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The toxic effects of neem extract and azadirachtin on the brown planthopper, *Nilaparvata lugens* (Stål) (BPH) (Homoptera: Delphacidae)

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Abstract

Extracts of neem (*Azadirachta indica* A. Juss) are used in the developing world for many purposes including management of agricultural insect pests. The effects of different neem extracts (aqueous (NSKEaq), ethanol (NSKEeth) and hexane (NSKEhex)) on mortality, survival and weight of the brown planthopper, *Nilaparvata lugens* (Stål) (BPH) (Homoptera: Delphacidae) third and fourth nymphal instars were investigated. When fed rice plants treated with neem derivatives in bioassays, the survival of BPH nymphs is affected. Comparisons were made with the pure neem limonoid, azadirachtin (AZA) to ascertain its role as a compound responsible for these effects. AZA was most potent in all experiments and produced almost 100% nymphal mortality at 0.5 ppm and higher concentrations. When higher concentrations were applied, the effects appeared shortly after treatment and mortality was higher. Many insects died after remaining inactive for several days or during prolonged moulting. At lower concentrations, if moulting was achieved, disturbed growth and abnormalities were then likely to occur in the moulting process. Nymphs that were chronically exposed to neem extract showed a reduction in weight (45–60%). The results clearly indicate the simple NSKE (aqueous, ethanolic or both), containing low concentrations of AZA, can be used effectively to inhibit the growth and survival of BPH.

Keywords: Neem; Azadirachtin; *Nilaparvata*; Toxicity; Mortality; Survival; Weight

1. Introduction

Pollution of the environment by pesticides has been increasing due to their use to manage various pests. The organochlorines (OCs), including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), such as dichlorodiphenyl trichloroethane (DDT) and its metabolites and hexachlorocyclohexane (HCHs) isomers, are important environmental contaminants because they are toxic, bioaccumulative and widely distributed (Flaoyen et al., 2005). Also, the use of such pesticides does not satisfy the modern pest management and environmental considerations (Durmusoglu et al., 2003). Consequently, an intensive effort has been made to find alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable (Matthews, 1999). Also, botanical insecticides make existing integrated pest management programs (IPM) more effective and sustainable, while decreasing the reliance on synthetic insecticides (Ma et al., 2000; Zabel et al., 2002).

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The increasing amount of studies on plant-insect chemical interactions in the last few decades has unveiled the potential of utilising botanical insecticides in the form of secondary plant metabolites, or allelochemicals, as pest control agents. This interest in natural pesticides from the leaves or seed is providing an alternative in pest management programmes to the synthetic insecticides (Whitten, 1992; Martinez and van Emden, 2001).

Species of the plant families Meliaceae and Rutaceae have received much attention due to the fact that they are chemically characterized by triterpenes known as limonoids (Connolly, 1983). Many of these compounds have been demonstrated to affect insect growth and behavior, acting as antifeedants, toxins, and insect growth regulators (Champagne et al., 1992; Senthil Nathan et al., 2005a,b, 2006a,b).

Neem compounds, derived from *Azadirachta indica* A. Juss (Meliaceae), are the most promising phytochemicals from the entomological perspective (Schmutterer, 1990). The neem tree is native to the Indian subcontinent and grows in many countries of the world (Schmutterer, 1990; Ascher, 1993). During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. Limonoids are bitter tetranortriterpenes found predominantly in Meliaceae and Rutaceae (Champagne et al., 1992). It is generally believed that bioactivity of neem is due to the azadirachtin (complex limonoids) content in them (Butterworth and Morgan, 1971; Ley, 1994; Senthil Nathan et al., 2005a,b). Further research is needed on the chemistry of neem compounds, however.

Effects of these 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 effects on *Trissolcus basalis* Wollaston (Abudulai and Shepard, 2003).

Many previous studies have shown that extracts from the leaves and seeds of the neem tree have insecticidal activity against over 200 species of phytophagous insects from several orders, especially Lepidoptera and Orthoptera (Schmutterer, 1990). However, insufficient research has been done on hemipteran insect pests (Schmutterer, 1990; Durmusoglu et al., 2003).

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the most serious pests of rice, causing economic damage directly by feeding and producing symptoms commonly referred to as 'hopper burn' (Bae and Pathak, 1970; Shepard et al., 1991; Qiu et al., 2004). Unless management of this pest is executed properly from the early growth stage of the crop, this insect causes serious yield loss directly by feeding, and indirectly by transmission of the grassy stunt virus disease in the tropics (Saxena and Khan, 1985). The brown planthopper migrates to Korea every year from foreign breeding areas such as the mainland of China (Ma, 1993; Yoo et al., 2002). This insect undergoes two or three generations in paddy fields during the summer season in Korea (Yoo et al., 2002). Current strategies for the control of planthopper in Korea have relied heavily on pesticides, resulting in the development of resistance in BPH (Yoo et al., 2002). Also certain insecticides may stimulate BPH reproduction (Heinrichs et al., 1982; Reissig et al., 1982). It is not uncommon among progressive rice farmers of Asia to make four or five insecticide applications. Such usage, although aimed at one or two target pests, will usually have an adverse effect on other insects and on the ecosystem, including natural enemies. This may result in resurgence (Heinrichs et al., 1982) and development of insecticide resistance in this insect (Ahn et al., 1993; Yoo et al., 2002).

To avoid these problems, it is necessary to minimize the use of chemical insecticides for BPH control which should retard resistance development. Assuming that the control of BPH in the field by using neem is aimed at nymphs early in their development, bioassays utilising mid to late instars are poor indicators of efficacy of neem against early nymph or adult BPH (Saxena et al., 1984; Saxena and Khan, 1985). Bioassays using early instars may provide more practical predictions of efficacy in the field. We used third and fourth nymphal instars in the present study. Three different extracts of neem were compared with pure azadirachtin as to their effects on mortality and weight of *N. lugens*.

2. Materials and method

2.1. Laboratory mass culture of BPH

The susceptible strain of *N. lugens* has been maintained for more than 10 years in the laboratory of the Honam Agricultural Research Institute, Rural Development Administration (RDA), Iksan, South Korea without any exposure to insecticide. These insects were maintained on *Oryza sativa* L. seedlings (9–11 days after germination), in acrylic cages at 27 ± 1 °C, 40–60% RH, and a photoperiod of 16:8 (L:D) h.

2.2. Extraction of neem

Ripe neem fruits were collected locally (Erode District, Tamil Nadu, India) in August, 2005, depulped, and shade dried; dry seeds were stored at ambient temperature indoors until needed. For extract preparation, seeds were decorticated and the kernels were ground to a fine powder (NSK) in an electric grinder. The moisture content was 10%, three different neem seed kernel (NSKE) extracts, viz., aqueous (NSKEaqu), ethanolic (NSKEeth) and hexane (NSKEhex) extracts were used in this study. An aqueous suspension of NSKE was prepared by stirring 20 g of powdered NSK in a beaker containing 100 ml of distilled water for 3–4 h, followed by filtering through a