Ecotoxicology and Environmental Safety 72 (2009) 1707–1713

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicity and physiological effects of neem pesticides applied to rice on the *Nilaparvata lugens* Stål, the brown planthopper

Sengottayan Senthil-Nathan [a,b,*], Man-Young Choi [b,c], Chae-Hoon Paik [b], Hong-Yul Seo [b,d], Kandaswamy Kalaivani [a,b,☆,☆☆]

[a] Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi, 627 412 Tirunelveli, Tamil Nadu, India
[b] Plant Environment Division, Honam Agricultural Research Institute (HARI), National Institute of Crop Science (NICS), Rural Development Administration (RDA), #381 Songhak-dong, Iksan, Chonbuk 570-080, Republic of Korea
[c] Agricultural Pest Division, National Institute of Agricultural Sciences and Technology (NIAST), RDA, Seodoon dong, Suwon, Republic of Korea
[d] National Institute of Biological Resources, Environmental Research Complex, Gyoungseo-dong, Seo-gu, Incheon 404-170, Republic of Korea

ARTICLE INFO

Article history:
Received 12 January 2009
Received in revised form
14 April 2009
Accepted 18 April 2009
Available online 4 June 2009

Keywords:
Neem
BPH *Nilaparvata*
Toxicity
Mortality
Food assimilation
Ingestion

ABSTRACT

The effects of two different neem products (Parker Oil™ and Neema®) on mortality, food consumption and survival of the brown planthopper, *Nilaparvata lugens* Stål (BPH) (Homoptera: Delphacidae) were investigated. The LC_{50} (3.45 ml/L for nymph and 4.42 ml/L for adult in Parker Oil™ treatment; 4.18 ml/L for nymph and 5.63 ml/L for adult in Neema® treatment) and LC_{90} (8.72 ml/L for nymph and 11.1 ml/L for adult in Parker Oil™ treatment; 9.84 ml/L for nymph and 13.07 ml/L for adult in Neema® treatment) were identified by probit analysis. The LC_{90} (equal to recommended dose) was applied in the rice field. The effective concentration of both Parker Oil™ and Neema® took more than 48 h to kill 80% of the *N. lugens*. Fourth instar nymph and adult female *N. lugens* were caged on rice plants and exposed to a series (both LC_{50} and LC_{90}) of neem concentrations. Nymph and adult female *N. lugens* that were chronically exposed to neem pesticides showed immediate mortality after application in laboratory experiment. The quantity of food ingested and assimilated by *N. lugens* on neem-treated rice plants was significantly less than on control rice plants. The results clearly indicate the neem-based pesticide (Parker Oil™ and Neema®), containing low lethal concentration, can be used effectively to inhibit the growth and survival of *N. lugens*.

1. Introduction

Rice is the world's largest dietary crop and, especially in Asian countries, rice is the most important food crop (Smil, 2005). The brown planthopper, *Nilaparvata lugens* Stål (BPH), is one of the major pests of rice (Sogawa, 1982). Damage to the rice crop is caused directly by feeding on the phloem (Sogawa, 1982) and indirectly by transmitting plant viral diseases like grassy stunt and wilted stunt viruses (Powell et al., 1995).

Control of *N. lugens* is mainly dependent on chemical insecticides (Yoo et al., 2002). Although insecticides were useful for controlling *N. lugens*, their constant use has reduced the biological control efficacy, resulting in resurgence (Fabellar and Heinrichs, 1984; Heinrichs, 1994), insecticide resistance and environmental hazard (Alarm and Karim, 1986; Yoo et al., 1997; Campiche et al., 2006). Many types of synthetic insecticides have been used during the last five decades in rice fields to control the hoppers but they did not fulfill the requirements for rice pest management in Asia (Kiritani, 1979; Heinrichs, 1994; Tanaka et al., 2000). Also, pesticides usually have a negative effect on the *N. lugens* predators such as mirid bugs, dryinid wasps, *Anagrus* parasitoids, *Coccinella* predators, and *Miscrapis* sp. (Tanaka et al., 2000). The increasing amount of research on insect–plant chemical interactions has unveiled the potential of utilizing botanical insecticides in the form of secondary plant metabolites, or allelochemicals (Kubo, 2006). Natural pesticidal products have low toxicity to humans and natural enemies (Rahman and Siddiqui, 2004). Botanicals (also referred to as botanical

Abbreviations: BPH, brown planthopper; DAP, days after planting; DAT, days after transplanting; SEM, standard error mean

* Corresponding author at: Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi, 627 412 Tirunelveli, Tamil Nadu, India. Fax: +91 4634 283270.
E-mail addresses: senthilkalaidr@hotmail.com, senthil@ms-university.org (S. Senthil-Nathan).

☆ *Funding sources*: This research was fully supported by the Rural Development Administration, NICS, Republic of Korea as a part of the organic agriculture research consortium.

☆☆ *Authors assurance*: We assured that the present research work did not involve humans or other domestic animals in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

0147-6513/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2009.04.024

insecticides) are naturally occurring secondary metabolites extracted from plants. Botanical insecticides break down very quickly in the environment and do not accumulate in plant or animal tissue (Jacobson, 1975; Saxena, 1987; Schmutterer, 1990; Copping and Menn, 2000). Such materials have the potential to enhance the management of *N. lugens* populations in the rice ecosystem (Senthil-Nathan et al., 2007).

Plant families with insecticidal species include Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Oocystaceae, and Rutaceae (Sukumar et al., 1991). Particular species of the plant families Meliaceae and Rutaceae have attracted most of the entomologists' and phytochemists' attention because they produce chemicals such as triterpenes (often referred to as limonoids) (Connolly, 1983). These substances affect pest insects by various modes of action such as growth regulation and antifeedant activity (Senthil-Nathan et al., 2005a, b, 2006a, b). In the Meliaceae family the neem tree is native to the Indian subcontinent and grows in many countries of the world, including subtropical countries such as Africa, Central and South America and Australia (Schmutterer, 1990). During the last five decades, considerable progress has been made regarding the biological activity and medicinal applications of neem. Azadirachtin (hereafter AZA), derived primarily from *Azadirachta indica* A. Juss and *Melia azedarach* L. (Meliaceae), is the most promising phytochemical for pest control (Athanassiou et al., 2005; Senthil-Nathan et al., 2005a, b, 2008, 2009; Kavallieratos et al., 2007). In addition to AZA, which is considered the primary active principle, numerous other compounds in neem have insect growth regulator effects (Schmutterer, 1990; Senthil-Nathan et al., 2007).

Effects of neem preparations on beneficial arthropods are generally considered to be minimal. Some laboratory and field studies have found neem extracts to be compatible with biological control (Goudegnon et al., 2000; Abudulai and Shepard, 2003; Mitchell et al., 2004). For example, a commercial neem formulation had no detrimental effect on *Diadegma mollipla* (Holmgren) on *Plutella xylostella* (L.). (Akol et al., 2002), *Opius concolor* Szeapligeti, an endoparasitoid, *Chrysoperla carnea* Stephens, or *Podisus maculiventris* Say, a generalist predator (Vinuela et al., 2000). Furthermore, azadirachtin-based insecticides can be used as grain protectants as well where abiotic (i.e. formulation, temperature, RH) and biotic factors (i.e. target pest, commodity) play an important role in their efficacy (Kontodimas et al., 2004; Athanassiou et al., 2005; Kavallieratos et al., 2007). Control of *N. lugens* in the field using neem-based pesticides is aimed at the nymphal stage because the *N. lugens* is most susceptible in early developmental stages (Saxena et al., 1984; Saxena and Khan, 1985).

Our objective is to determine the biological activity of the two neem pesticides against the nymph and adult of *N. lugens* as measured by mortality and food utilization parameters on foliar-treated rice plants in field and laboratory experiments.

2. Materials and methods

2.1. Laboratory mass culture of N. lugens

A susceptible strain of *N. lugens* has been maintained for more than 10 years in the laboratory of Honam Agricultural Research Institute, Rural Development Administration (RDA), Iksan, South Korea, without any exposure to insecticide. These insects were maintained on the cultivar 'Taebaegbyeo' (*Oryza sativa* L.) seedlings (9–11 days after germination), in acrylic cages at $27\pm1\,^{\circ}C$, 40–60% RH, and a photoperiod of 16:8 (L:D) h.

2.2. Bioassay

Ten fourth instar nymphs and ten adult female (1 day old) *N. lugens* were transferred into an individual experimental cage (48 cm height and 34 cm width with both sides covered with 20 cm^2 mesh for aeration) containing two Taebaegbyeo rice seedlings (15–18 days after germination). Two commercial neem products (Parker™ (Neem Korea-0.2% AZA) and Neema® (0.25% AZA)) were purchased from Neem Korea Inc., Seoul, and Bicosys Inc. Daegu, Korea, respectively. Each neem pesticide treatment (0.5, 1, 2.5 and 5 ml/L) was dissolved in water with emulsifier, Triton X-100™ (Sigma Chemical, USA) (1%). Controls received only water+Triton X-100 solution (1%). Two ml of test material solutions was applied by a regulator-controlled spray applicator (Komex, Korea) (air/supply pressure less than 24 psi; fluid/output pressure less than 87 psi) for each plant. Forty nymphs and adult female/concentration were used for all the experiments (10 insects with four replicates). Mortality was recorded every 12 h, final mortality was recorded after 48 h.

2.3. Field experiments

The field experiments were conducted on 1 ha of rice at the one site in Honam Research Institute, 355′6″N & 126′55″E Iksan, Republic of Korea (Fig. 1). Two

Fig. 1. Experimental site at Iksan with exclusion cages in position.

(Parker Oil™ and Neema®) pesticide treatments were used with three replicates with five plots. Each plot had dimensions of 21.4 m (wide) × 10.7 m (length). The experiments were conducted with transplanted ~40-day-old (DAP) seedlings.

In each plot, three permanent cages (modified from Claridge et al., 2002) (0.36 m × 1.5 m) (Fig. 2) were used to enclose four plantations. Spacing between plantations (hills) was approximately 20 cm. All arthropods inside the cages were removed by careful inspection to make the cage as free as possible from natural enemies. The experimental field cage-enclosures were made from iron frames and measured 1.5 m high by 0.36 m square. All cages were roofed with a fine mesh gauze, which extended 0.1 m down each side with a nylon mesh side zipper door. The remaining sides were covered with appropriate mesh gauze. The cages were set out in the field with the plastic skirt immersed in the paddy water and mud to preclude entry of aerial predators and parasitoids from below. The cages were infested for 1 week with 20 gravid female *N. lugens*. Neem pesticides were applied once at 40 DAP. The neem formulations were applied at a rate of 2 L/ha with a hand sprayer (Model-HDKS-2, Haechoung Machinery Co., Ltd., Korea). The neem pesticides were sprayed during morning time. Observations on *N. lugens* (both nymph and adult) were recorded by visual counting by using a convex lens as a magnifier at intervals (24 h, 48 h, 4 days, 6 days and 9 days) on all caged hills in the central area (2.5 m^2) of each plot.

2.4. Ingestion and assimilation of food

To determine the quantity of food ingested and assimilated, newly emerged females starved for 3 h were weighed individually on a microbalance (Sartorius, CP2245). Each test insect was placed within an airtight parafilm sachet on the stem of 25-day-old test plants. After 24 h, the weight of each female and its excreta were recorded. Similarly control insects were individually weighed and were given access to a wet cotton swab inside a parafilm sachet to prevent desiccation. The amount of food ingested and assimilated by the insect was calculated as (Khan and Saxena, 1985; Velusamy et al., 1995)

$$\text{Food assimilated} = W1 \times \frac{C1 - C2}{C1} + W1 - W2 \quad (1)$$

where $W1$ is the initial weight of test insect, $W2$ the final weight of test insect, $C1$ the initial weight of control insect and $C2$ the final weight of control insect. Food ingested = food assimilated+weight of excreta. There were three replications for each treatment including the control. Each replicate was composed of five females held individually in parafilm sachets on five different plants.

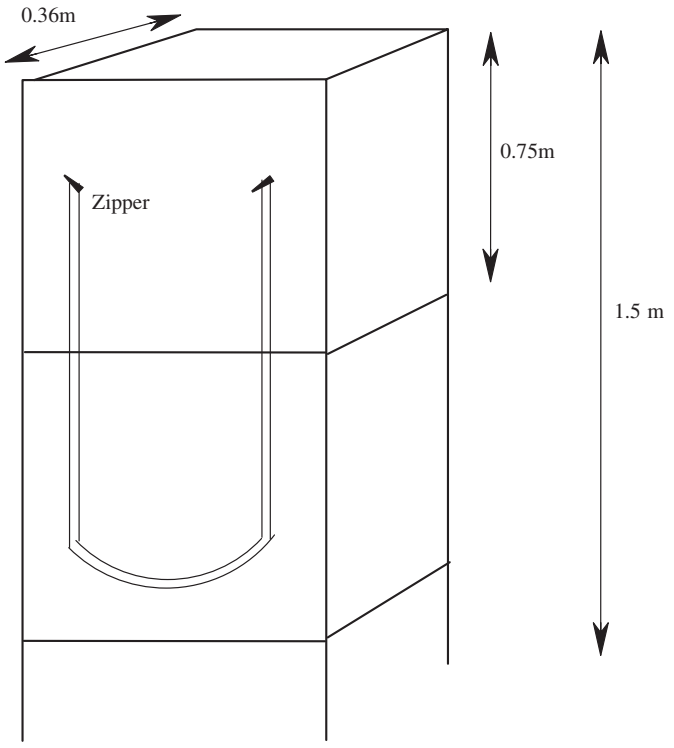

Fig. 2. Schematic diagram of field cage with frame measurements.

2.5. Statistical analysis

Data from food assimilation and ingestion were expressed as the mean of three replications and normalized by arcsine-square root transformation of percentages. The transformed percentages were subjected to analysis of variance (ANOVA). Differences between the five treatments were determined by the Tukey–Kramer HSD test ($P \leq 0.05$) (Snedecor and Cochran, 1989; SAS Institute, 2001). Mortality was corrected using Abbott's (1925) formula. The lethal concentrations (both LC_{50} and LC_{90}) were calculated using probit analysis (Proc PROBIT) (Finney, 1971), and values were expressed as mean ± standard error (SEM) of three replicates.

3. Results

Ingestion of Neema® resulted in significant mortality of nymphs ($F = 2949.78$; d.f. = 18; $P < 0.001$) and adult females ($F = 8898.14$; d.f. = 18; $P < 0.001$) within 24 h after feeding was initiated (Fig. 3). Results of the field study also demonstrate that the neem pesticides were effective under field conditions for control of *N. lugens*. The *N. lugens* nymphs and adult females exposed more than 5 ml/L of Parker Oil™ and Neema®, neem pesticide showed 88% and 82% mortality, respectively (Fig. 4). Daily mean percent mortality of the *N. lugens* in the control remained below 10% for 24 h ($F = 12331.53$; d.f. = 11; $P < 0.001$) (Fig. 4). At an effective concentration of Parker Oil™, 40% of the *N. lugens* died within 24 h, and the mortality increased to 82.5% by 48 h. At an effective concentration of Neema®, 37.5% of the larvae died within 24 h and 70.5% by 48 h ($F = 273.68$; d.f. = 18; $P < 0.001$)(Fig. 4).

During the field experiment, the numbers of fourth instar nymphs and adult female *N. lugens* in the beginning, on neem-treated plots, were much lower than in the controls (Fig. 4). The LC_{50} and LC_{90} values of neem products for the brown planthopper nymph and adult female are shown in Fig. 5 and 6.

Neem pesticides on food utilization and mortality of the *N. lugens* could be observed at the highest concentration (1 ml/L) within 24 h and are significantly different from those of other treatment doses ($P < 0.05$). All treatments reduced the survival of both adult and nymphal *N. lugens*. Lethal times for the *N. lugens* nymphs and adults were concentration dependent. *N. lugens* adult females and nymphs died sooner in treatments with higher concentrations of neem pesticides than those with lower treatment concentrations.

Results of the present study indicate that the feeding (food ingested and assimilated) is significantly reduced in *N. lugens* following neem pesticide application to rice foliage (Tables 1 and 2). Data of Tables 1 and 2 show a negative effect on the food intake and consumption of adult female insects. The calculated values of food utilization parameters were less than those of control larvae and decreased gradually as the neem pesticides' concentration level was increased. For example, the food assimilated by the control adult female *N. lugens* was 1.31 mg/day and decreased to 0.976 mg/day with 1 ml/L treatment of Parker Oil™ ($F = 2984.56$; d.f. = 11; $P < 0.001$). Also the food assimilated by the control adult female *N. lugens* was 1. 250 mg/day and decreased to 1.082 mg/day with 1 ml/L treatment of Neema® and was further reduced to 0.857 in 2.5 ml/L concentration of the same treatment ($F = 18333.20$; d.f. = 11; $P < 0.001$). The food ingested by the control female adult insects was 32.46 mg/day and decreased to 24.66 mg/day with 1 ml/L treatment of Neema® and was further reduced to 11.28 in. 5 ml/L concentration ($F = 23953.02$; d.f. = 11; $P < 0.001$). The food ingested by the control female adult insects was 31.66 mg/day and decreased to 16.96 mg/day with 2.5 ml/L treatment of Parker Oil™ and was further reduced to 10.43 in 5 ml/L concentration of the same treatment ($F = 8577.29$; d.f. = 11; $P < 0.001$). The highest ingestion and assimilation were recorded in the control.

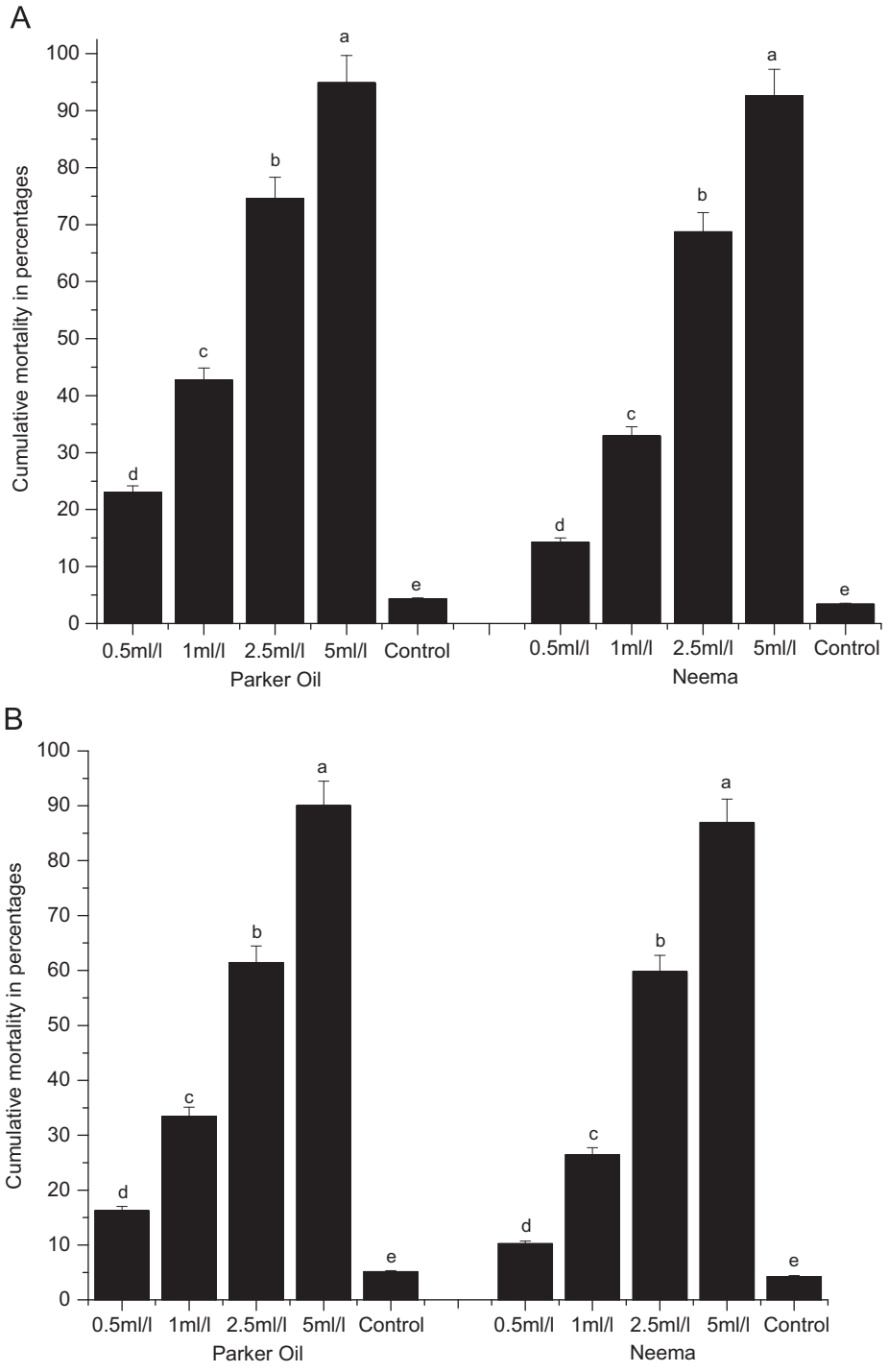

Fig. 3. Cumulative mortality (±SEM) of fourth instar nymphal (A) and adult female (B) BPH after treatment with Parker Oil™ and Neema®. Mean (±SEM) followed by the same letters above bars in an individual pesticide treatment indicate no significant difference ($P \leq 0.05$) according to the Tukey test.

The results also showed that the food ingestion and assimilation were significantly reduced for both neem product treatments.

4. Discussion

The effects of neem derivatives including azadirachtin have already been assayed on several rice insect pests of different classes to evaluate them in rice pest management programs including the small rice stinkbug, *Oebalus poecilus* (Dallas) (Sutherland et al., 2002), rice leaffolder, *Cnaphalocrocis medinalis* Guenée (Saxena et al., 1981; Senthil-Nathan et al., 2005a, b), the BPH, *N. lugens* (Saxena and Khan, 1985; Senthil-Nathan et al., 2007), green rice leafhopper (GRL), *Nephotettix virescens* Distant (Saxena et al., 1987), rice thrips, *Stenchaetothrips biformis* Bagnall (Pillai and Pooniah, 1988), paddy armyworm, *Spodoptera mauritia acronyctoides* Guenée (Jagannadh and Nair, 1992), white planthopper (WBH) *Sogatella furcifera* Horvath and rice bug *Leptocorisa oratorius* Fabr. (Heyde et al., 1984) *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Tribolium confusum* Jacquelin du Val (Kontodimas et al., 2004; Athanassiou et al., 2005; Kavallieratos et al., 2007). However, insufficient work has been conducted

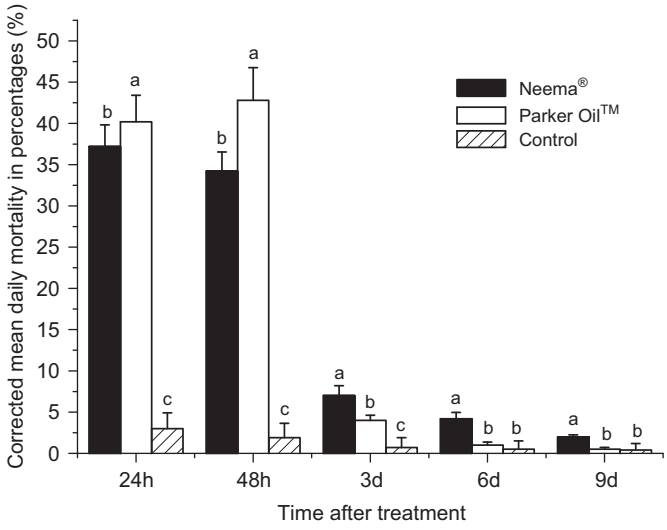

Fig. 4. Daily mean (±SEM) percentage of corrected mortality of the BPH after treatment with Parker Oil™ and Neema® in field condition. Mean (±SEM) followed by the same letters above bars in a particular treatment time indicate no significant difference ($P \leq 0.05$) according to the Tukey test.

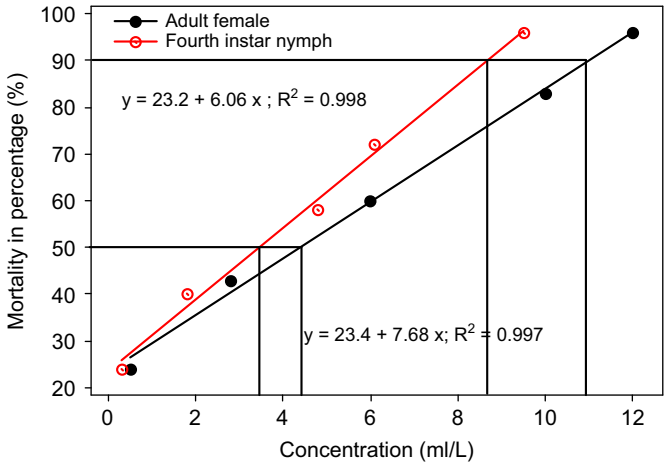

Fig. 5. Probit regression analysis with LC_{50} (3.4 ml/L for fourth instar nymph and 4.4 ml/L for adult female, respectively) and LC_{90} (8.7 ml/L for fourth instar nymph and 11.1 ml/L for adult female, respectively) values (the data were fitted with simple linear regression) after treatment with Parker Oil™.

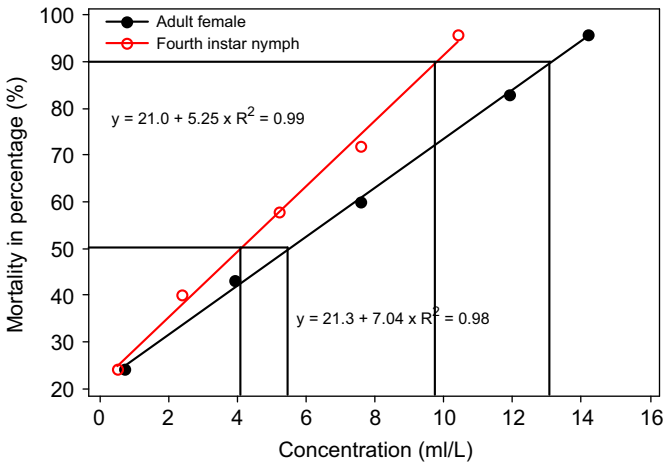

Fig. 6. Probit regression analysis with LC_{50} (4.1 ml/L for fourth instar nymph and 5.6 ml/L for adult female, respectively) and LC_{90} (9.8 ml/L for fourth instar nymph and 13 ml/L for adult female, respectively) values (the data were fitted with simple linear regression) after treatment with Neema®.

Table 1
Food assimilation of adult female BPH after treatment with Parker Oil™ and Neema®.

Sample	Food assimilated/female/24 h (mg)			
	1 ml/L	2.5 ml/L	5 ml/L	Control
Neema®	1.082 ± 0.0061^{b}	0.857 ± 0.0040^{c}	0.433 ± 0.0070^{d}	1.250 ± 0.0200^{a}
Parker Oil™	0.976 ± 0.0050^{b}	0.769 ± 0.0060^{c}	0.351 ± 0.0045^{d}	1.318 ± 0.0050^{a}

Mean (±SD) followed by the same letters in a row indicate no significant difference ($P \leq 0.05$) according to the Tukey test.

Table 2
Food ingestion of adult female BPH after treatment with Parker Oil™ and Neema®.

Sample	Food ingested/female/24 h (mg)			
	1 ml/L	2.5 ml/L	5 ml/L	Control
Neema®	24.66 ± 0.208^{b}	19.09 ± 0.060^{c}	11.38 ± 0.025^{d}	32.46 ± 0.252^{a}
Parker Oil™	22.80 ± 0.045^{b}	16.96 ± 0.136^{c}	10.43 ± 0.586^{d}	31.66 ± 0.208^{a}

Mean (±SD) followed by the same letters in a row indicate no significant difference ($P \leq 0.05$) according to the Tukey test.

regarding insecticidal effects of neem on important Hemipteran pests. Most of these studies were concentrated on the antifeedant action of neem on Heteropteran pests (Durmusoglu et al., 2003). Therefore, studies on the competence of neem products have become obligatory to identify its prospective impact on the *N. lugens*.

The active principles in neem constitute an array of complex limonoids that have diverse behavioral and physiological effects against a variety of insect pests (Schmutterer, 1990). Neem and its derivatives work as systemic insecticides; it is absorbed into the plant and carried throughout the tissues to be ingested by insects when they feed on the plant. This may make it effective against phloem feeders like *N. lugens* (Heyde et al., 1984; Senthil-Nathan et al., 2008). Unlike other synthetic or chemical pesticides, the possibility of a pest species developing resistance to a complex chemical is not possible (Jilani and Sexena, 1990).

N. lugens responses such as food intake, assimilation of ingested food, and growth and development were inhibited in all neem treatments especially at doses over 2.5 ml/L. Adverse effects of neem-treated rice plant on *N. lugens* responses and mortality have been reported earlier by Heyde et al. (1984), Saxena and Khan (1985) and Senthil-Nathan et al. (2007) but apparently this is the first study on commercial neem insecticides against *N. lugens* with dose recommendation.

Previous studies have shown that neem and AZA cause feeding deterrence in many Hemipteran insect species. Nymphal and adult insects are inhibited from feeding on neem-treated plant parts (Heyde et al., 1984; Tuncer and Niazee, 1998; Durmusoglu et al., 2003). The feeding inhibition and mortality effects depend on the neem concentration and the stage of insect species tested (Heyde et al., 1984; Athanassiou et al., 2005; Kavallieratos et al., 2007; Senthil-Nathan et al., 2007). Results from this study suggest that an emulsifiable concentrate neem product at a low concentration (0.5 ml/L) does not cause significant mortality on the *N. lugens* nymphs and adults, but high mortality was observed at concentrations of 2.5 and 5 ml/L.

Feeding reduction (ingestion and assimilation) in *N. lugens* and adult females that were exposed to neem products (Parker Oil™ and Neema®) is probably useful in rice ecosystems. Some

researchers have found neem extracts to be compatible with natural enemies (Hoelmer et al., 1990; Goudegnon et al., 2000; Abudulai and Shepard, 2003). Saxena and Khan (1985) found that *N. lugens* is very sensitive to neem oil. But so far, no studies have been conducted for commercial neem insecticides against *N. lugens*, especially for nutritional indices. Concentration-dependent mortality and reduction in food intake were evident. Neem causes reduced food intake in the *N. lugens*; however, its potential is affected by the rate and frequency of application (Senthil-Nathan et al., 2007).

Results from this research work and previously published work (Senthil-Nathan et al., 2007) clearly indicate the capability of neem pesticides in controlling *N. lugens*. Parker Oil™ and Neema® cause both mortality and feeding inhibition of nymphs and adult females of *N. lugens*. At a concentration of 5 ml/L, nymphs and adult females died immediately after foliar application. The strong sublethal effects on *N. lugens* at low concentrations decrease their food consumption (digestion and assimilation). The same effect was observed in Neemix®-treated sawfly *Neodiprion abietis* Harris (Li et al., 2003). Our studies show that the neem pesticides are effective against *N. lugens* adult females and nymphs. According to these results, the neem-based pesticide may be a good candidate for the control of *N. lugens*. A significant advantage in the use of neem-based insecticides for *N. lugens* control would be the decreased reliance on conventional synthetic insecticides.

5. Conclusion

To conclude, both neem pesticides (Parker Oil™ and Neema®) inhibited the survival of nymph and adult female *N. lugens*. Results from nutritional indices and field experiments suggest that the neem pesticides directly affect the survival and food intake of *N. lugens*. Our laboratory and field experiment observations support this hypothesis. Utilization of neem-based pesticides early in the rice-growing season when young nymphs are the predominant life stage would provide effective control and, due to their minimal effects on natural enemies, reduce the resurgence problem seen when insecticides are used early in the rice-growing season.

Acknowledgments

This research was supported by the Rural Development Administration, NICS, Korea, as a part of the organic agriculture research consortium. The authors express gratitude to Prof. J. Powell Smith for his critical review and advice of an earlier draft of the manuscript. We thank Profs. B. Merle Shepard and Richard W Mankin for providing valuable reprints and related articles. We thank (Mrs.) S. S. Kang, S. M. Kang, H. S. Park and S. H. Han for insect rearing and field experiments assistance. The authors thank the two anonymous experts for their thorough and constructive comments.

References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Abudulai, M., Shepard, B.M., 2003. Effects of neem (*Azadirachta indica* A. Juss) on *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), a parasitoid *of Nezara viridula* (L.) (Hemiptera: Pentatomidae). J. Entomol. Sci. 38, 386–397.

Akol, A.M., Sithanantham, S., Njagi, P.G.N., Varela, A., Mueke, J.M., 2002. Relative safety of sprays of two neem insecticides to *Diadegma mollipla* (Holmgren) a parasitoid of the diamondback moth: effects on adult longevity and foraging behaviour. Crop Prot. 21, 853–859.

Alarm, S., Karim, A., 1986. Brown planthopper situation and its management in Bangladesh. In: Proceedings of the Workshop on Experiences with Modern Rice Cultivation in Bangladesh, BRRI, Gazipur, Bangladesh, p. 218.

Athanassiou, C.G., Kontodimas, D.C., Kavallieratos, N.G., Anagnou-Veroniki, M., 2005. Insecticidal effect of Neem-Azal against three stored-product beetle species on rye and oats. J. Econ. Entomol. 98, 1499–1505.

Campiche, S., Slooten, K.P., Ridreau, C., Tarradellas, J., 2006. Effects of insect growth regulators on the nontarget soil arthropod Folsomia *candida* (Collembola). Ecotoxicol. Environ. Saf. 63, 216–225.

Connolly, J.D., 1983. Chemistry of the *Meliaceae* and *Cneoraceae*. In: Waterman, P.G., Grunden, M.F. (Eds.), Chemistry and Chemical Taxonomy of the Rutales. Academic Press, London, pp. 175–213.

Copping, L.G., Menn, J.J., 2000. Biopesticides: a review of their action, applications and efficacy. Pest Manage. Sci. 56, 551–576.

Claridge, M.F., Morgan, J.C., Steenkiste, A.E., Iman, M., Damyanti, D., 2002. Experimental field studies on predation and egg parasitism of rice brown planthopper in Indonesia. Agric. Forest Entomol. 4, 203–209.

Durmusoglu, E., Karsavuran, Y., Ozgen, I., Guncan, A., 2003. Effects of two different neem products on different stages of *Nezara viridula* (L.) (Heteroptera, Pentatomidae). J. Pest Sci. 76, 151–154.

Fabellar, L.T., Heinrichs, E.A., 1984. Toxicity of insecticides to predators of rice brown planthopper *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae). Environ. Entomol. 13, 832–837.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London, UK, p. 383.

Goudegnon, A.E., Kirk, A.A., Schiffers, B., Bordat, D., 2000. Comparative effects of deltamethrin and neem kernel solution treatments on diamond backmoth and *Cotesia plutellae* (Hym., Braconidae) parasitoid populations in the Cotonou peri-urban area in Benin. J. Appl. Entomol. 124, 141–144.

Heinrichs, E.A., 1994. Host plant resistance. In: Heinrichs, E.A. (Ed.), Biology and Management of Rice Insects. Wiley, New York, pp. 517–547.

Heyde, V.D.J., Saxena, R. C., Schmutterer, H., 1984. Neem oil and neem extracts as potential insecticides for control of hemipterous rice pests. In: Schmutterer, H., Ascher, K.R.S. (Eds.), Natural Pesticides from the Neem Tree and Other Tropical Plants. Proceedings of the Second International Neem Conference, Berlin, Germany, pp. 379–399.

Hoelmer, K.A., Osborne, L.S., Yokomi, R.K., 1990. Effects of neem extract on beneficial insects in greenhouse culture. In: Locke, J.C., Lawson, R.H. (Eds.), Proceedings of a Workshop on Neem's Potential in Pest Management Programmes. USDA-ARS, Beltsville, MD ARS-86.

Jacobson, M., 1975. Insecticides from plants a review of the literature, 1954–1971. In: Agriculture Handbook 461. USDA, Washington, DC, p. 138.

Jagannadh, V., Nair, V.S.K., 1992. Azadirachtin-induced effects on larval–pupal transformation of *Spodoptera mauritia*. Physiol. Entomol. 17, 56–61.

Jilani, G., Sexena, R.C., 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil and a neem insecticide against lesser grain borer (Coleoptera: Bostrychidae). J. Econ. Entomol. 83, 629–634.

Kavallieratos, N.G., Athanassiou, C.G., Saitanis, C.J., Kontodimas, D.C., Roussos, A.N., Tsoutsa, M.S., Anastassopoulou, U.A., 2007. Effect of two azadirachtin formulations against adults of *Sitophilus oryzae* and *Tribolium confusum* on different grain commodities. J. Food Prot. 70, 1627–1632.

Khan, Z.R., Saxena, R.C., 1985. Mode of feeding and growth of *Nephotettix verescens* (Homoptera: Cicadellidae) on selected resistant and susceptible rice varieties. J. Econ. Entomol. 78, 583–587.

Kiritani, K., 1979. Pest management in rice. Annu. Rev. Entomol. 24, 279–312.

Kontodimas, D., Kavallieratos, N.G., Mantzoukas, S., Athanassiou, C.G., Anagnou-Veroniki, M., 2004. Effect of *Beauveria bassiana*, *Vertcillium lecanii*, *Bacillus thuringiensis* ssp. *tenebrionis* and azadiractin compounds on *Sitophilus oryzae* (L.) and *Tribolium confusum* Jacquelin du Val in stored rye. In: Seventh International Conference on *Bacillus thuringiensis*, Helsinki, Finland, 1–6 September 2004, pp. 94–95.

Kubo, I., 2006. New concept to search for alternate insect control agents from plants. In: Rai, M., Carpinella, M. (Eds.), Naturally Occurring Bioactive Compounds 3. Elsevier, Amsterdam, pp. 61–80.

Li, S.Y., Skinner, A.C., Rideout, T., Stone, D.M., Crummey, H., Holloway, G., 2003. Lethal and sublethal effects of a neem-based insecticide on balsam fir sawfly (Hymenoptera: Diprionidae). J. Econ. Entomol. 96, 35–42.

Mitchell, P.L., Gupta, R.K., Singh, A.K.K., Kumar, P., 2004. Behavioral and developmental effects of neem extracts on *Clavigralla scutellaris* (Hemiptera: Heteroptera: Coreidae) and its egg parasitoid, *Gryon fulviventre* (Hymenoptera: Scelionidae). J. Econ. Entomol. 97, 916–923.

Pillai, M.A.K., Pooniah, S., 1988. Neem for control of rice thrips. Int. Rice Res. Newsl. 13, 33–34.

Powell, K.S., Gatehouse, A.M.R., Hilder, V.A., Gatehouse, J.A., 1995. Antifeedant effects of plant lectins and an enzyme on the adult stage of the rice brown planthopper, *Nilaparvata lugens*. Entomol. Exp. Appl. 75, 51–59.

Rahman, M.F., Siddiqui, M.K.J., 2004. Biochemical effects of vepacide (from *Azadirachta indica*) on Wistar rats during subchronic exposure. Ecotoxicol. Environ. Saf. 59, 332–339.

Saxena, R.C., 1987. Neem seed derivatives for management of rice insect pest—a review of recent studies. In: Schmutterer, H., Ascher, K.R.S. (Eds.), Proceedings of the Third International Neem Conference, Nairob, Kenya, GTZ, Eschborn, p. 689.

Saxena, R.C., Khan, Z.R., Bajet, N.B., 1987. Reduction of tungro virus transmission by *Nephotettix virescens* (Homoptera: Cicadellidae) in neem-cake treated seedlings. J. Econ. Entomol. 80, 1079–1082.

Saxena, R.C., Khan, Z.R., 1985. Effect of neem oil on survival of *Nilaparvata lugens* (Homoptera: Delphacidae) and on grassy stunt and ragged stunt virus transmission. J. Econ. Entomol. 78, 647–651.

Saxena, R.C., Justo, H.-D., Epino, P.B., 1984. Evaluation and utilisation of neem cake against the brown planthopper, *Nilaparvata lugens*. In: Natural Pesticides from the Neem Tree and other Tropical Plants. Second International Neem Conference, Rauischholzhausen, pp. 391–402.

Saxena, R.C., Waldbauer, G.P., Liquido, N.J., Puma, B.C., 1981. Effect of neem seed oil on rice leaffolder, *Cnaphalocrocis medinalis*. In: Proceedings of the Second International Neem Conference, Rottach-Engern, pp. 189–204.

SAS Institute, 2001. The SAS System for Windows, release 8.1. Cary, NC

Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35, 271–297.

Senthil-Nathan, S., Choi, M.Y., Paik, C.H., Seo, H.Y., Kim, J.D., 2009. Toxicity and behavioral effect of 3β, 24, 25-trihydroxycycloartane and beddomei lactone on the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). Ecotoxicol. Environ. Saf.

Senthil-Nathan, S., Choi, M.Y., Paik, C.H., Seo, H.Y., Kalaivani, K., Kim, J.D., 2008. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål). Ecotoxicol. Environ. Saf. 70, 244–250.

Senthil-Nathan, S., Choi, M.Y., Paik, C.H., Seo, H.Y., Kim, J.D., Kang, S.M., 2007. The toxic effects of neem extract and azadirachtin on the brown planthopper, *Nilaparvata lugens* (Stål) (BPH) (Homoptera: Delphacidae). Chemosphere 67, 80–88.

Senthil-Nathan, S., Chung, P.G., Murugan, K., 2006a. Combined effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocis medinalis* (Guenée) (Rice leaffolder) (Insecta: Lepidoptera: Pyralidae). Ecotoxicol. Environ. Saf. 64, 382–389.

Senthil-Nathan, S., Kalaivani, K., Murugan, K., 2006b. Effect of biopesticides on the lactate dehydrogenase (LDH) of the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) (Insecta: Lepidoptera: Pyralidae). Ecotoxicol. Environ. Saf. 65, 102–107.

Senthil-Nathan, S., Kalaivani, K., Murugan, K., Chung, P.G., 2005a. Efficacy of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) the rice leaffolder. Crop Prot. 24, 760–763.

Senthil-Nathan, S., Kalaivani, K., Murugan, K., Chung, G., 2005b. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leaffolder. Pestic. Biochem. Physiol. 81 (2), 113–122.

Sogawa, K., 1982. The rice brown planthopper: feeding physiology and host plant interactions. Annu. Rev. Entomol. 27, 49–73.

Smil, V., 2005. Feeding the world: how much more rice do we need? In: Toriyama, K., Heong, K.L., Hardy, B., Toriyama, K., Heong, K.L., Hardy, B. (Eds.), Rice is Life: Scientific Perspectives for the 21st Century. Proceedings of the World Rice Research Conference held in Tokyo and Tsukuba, Japan, 4–7 November 2004, pp. 21–23.

Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods, eighth ed. Iowa State University Press, Ames, IA.

Sutherland, J.P., Baharally, V., Permaul, D., 2002. Use of the botanical insecticide, neem to control the small rice stinkbug *Oebalus poecilus* (Dallas, 1851) (Hemiptera: Pentatomidae) in Guyana. Entomotropica 17, 97–101.

Sukumar, K., Perich, M.J., Boobar, L.R., 1991. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Assoc. 7, 210–237.

Tanaka, K., Endo, S., Kazano, H., 2000. Toxicity of insecticides to predators of rice planthoppers: spiders, the mirid bug and the dryinid wasp. Appl. Entomol. Zool. 35, 177–187.

Tuncer, C., Niazee, M.T.A., 1998. Acute and chronic effects of neem on *Myzocallis coryli* (Homoptera: Aphididae). Int. J. Pest Manage. 44, 53–58.

Velusamy, R., Kumar, M.G., Edward, Y.S.J.T., 1995. Mechanisms of resistance to the brown planthopper *Nilaparvata lugens* in wild rice (*Oryza* spp.) cultivars. Entomol. Exp. Appl. 74, 245–251.

Vinuela, E., Adan, A., Smagghe, G., Gonzalez, M., Medina, M.P., Budia, F., Vogt, H., Estal, P.D., 2000. Laboratory effects of ingestion of azadirachtin by two pests (*Ceratitis capitata* and *Spodoptera exigua*) and three natural enemies (Chrysoperla *carnea*, *Opius concolor* and *Podisus maculiventris*). Biocon. Sci. Tech. 10, 165–177.

Yoo, J.K., Park, H.M., Choi, B.R., Lee, S.G., Ahn, Y.J., 1997. Resistance monitoring and cross-resistance of brown planthopper (Homoptera: Delphacidae), *Nilaparvata Lugens* Stal in Korea. Korea. J. Pest. Sci. 1, 41–47.

Yoo, J.K., Lee, S.W., Ahn, Y.J., Nagata, T., Shono, T., 2002. Altered acetylcholinesterase as a resistance mechanism in the brown planthopper (Homoptera: Delphacidae), *Nilaparvata lugens* Stål. App. Entomol. Zool. 37, 37–41.