haque, M. Fazle Rabbi, A.N.M. Rezaul Karim, Mainul Haq & M.A. Chowdhury
Entomology Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh

Abstract: Probit analysis showed that the LC_{50} of Neem oil (NO) 50% EC for two sets of brown planthopper (BPH), *Nilaparvata lugens* (Stål) (mother population) were 0.202 and 0.224%. The LC_{50} values of NO 50 % EC to BPH increased by 1.65 folds in the 10th generation of the first set, and also increased 1.63 folds in the 5th and 1.54 folds in 7th generation of the second set. These increases may be caused either due to loss of bio-efficacy or inconsistency in killing efficacy of neem oil. After three applications of the treatments, the population of BPH increased in monocrotophos and as well as neem treated plots. However, resurgence ratios among different treatments (NO 3 ml/litre of water, NO 4.5ml/litre of water, monocrotophos and deltamethrin) showed no significant differences. It may be concluded that further studies are needed to recommend neem oil for use commercially to control BPH.

Key words: Neem oil, *Nilaparvata lugens*, rice brown planthopper, deltamethrin, monocrotophos

Introduction

Insect pests and diseases are the important limiting factors of rice production in Bangladesh. There are more than 175 insects and several vertebrate pest species, which cause damage to the rice plants (Anonymous, 1995). Out of this large complex, about 20-30 species may be considered as the major pests and these have the potential to cause significant yield loss (Islam *et al.*, 2001). Use of the chemical pesticides is still the dominant pest control mean in Bangladesh. But the pattern of pesticide use in Bangladesh is alarming. Among pesticides, insecticides share is over 90% (Islam *et al.*, 2001). Insecticide use in the sub-lethal doses is the most common in Bangladesh. Sub-lethal doses of insecticide use, pose threats of resurgence of some insects and development of resistance against insecticides (Islam *et al.*, 2001). Insecticide resistance is an increasing problem in the efficient control of agricultural pests (Symondson and Hemingway, 1997). Effective resistance management depends on early detection of the problem and rapid assimilation of information on the resistant insect population, so that rational pesticide choice can be made. Insect resistance to insecticides, which has occurred in limited cases, is expected to increase with the increased use of insecticides in the tropics.

Brown planthopper (BPH), *Nilaparvata lugens* (Stål) is presently one of the most menacing insect pests of rice in Bangladesh. Rice crops worth at least US$ 8.1 millions were lost to it (BPH) during the last three widespread outbreaks in 1976, 1978 and 1983 (Alam and Karim, 1986). The most commonly practical method of controlling the BPH is through the application of insecticides. Among the various leafhopper and planthopper species that attack rice, BPH is one of the most difficult ones to control (Heinrichs, 1979). Resurgences of the BPH that result in total of the yield loss occur when certain insecticides are excessively used (Heinrichs, 1979). Reports of BPH resistance to insecticides in the tropics are rare, most likely because of the low level of insecticides use (Heinrichs, 1979). Brown plant hopper (BPH) resurgence, after treatment of some spray able insecticides, has been recorded at IRRI (Heinrichs, 1979; Reissing *et al.*, 1982). The degree of resurgence is influenced by insecticide management practices (application rate, number, method, volume of spray and timing of application) and the level of varietal resistance to BPH (Alam and Karim, 1986).

It has, therefore, become necessary to complement our reliance on synthetic pesticides with less hazardous, safe and biodegradable substitutes. Evaluation of neem (*Azadiracta indica* A. Juss.) seed oil in laboratory and insectary tests showed the oil's potential as an antifeedant for the control of the brown planthopper, *Nilaparvata lugens* (Stål); the white-backed planthopper, *Sogatella furcifera* (Horvath); and the green leafhopper, *Nephotettix virescens* (Distant) (Heyde *et al.*, 1984). The increasingly serious problems of pest resistance to pesticides and of contamination of the biosphere associated with the large-scale use of broad-spectrum synthetic pesticides have enhanced the need for effective biodegradable pesticides with greater selectivity (Saxena, 1983). This awareness has created a world-wide interest in the re-evaluation and use of age-old, traditional botanical pest control agents (Heyde *et al.*, 1984).

Environmental impact of neem products needs to be investigated before these products are recommended for large-scale use in rice pest control. The evaluation aspects of neem products should include their effects on natural enemies and the possibility of resistance development and resurgence in target pest due to their intensive use (Krishnaiah and Kalode, 1992). Neem oil and its other formulations have been found effective against rice plant sucking insects. The present studies have been taken to evaluate Neem oil 50 % EC i.e. whether it creates pest resistance or resurgence against brown planthopper.

Materials and Methods

Mass culture of BPH: To obtain BPH adults for the experiments the insect was mass reared in Bangladesh Rice Research Institute (BRRI) greenhouse at about 32°C with 60-80% RH during 1992-93. Four-six weeks old potted BR3 plants with 10-12 tillers were used for this purpose. The plants were cleaned and the outer leaf sheaths of the potted plants were removed to destroy the eggs of other insects if any and placed in iron framed (150 x 66 x 76 cm^3) rearing cages covered with fine mesh wire net. Gravid females of BPH were released on the potted plants for oviposition. On each plant 10 to 12 gravid BPH were released and allowed to lay eggs for one day and then the insects were shifted to other plants. Thus a series of plants with BPH eggs were maintained. This was done to ensure supply of the insects of about the same age at a time. Regular observation was done to keep the culture free from predators. After hatching, the host plants were changed at 3-4 days interval to provide sufficient food for the development of the nymphs to adulthood. This cycle was maintained to obtain the required number of BPH adults and nymphs as and when needed for the experiment.

Resistance studies of NO 50% EC on BPH

Bioassay study- Seedling dipping method: All treatment dosage dilutions were made with acetone. The test insecticide (NO 50% EC) was dissolved in acetone to make a percent weight/volume stock solution. Preliminary range test were conducted with a number of concentrations before the final test. Seven concentrations were used in the final bioassay (Table 1). The serial dilutions were made to a half concentration gradually from higher to lower level.

Experimental procedure: Thirty to forty days old BR3 tillers were treated with different concentrations of neem oil 50% EC formulation by dipping the tiller for 15 seconds. After dipping, the treated tiller (Seedlings) were kept on aluminum foil to dry up for 15 minutes and then placed in the 70 ml capacity test tube with the help of forceps and were kept moistened by placing wet cotton in the bottom of the tube. Fifteen 4th instar BPH nymphs were released in each tube and kept in contact with the treated surface for 24 hours.

All treatments were replicated 10 times except mother populations, which were replicated 3 times. After that the mortality counts were taken from every treatment. Moribund insects were assumed to be dead. Mortality in all treatments was corrected by Abbott's formula (Abbott, 1925).

$$\text{Corrected \% Mortality} = \frac{\text{\% Mortality in treatment} - \text{\% Mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

Tillers (seedlings) treated with acetone alone served as the control. The LC_{50} value was calculated by analyzing the data using a computer based Probit analysis programme developed in the USA. The BPH populations treated with LC_{50} values of NO 50% EC were compared with that of non-treated BPH to determine whether BPH could develop resistance to NO 50% EC applications. The insects that survived LC_{50} treatments were reared in greenhouse for subsequent generations and the progenies at each generation were treated with NO 50 % EC twice at a 10 day interval. BPH populations were reared for 10 generations for the first set and seven generations for the second set. The LC_{50} values of the treated population were also calculated at 1, 2, 5,7 and 10th generations in set 1 and 1, 2, 5 and 7th generations in set 2.

Resurgence studies of NO 50% EC on BPH: One field experiment and three greenhouse experiments were conducted to determine whether neem materials induce BPH resurgence.

Field trial: Thirty days old seedlings of high yielding dwarf variety (BR 3) susceptible to BPH were transplanted in the field at a spacing of $10 \times 15 cm^2$. The trial was conducted in RCBD with five replications and unit plot size was $5 \times 5 m^2$.

The treatments were:

T_1 = NO 50% EC @ 3 ml^{-1} of water,

T_2 = NO 50% EC @ 4.5 ml^{-1} of water,

T_3 = Deltamethrin 50 g a. I. (active ingredient) ha^{-1},

T_4 = Monocrotophos 400 g a. I. ha^{-1}

T_5 = Untreated control.

The treatments were applied at 10 day intervals beginning from 50 days after transplanting (DAT) at the dosages mentioned above. For population build up, artificial inoculation of BPH was done at 25 DAT (10 gravid BPH were caged in two places per plot). Observations were made on BPH population, other insect pests and natural enemies by visual sampling in 20 hills in each plot before each treatment and after 24 h of each treatment. After each treatment, the resurgence ratio was calculated by adopting following formula (Jayaraj, 1987):

$$\text{Resurgence ratio} = \left\{\left(\frac{Ts}{Cs} \times \frac{Cb}{Tb}\right) - 1\right\} \times 100$$

Where,

Ts = BPH number in treated plot after treatment,

Tb = BPH number in treated plot before treatment,

Cs = BPH number in untreated control plot after treatment and

Cb = BPH number in untreated control plot before treatment.

The data was analyzed by analysis of variance, Duncan's multiple range (DMR) test and also by combined analysis.

Greenhouse trial: Forty-five days old BR3 plants were grown in 15 cm diameter earthen pots. Ten 4-5th instar BPH nymphs infested the potted plants. Each pot served as replications. There were five replications for each treatment. The potted plants with BPH were caged by Mylar film (12 cm diameter x 60 cm length) and then the caged plants with BPH nymphs were sprayed with neem oil 50% EC (at the rate of 3 ml and 4.5 ml per litre of water), deltamethrin (50 g a.i. hectare) and monocrotophos (400 g a.i. /hectare). If there was 100% mortality in any of the treatment, replacement of nymphs was done uniformly in all treatments. Population build-up of BPH in the treated plants was compared with that of the untreated ones up to second generation. In greenhouse trial, the resurgence ratio was calculated by using two formula; formula-1 (Jayaraj, 1987) as mentioned earlier and formula-2 (Heinrichs *et al.*, 1981) given below:

$$\text{Resurgence ratio} = \frac{\text{No. of insects on treated plants}}{\text{No. of insects on untreated plots}}$$

The data was analyzed by analysis of variance and DMR test.

Results and Discussion

Development of resistance of BPH to neem oil 50% EC: To test whether neem oil develops resistance to brown planthopper in the rice field, LC_{50} value of NO 50% EC for the two sets of BPH was studied in the laboratory and greenhouse by seedling dipping method. Percent mortality due to different dosages of neem oil 50% EC for the set 1 and set 2 BPH populations has been shown in Tables 1 and 2 respectively. In set 1 and set 2 higher concentration (1.6%) of neem oil gave higher percent mortality of BPH (100 and 88%, respectively) and in both sets the LC_{50} of NO 50% EC lies in between 0.2 and 0.4% concentrations of neem oil 50% EC. Probit analysis showed that the LC_{50} of NO 50% EC BPH (mother population) were 0.202% (lower and upper confidence limits 0.158 and 0.259%, respectively) and 0.224% (lower and upper confidence limits 0.192 and 0.262%, respectively) respectively for set 1 and set 2 populations (Table 3). Krishnaiah and Kalode (1992) reported that the lower LC_{50} value of NO 1 and NO 4% were 0.967 and 1.212%, respectively. In comparing with Krishnaiah and Kalode (1992) the LC_{50} value of NO 1 and NO 4% were lower than the tested LC_{50} value of the present study because of higher concentration of neem oil.

LC_{50} values of NO 50% EC for BPH increased by 1.65 (0.33/0.20) folds in the 10th generation of the first set, and also increased 1.63 (0.36/0.22) folds in the 5th and 1.54 (0.34/0.22) folds in the 7th generation of the other set (Table 4). However, the results do not yet clearly indicate the development of resistance in BPH to NO

Table 1: Responses of different dosages of Neem oil 50% EC to BPH (mother population) nymphs, *Nilaparvata lugens* (Stål) Set 1

Treatments	Treatment concentrations (%)	Response of BPH nymphs (no. of dead /no. of tested)	%Corrected mortality
1	0.025	4/45	8.89
2	0.05	8/45	17.78
3	0.1	18/45	40.00
4	0.2	21/45	46.66
5	0.4	26/45	57.78
6	0.8	34/45	75.56
7	1.6	45/45	100.00
8	Control	0/45	0.0

Table 2: Responses of different dosages of Neem oil 50% EC to BPH (mother population) nymphs, *Nilaparvata lugens* (Stål), Set 2

Treatments	Treatment concentrations (%)	Response of BPH nymphs (no. of dead /no. of tested)	%Corrected mortality
1	0.025	20/150	13.33
2	0.05	33/150	22.00
3	0.1	44/150	29.33
4	0.2	73/150	48.66
5	0.4	89/150	59.33
6	0.8	113/150	75.33
7	1.6	132/150	88.00
8	control	0/150	0.0

Table 3: Probit analysis for the tested dosages and their $LC_{1\text{-}99}$ values and 95 % confidence limits for NO 50% EC of the two sets of BPH, *Nilaparvata lugens* (Stål)

Set 1			Set 2		
LC[a]	Dose (%)	LCL-UCL (%)	Lc[a]	Dose(%)	LCL-UCL (%)
1	0.005	0.002-0.011	1	0.003	0.001-0.005
10	0.028	0.016-0.042	10	0.021	0.014-0.028
20	0.056	0.037-0.076	20	0.047	0.036-0.059
30	0.091	0.065-0.118	30	0.085	0.069-0.101
40	0.138	0.153-0.250	40	0.140	0.118-0.164
50	0.202	0.158-0.259	50	0.224	0.192-0.262
60	0.298	0.223-0.392	60	0.357	0.304-0.426
70	0.450	0.345-0.626	70	0.583	0.489-0.730
80	0.730	0.535-1.104	80	1.057	0.841-1.393
90	1.426	0.963-2.476	90	2.380	1.762-3.453
99	7.002	3.745-17.456	99	16.338	9.991-30.393

Lc[a]-Lethal concentration, LCL-lower 95% confidence limits and UCL-upper 95% confidence limits.

Table 4: LC_{50} values of neem oil 50% EC for different generations of BPH, BRRI greenhouse, Gazipur, 1992-93

BPH Generations (No.)	Set 1			Set 2		
	LC_{50}	ULC	LCL	LC_{50}	ULC	LCL
1 (Mother population)	0.20	0.26	0.16	0.22	0.26	0.19
2	0.18	0.22	0.15	0.23	0.27	0.21
5	0.15	0.17	0.13	0.36	0.44	0.31
7	0.28	0.3	0.24	0.34	0.39	0.29
10	0.33	0.42	0.26	-	-	-

Table 5: Resurgence ratios for BPH on rice variety BR3 against NO 50% EC and insecticide treatments under field conditions

Treatments	Resurgence ratio		
	50 DAT	60DAT	70DAT
T_1	-45.85aA	-18.91aA	34.24aA
T_2	43.97aA	7.04aA	49.50aA
T_3	-58.97aA	-30.67aA	-24.50aA
T_4	-2.30aB	-33.79aA	60.38aA
Mean	-15.80B	-19.08aB	29.91A

Table 6: Resurgence ratios for BPH in 2nd generation on rice variety BR3 for NO 50% EC and insecticide treatments, Trial-1, BRRI greenhouse, Aus, 1992

	Resurgence ratio			
	Formula 1		Formula 2	
Treatments	(22DAT)	(40DAT)	(22DAT)	(40DAT)
T_1	300.oaA	-20.0aA	2.2a	0.80a
T_2	140.0aA	130.0aA	1.8a	0.83a
T_3	-80.0aB	480.0aA	0.01a	0.14b
T_4	20.0aA	80.0aA	0.3a	0.54a

Table 7: Resurgence ratios for BPH in 2nd generation on rice variety BR3 for NO 50% EC and insecticide treatments, Trial-2,

	Resurgence ratio			
	Formula 1		Formula 2	
Treatments	(25DAT)	(52DAT)	(25DAT)	(52DAT)
T_1	-180.0aA	604.0aA	43.0aA	13.0aA
T_2	152.0aA	298.0aA	83.0aA	106.0aA
T_3	254.0aA	-44.0aA	81.0aA	68.0aA
T_4	0.97aA	28.0aA	134.0aA	94.0aA

Means in each column and rows followed by the same letter are not significantly different at 5% level by DMRT. Formula 1 = Joyaraj, (1987) and Formula 2 = Heinrichs *et al.* (1981). Small and capital letters indicate comparison within column and rows respectively.

50% EC application. These increases in LC_{50} values might be due to loss of bio-efficacy of neem oil. Krishnaiah and Kalode (1992) reported that when BPH was sprayed with Neem oil for eight generations under the glasshouse condition or sprayed six times at 10 days interval in the field, no sign of resistance development appeared. However, a strain of brown planthopper (*N. lugens*) exposed to Neemgold showed considerable resistance to neem formulations (Sarupa *et al.*, 1999). Therefore, more and further long-term studies are needed to verify the resistance development possibility of BPH against neem oil.

Table 8: Resurgence ratios for BPH in 3rd generations on rice variety BR3 for NO 50% EC and insecticide treatments, Trial-3, BRRI greenhouse,

	Resurgence ratio	
Treatments	Formula 1	Formula 2
T_1	572.0 a	1.87 a
T_2	126.5 a	1.48 a
T_3	592.7 a	0.82 a
T_4	1150.0a	2.15 a

In a column, means followed by the same letter are not significantly different at 5% level by DMRT. Formula 1 = Joyaraj, (1987) and Formula 2 = Heinrichs *et al.* (1981). All the treatment values have been adjusted with control values, this why it is not mentioned in the text and table.

Neem oil induced resurgence on BPH

Field trial: Two rates of NO 50% EC were compared with two synthetic insecticides to determine whether neem materials can cause BPH resurgence. Three applications were made at 10 day intervals starting at 50 DAT. Brown plant hopper BPH) progenies from neem oil and monocrotophos treated populations increased (49.50 and 60.38% at 70 DAT, respectively) in number although the difference in number the generations was not significant even after 3 applications (Table 5). The positive values indicate an increase in the number of BPH (i.e. resurgence occurred) and negative values indicate no resurgence at all. Similarly, in a preliminary study at BRRI, 3 commercial insecticides i.e. Diazinon 60EC (diazinon), Azodrin 40 WSC (monocrotophos), Lebaycid 50 EC (malathion) were found to cause higher fecundity when applied at 0.04% concentration using 1000 litre spray volume per hectare. This means that these insecticides may cause BPH resurgence (Alam and Karim, 1986). Chelliah (1980) found that the BPH populations were highest on plots sprayed 3 times with deltamethrin. Jayaraj (1992), found no resurgence effect of the neem oil on white backed planthopper in rice. While there was marked resurgence in insects treated with methyl parathion, deltamethrin, quinalphos and fenvalerate.

Greenhouse trial: Three trials were conducted with 2 concentrations of NO 50% EC. Potted plants infested with 4th and 5th instar BPH nymphs were sprayed with neem oil 50% EC (3 and 4.5 ml per litre of water), deltamethrin (50 g a.i. ha^{-1}) and monocrotophos (400 g a.i. ha^{-1}).

Population build-up of BPH in the treated plants was compared with that of the untreated ones up to 3rd generation. Although the resurgence ratio were statistically insignificant but the neem treated and monocrotophos treated population appeared to be higher in the first (300 and 20% respectively) and second trial (604 and 28% respectively) in the second generations, respectively (Table 6 and 7) and only statistically significant was found at 1st trial (0.80 and 0.54 respectively) at 40 DAT by the second formula where as insignificant at 22 DAT (Table 6), but in the 3rd generations of the second trials neem treated as well as insecticides treated population appeared to be higher compared to deltamethrin (Table 8), although the resurgence ratios among the treatments were insignificant (Table 6, 7, and 8) because of very high coefficient of variation (>100%). More studies are needed for confirmation.

Krishnaiah and Kalode (1992) found highest BPH population in the deltamethrin treated plots and complete hopperburn occurred after four sprays causing total yield loss. Moderate hopperburn was caused in the monocrotophos treated plots. However, the neem oil treatments did not cause BPH resurgence.

Krishnaiah and Kalode (1987) reported that resurgence under field conditions could be due to reduction in population of natural enemies and low persistent toxicity of the insecticides at the concentration applied. They also reported that monocrothophos, phosalone and phosphamidon at recommended dosage controlled BPH but monocrotophos and phosphamidon at sub optimal level exhibited a tendency for increased pest population. Deltamethrin caused high degree of resurgence while methyl parathion, fenvalarate and quinalphos were moderate in inducing resurgence (Krishnaiah and Kalode, 1987).

Investigations have indicated that many factors are involved in inducing BPH resurgence. Some insecticides contribute to a favourable environment in the rice ecosystem for the BPH to congregate, feed and survive. This stimulates BPH reproduction leading to a high population build-up and severe damage. To prevent outbreaks, a more natural pesticide management programme must be adopted (Chelliah and Heinrichs, 1984). The use of chemicals with low human and fish toxicity and/ or low cost such as botanicals, microbials, insectistatics (compound that decimate insect population by suppressing growth and reproduction rather than by causing rapid mortality), pheromones, etc., should be encouraged when ever practical (Anonymous, 1984). From the studies it may be concluded that further studies are needed to recommend neem oil for commercial use to control BPH.

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