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Insecticidal Activity of Leaf Extracts of Neem (*Azadirachta indica* A. Juss.)

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ABSTRACT. *Neem, (Azadirachta indica A. Juss.), is a multipurpose tree which contains secondary metabolites with potent effects on insects. Even though its plant parts have been used by farmers for insect pest control since ancient times, the main interest of the scientists have focussed on the major active ingredient, pure azadirachtin, and the products derived from seeds. The production of seed in the tree is seasonal but other plant parts are available all round the year and have the potential for regular use.*

Extracts were derived using solvents with different polarities from leaves of a laboratory grown neem tree. The insecticidal activity of the extracts was assessed in terms of insect antifeedancy and growth regulation.

The metabolites were extracted using four solvents i.e. petroleum ether, acetone, methanol and water. Insect antifeedancy of the four extracts was assessed using 3–4 d old fifth instar nymphs of the desert locust, Schistocerca gregaria. Out of the four extracts tested, petroleum ether hardly had any antifeedancy. The other three fractions showed dose dependant antifeedant effects. Acetone fraction was the most active. The ED_{50} *of the three fractions, acetone, methanol and water were* 2×10^{-4}, 1.59×10^{-3} *and* 5.71×10^{-3} *mg* ml^{-1}, *respectively. Growth regulatory assays of acetone extract on large milkweed bug, Oncopeltus fasciatus, showed that the effects enhanced with increases in the dosage of the extract in a dose dependant manner.*

Insect growth regulatory effect together with the antifeedancy indicates the potential usefulness of these extracts in pest control.

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INTRODUCTION

The neem tree, *Azadirachta indica* A. Juss. is a member of the Mahogany family, *Meliaceae*. It is a fast growing tree of multipurpose use (Schmutterer, 1995). One of the important uses of neem is its use as a source of botanical pesticides. As far as its use as a source of insecticides is concerned the main interest of the scientists has focussed on the products derived from neem seed kernels and the pure compound azadirachtin. Although the tree begins fruiting in 3–5 years full productivity is not reached until 10 years of growth [Mordue (Luntz) *et al.*, 1995]. Further, it fruits generally only once a year. Other plant parts such as leaves are available all round the year from this evergreen tree and farmers in Asia have used these against insect pests for centuries (Duke and duCellier, 1993). Using many solvents, extracts derived from various parts such as roots, twigs and bark of the tree have been proven to be active against a number of species of insects (Schmutterer, 1995). Indiscriminate use of harmful pesticides has resulted in such problems as development of resistance and secondary pest outbreaks. Plant derivatives such as neem extracts would provide an alternative, environmentally sound, and safe pesticides for farmers in the tropics.

The study was designed to extract active metabolites from neem leaves using solvents with different polarities and to assess and compare their insecticidal activity in terms of insect antifeedancy and growth regulation.

MATERIALS AND METHODS

Plant material

Seeds obtained from a labelled tree in Dambulla were deshelled and kernels were surface sterilized by soaking in 10% Domestos (Uniliver, U.K.) for 1 min. Subsequently seeds were washed with sterile distilled water until the Domestos was completely removed. Six autoclaved filter papers (Whatman No. 1; 13.8 cm dia.) were laid in a Petri dish (13.8 cm dia.) and moistened with 0.1% benlate solution. The seeds were sown on filter papers (about 25 seeds per Petri dish). The Petri dish was wrapped in aluminium foil to prevent light from entering and incubated at 24–26°C. Observations were made for germination every day.

Seedlings obtained from germination were transferred to a Twyford box with an autoclaved mixture of sand:compost (50:50). The box was kept in a growth cabinet (Conviron, Canada). After growing for two weeks,

providing adequate moisture, seedlings were transferred to individual pots. The plants were grown for seven months until they were ready to be harvested.

Leaves were harvested after seven months. Harvested leaf material was thoroughly washed with water and kept at -70°C until freeze-drying. The material was finally dried in freeze drier. Freeze dried material was stored at -20°C until extraction.

Extraction

Freeze dried leaf material was ground to a fine powder using a pestle and mortar. Thereafter, 0.5 g material was weighed and transferred to a cellulose extraction thimble (Whatman, UK). This material was extracted with 75 ml petroleum ether (40–60°C) for 30 min in a Soxhlet apparatus. The extract was decanted from the flask and the remains in the thimble were air-dried. Subsequently, in the same thimble, extraction was continued using same volume of acetone and methanol, respectively. All three extracts were separately concentrated in the Soxhlet apparatus and dried in a vacuum concentrator (Savant Instruments Inc. U.S.A.). Finally, the remains were carefully taken out from the thimble and extracted by a cold water extraction method adapted from Morgan (pers. comm.) and Zounos (1994). Thus the material was homogenised in 12.5 ml distilled water in a MSE homogeniser for 1 min. This material was then centrifuged (4600 rpm, MSE Chilspin, UK) for 10 min to separate the meal and the water. The supernatant was decanted. Homogenisation and centrifugation were repeated 3 times using 12.5 ml water each time. The pooled extracts, including washings from tubes, *etc.*, were made up with distilled water to a final volume of 60 ml. The pellet was discarded. The pooled aqueous extract was evaporated to dryness in a rotary evaporator (Buchi, Switzerland) at a temperature not exceeding 35°C. The dried extract was re-suspended in 4 ml ethanol and where necessary an ultrasonic bath (Decon Ultrasonic Ltd, UK) was used to aid the solubility of extract. The samples were then evaporated to dryness to estimate the dry weight of the extracts in a Bijoux bottle in a vacuum oven (Gallenkamp, UK) at 35°C. The dried extracts were stored at -20°C (Electrolux, Sweden). The dried extract was taken up in 10 ml of the respective solvent to get 50 mg ml^{-1} concentration. Further dilutions were made for bioassays.

Bioassays

Antifeedancy

Choice bioassays were conducted to determine the antifeedancy of *Schistocerca gregaria* for each extract. The methods of Nasiruddin (1993) were followed and developed in the assays. Thus *S. gregaria* nymphs were reared on bran, barley seedlings and lettuce at 27°C at 16:8 light:dark regime. Fifth instar nymphs were selected from the cages and transferred to cylindrical jars (38 cm height × 20 cm dia.) until the insects were at the correct age for use. Three to four days after emergence, male and female fifth instar nymphs were used in the bioassays. Prior to the bioassay, the hunger of the insects was standardized firstly by depriving the insects of food for 16 h overnight and then allowing the insects to feed on lettuce for 1 h. Subsequently the insects were kept deprived of food for another 3 h. A choice bioassay was used whereby the insects were presented with 2 glass fibre discs (Whatman 3.7 cm dia.), a control disc and a treatment disc. The discs were pretreated with 350 µl of 50 mM sucrose as a phagostimulant and oven dried at 31°C. The discs were then impregnated with a further aliquot of appropriate extract or the carrier solvent (*i.e.* petroleum ether, acetone, methanol or water) and dried again. Control experiments were run whereby two discs with sucrose and the respective carrier solvent were used.

The bioassay was complete when approximately half of the control disc had been eaten, between 2½–4 h at 27°C, when each test insect was removed from the assay. The discs were air dried overnight. Weight of the discs before and after the assay was recorded using a fine balance (Mettler AE). The antifeedancy index (Blaney *et al.*, 1990; Nasiruddin, 1993) was calculated using the following equation:

$$\%\ antifeedancy = \frac{Weight\ of\ the\ control\ disc\ eaten - Weight\ of\ the\ test\ disc\ eaten}{Weight\ of\ the\ control\ disc\ eaten + Weight\ of\ the\ test\ desc\ eaten} \times 100\%$$

Probit transformation was carried out to get linear relationships between antifeedancy and the dosage in order to determine the ED_{50} values of the different fractions and compare the different fractions.

Insect growth regulation (IGR)

Large milkweed bugs (*Oncopeltus fasciatus*) were reared on dehusked sunflower seeds in the laboratory at 27°C and 16:8 light:dark

regime. Newly moulted 5th instar nymphs were used in the IGR assay as in the methods of Isman *et al.* (1990). Extract, dissolved in acetone, was applied to the abdominal dorsum of the nymph using a calibrated 10 µl Hamilton syringe. Acetone alone (for control) or 1 µl of the appropriate test solution was applied on insects. After the application, nymphs were kept in 10 cm diameter Petri dishes (10 nymphs per dish) and were provided with sunflower seeds and damp cotton wool. Moulting of the nymphs was monitored over a period of 10 days at which stage the assay was terminated.

RESULTS AND DISCUSSION

Antifeedancy

Concentrations ranging from 5×10^{-4} to 5 mg ml^{-1} were tested for antifeedancy of *S. gregaria* for all extracts. The antifeedancy values at each dose were plotted against the respective dose to build up the response curves for all extracts (Figure 1.). This shows that with the increase in the dosage of the extract, except for petroleum ether extract, the antifeedancy increases in a dose dependant manner.

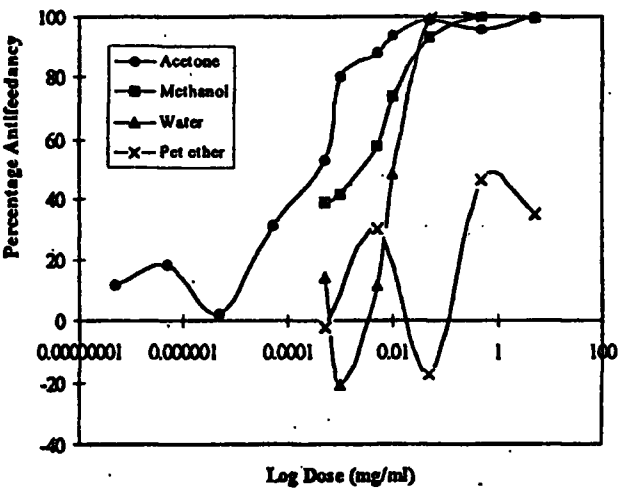

Figure 1. **Percentage Antifeedancy of *Schistocerca gregaria* fifth instar nymphs presented with glass fibre discs with leaf extract and sucrose (50 mM) or sucrose and respective solvent in a choice bioassay (n = 8–15).**

Petroleum ether extract did not show a good antifeedancy at any dosage. Out of the other three extracts tested, acetone extract had the lowest ED_{50} value (2×10^{-4} mg ml^{-1}) thus showing the best activity against *S. gregaria* (Figure 2).

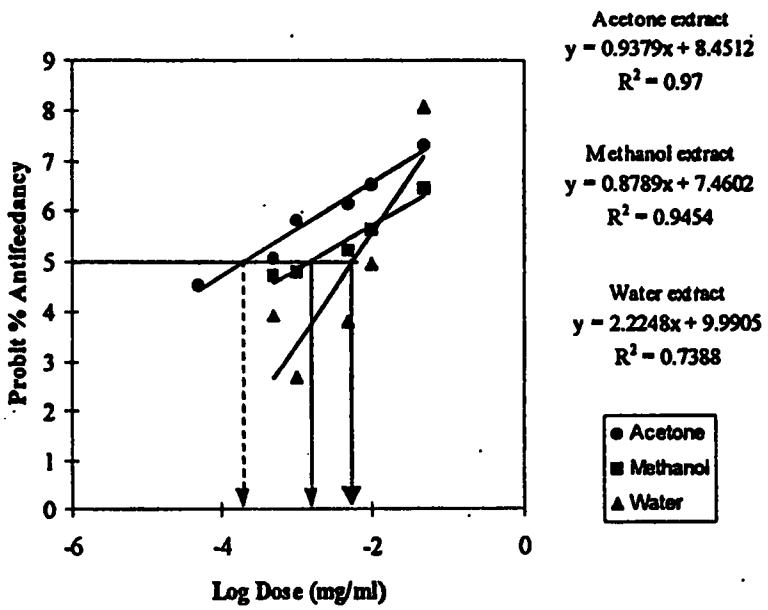

Figure 2. Probit %antifeedancy of *S. gregaria* fifth instar nymphs presented with glass fibre discs with leaf extract and sucrose (50 mM) or sucrose and respective solvent in a choice bioassay (n = 8–15).
[Note: ED_{50} acetone = 2×10^{-4} mg ml^{-1}; ED_{50} methanol = 1.59×10^{-3} mg ml^{-1}; ED_{50} water = 5.71×10^{-3} mg ml^{-1}].

Insect growth regulation (IGR)

Acetone leaf extract doses ranging from 0.5–50 µg μl^{-1} were selected for the IGR assay. The results of the IGR assay showed that the growth regulatory effects of the acetone extract enhanced with the increases in the dosage in a dose dependent manner (Figure 3). A range of abnormalities was observed.

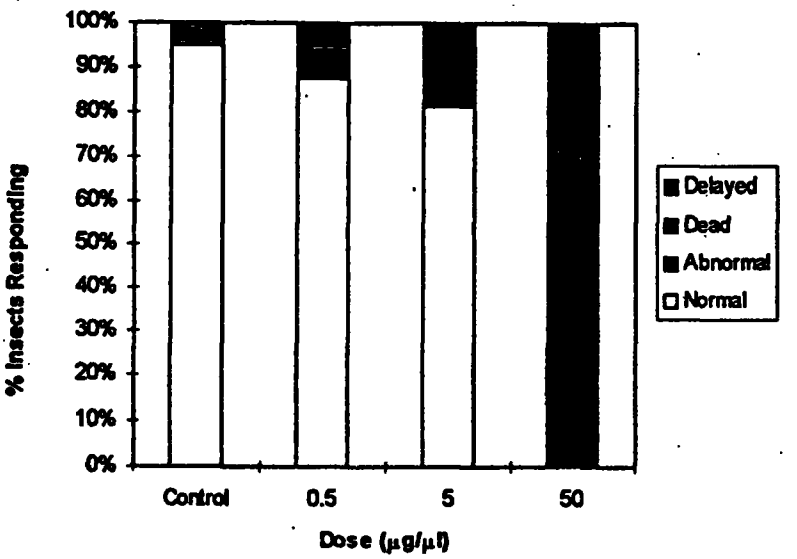

Figure 3. **The IGR effect of *Oncopeltus fasciatus* fifth instar nymphs treated with acetone leaf extract on day 1 of the fifth instar. n = 20–40/dose.**

The activity in the petroleum ether fraction was very low indicating the lack of any significant antifeedant compounds. The acetone fraction was the most active even at low doses (*e.g.* 5×10^{-4} mg ml^{-1}). Activity of extracts derived from neem against different insect species has been demonstrated by many authors. Ascher *et al.* (1989) showed that methanolic neem seed kernel extract was antifeedant against fourth instar nymphs of *Eyprepocnemis plorans*. Passerini and Hill (1993) reported that aqueous neem seed extract acted as an antifeedant against Sahelian grass hopper *Kraussaria angulifera* (Krauss). Application of aqueous neem seed extract resulted in growth regulatory effects which were dose dependant on African army worm *Spodoptera exempta* (Tanzubil and McCattery, 1990). These findings are in agreement with the results of the present experiments. The acetone extract, which showed the highest antifeedancy even at lower doses, was selected for the IGR assay. Absence of any normal moults at a lower dose such as 50 µg $µl^{-1}$ indicates the potential usefulness of this as an insect growth regulator. The IGR effect, together with antifeedancy shows the suitability of these extracts in pest control. Compounds having a mixture of effects on insects are

more suitable for pest control since it is difficult for the insects to develop resistance against such compounds.

Experiments should be continued to find the effectiveness of other plant parts of neem such as roots against insects. Even though the highest amount of active compounds (*i.e.* azadirachtin 2–4 mg g^{-1} seed kernel, National Research Council, 1992) are present in the seed kernel, which is the storage area for neem metabolites, other plant parts also contain active metabolites (Soon and Botrell, 1994). Further, the exact location of synthesis of these compounds is still entirely unknown. Neem is a tree which generally bears fruits only once a year and provides a limited annual supply of seeds, but other plant parts are available throughout the year. These may provide a valuable source of environmentally sound, safe pesticides for small scale farmers in developing countries such as Sri Lanka where the farmers themselves may grow neem in their gardens.

CONCLUSIONS

Neem leaves contain metabolites, which are effective against *S. gregaria* and *O. fasciatus*. Among the four solvents used for this experiment, acetone is the best solvent to extract antifeedant compounds from neem leaf material. Methanol and water extracts are also active. Petroleum ether extract is not active and hence is not suitable to extract antifeedant compounds from neem leaf material. Antifeedant effect of acetone, methanol and water extracts on *S. gregaria* is dose dependant. Growth regulatory effect of acetone extract on *O. fasciatus* is also dose dependant.

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