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Short communication

Toxic effects of neem-based insecticides on *Pieris brassicae* (Linn.)

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ABSTRACT

Four neem-based insecticides, Neemix® (0.25% EC @ 20 mg azadirachtin/liter), Ecozin® (3% EC @ 20 mg azadirachtin/liter), Agroneem® (0.15% EC @ 4.8 mg azadirachtin/liter) and Neem oil (0.25% EC azadirachtin @ 20 mg azadirachtin/liter) and a non-commercial neem leaf powder, were evaluated for oviposition deterrence, antifeedant effect to larvae and toxicity to eggs and larvae of *Pieris brassicae* (Linn.) on cabbage leaves in the laboratory. The concentrations tested were with in the ranges of recommended field rates. Oviposition deterrence in no-choice, two-choice and six-choice assays, was observed for all the treatments. They exhibited significant ($P < 0.01$) oviposition deterrence on *P. brassicae* when compared with a non-treated control. Cabbage leaves treated with the neem-based insecticides were used as an egg-laying substrate. Numbers of eggs oviposited by *P. brassicae* adults on treated cabbage leaves were significantly lower then those treated with water, but no significant differences were detected among the neem insecticides. They also deterred feeding by *Pieris* larvae and exhibited significant antifeedant effects. Larvae of *P. brassicae* on treated leaves stopped feeding and dropped from the leaf, resulting in no or minimal damage. Direct contact with neem-based insecticides decreased the survival of eggs. Survival of larvae fed for 9 days on leaves treated with neem-based insecticides was reduced to 51%, 49%, 48%, 24% and 18% in the Neem oil, Neemix, Agroneem, Ecozin and neem leaf powder treatments, respectively. Therefore, it can be concluded from the present investigations that neem-based insecticides had oviposition deterrence, antifeedant and toxic effect to *P. brassicae*.

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1. Introduction

Cabbage (*Brassica oleracea* L. var. *capitata*) is a major vegetable produced and consumed in India. In 2007, cabbage accounted for 7.3%, of India's total vegetable production and India was the second largest producer worldwide (FAO, 2009). Cabbage and cauliflower are considerable economic importance, and are often produced under smallholder conditions (Weinberger and Srinivasan, 2009). Over the years, they have been cultivated more intensively, which has resulted in high pest infestation (Srinivasan and Murthy, 1991; Chaudhuri et al., 2001; Hasan and Ansari, 2010a,b). About 38 insect pests of cole crops are reported. Among the plethora of insect pests, cabbage white butterfly, *Pieris brassicae* (Linn.), is one of the most destructive causing damage at all the growth stages including seedling, vegetative growth and flowering (Lal and Ram, 2004; Younas et al., 2004; Hasan, 2008; Hasan and Ansari, 2010a, b). It is an oligophagous pest with a wide host range and is known to infest 83 species of food plants belonging to the Cruciferae (Hasan and Ansari, 2010a). It has a Palearctic distribution from North Africa across Europe and from Asia to the HimalayanMountains (Jainulabdeen and Prasad, 2004). In India, it passes winter in the plains and migrates to hilly regions during summer. The young caterpillars feed gregariously on leaves, resulting in defoliation of the plants (Younas et al., 2004; Jainulabdeen and Prasad, 2004; Hasan, 2008; Hasan and Ansari, 2010b). On cabbage and cauliflower, the caterpillar sometime bores into the heads and is most destructive. During development, single larvae of *P. brassicae* consume 74–80 cm^2 of leaf area (Younas et al., 2004). Insecticide application against the larval stage is the primary method of control of *P. brassicae*, but high tolerance to most insecticides and associated environmental problems may jeopardize their continued use (Grisakova et al., 2006). Long-term use of broad-spectrum pesticides may result in outbreaks of pests by destruction of their natural enemies (Hossain and Poehling, 2006). These drawbacks of synthetic pesticides have increased consumers' and growers' interest in natural insecticides originating from plants and their usage has increased in recent years. Therefore, control programs should rely to the maximum extent possible on alternative tactics that prevent the development of population outbreaks or reduce the cost of management.

Botanical products are useful and desirable tools in most pest management programs because they can be effective and often

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complement the actions of natural enemies, breakdown rapidly in the environment, have low human toxicity and have a lower risk of selection of resistant pest biotypes (Schmutterer, 1990, 1995; Liang et al., 2003; Greenberg et al., 2005). Neem [*Azadirachta indica* A. Juss (Meliaceae)]-based insecticides containing azadirachtin have been reported to control >400 species of insects, including important pests, such as *P. brassicae*, *Plutella xylostella* L., *Spodoptera* spp., leafminers, aphids, and whiteflies (Schmutterer, 1990; Isman, 1999; Walter, 1999; Pineda et al., 2009). The compounds from neem have a number of properties useful for insect pest management (Isman, 2006). These include toxicity, repellence, feeding and oviposition deterrence, insect growth regulator activity, etc. (Schmutterer 1990; Koul, 2004; Mordue (Luntz), 2004; Isman, 2006). The key insecticidal ingredient found in the neem tree is azadirachtin, a steroid like tetranortriterpenoid, responsible for both antifeedant and toxic effects in insects (Mordue (Luntz) and Nisbet, 2000). Higher doses are toxic and larvae may be killed by direct contact with the spray. Neem possesses the ability to function at hormonal concentrations and produce ecdysone-type effects in susceptible insects. (Ascher and Meisner, 1989; Schmutterer, 1990; Mordue (Luntz) and Blackwell, 1993; Govindachari et al., 2004). In addition to controlling pests, some neem-based insecticides have negligible effects on beneficial insects and low environmental impacts (Schmutterer, 1995; Haseeb et al., 2004; Greenberg et al., 2005; Isman, 2006). This research addresses the need for finding effective options for managing the cruciferous insect pest *P. brassicae* in India in the face of the declining popularity of conventional chemical insecticides.

The aims of the current investigations were to determine the feeding and oviposition deterrency and lethal effects on eggs and larvae of commercial formulation of neem-based insecticides on of *P. brassicae*.

2. Materials and methods

2.1. Experimental conditions

Cabbage (cv. Golden Acre) was raised in the winter season of year 2007–2008 at experimental field of the Department of Plant Protection, Faculty of Agricultural Sciences, AligarhMuslim University, Aligarh, India. The seedlings of cabbage were sown in plots size 3 × 3 m. For feeding and oviposition trials, we used fully expanded leaves excised from the plant of 60–65 day-old. Experiments were conducted in the laboratory at 26 ± 2 °C, 60 ± 5% RH, and the photoperiod was 12:12 (L:D) h. *P. brassicae* adults, and first, second, third, fourth and fifth instars were obtained from a colony maintained in the laboratory. Four commercial neem-based insecticides were used: Agroneem® (0.15% azadirachtin), Ecozin® (3% azadirachtin), Neemix® (0.25% azadirachtin), Neem oil (0.25% azadirachtin); Ajay Bio-Tech, Pune, India. The concentrations of these four materials used in this study were with in the ranges of recommended field rates, Agroneem at 4.8 mg azadirachtin/liter, Ecozin at 20 mg azadirachtin/liter, Neemix at 20 mg azadirachtin/liter and Neem oil at 20 mg azadirachtin/liter. The dilution of each insecticide was prepared separately by slowly adding the material to a 500 mL glass beaker filled with an appropriate amount of water while stirring consistently on a magnetic plate. A neem leaf powder, supplied from the Parijat Agrochemicals, India, was diluted at 3% concentration (by mass) by mixing with water and stirring for 30 min at room temperature. Deionized water was used as control in all experiments. All neem dilutions were used on the same day they were prepared.

2.2. Oviposition preference

To determine the effect of the neem-based insecticides on oviposition preference, all five formulations and a non-treated control were used in no-choice, and two- and six-choice assays. Cabbage leaves were excised near the axil with a razor blade and was immediately placed in a plastic container (12 cm long and 6 cm diameter) with water and covered with a plastic lid with in the center. In the no-choice assay, five pairs of leaves were sprayed on both leaf surfaces with a plastic handheld sprayer until runoff, each pair with one neem-based insecticide, and a sixth pair of non-treated leaves served as a control. After air-drying for 1 h, each pair of leaves was placed in a ventilated cage (50 cm diameter × 1m tall). Pairs of newly emerged male and female *P. brassicae* were confined in each cage for 5 days. Adults were supplied with a cotton ball soaked with a 10% sucrose-water solution in a small cup, replenished daily. Numbers of clusters and eggs in each cluster laid on the plants and on the walls of each cage were counted daily. Each treatment was replicated four times. In the two-choice assays, all possible paired combinations of Agroneem, Ecozin, Neemix, neem leaf powder, Neem oil and the water control were assayed. Two excised leaves in water containers, each with a different treatment, were placed in a plastic cage. *P. brassicae* pairs, introduction, feeding, and oviposition monitoring were the same as described for the no-choice assay. Each treatment combination was replicated eight times. In the six-choice assay, each of five excised leaves in a water container was sprayed with a different neem-based insecticide, and a sixth non-treated leaf served as the control. *P. brassicae* were exposed to all six leaves in a cage as previously described. The proportions of eggs deposited on the plant surface to the total number of eggs laid in each container were calculated. Each treatment had 4 replicates.

2.3. Feeding preference

In the feeding preference experiment, the area of each cabbage leaf was individually measured using an LI-3100 Area Meter (LICOR, Lincoln, NB). The leaves were then individually dipped in a neem-based insecticide. After air-drying for 1 h, each leaf was placed in a separate 14.5 cm diameter Petri dish. One first, second, third, fourth and fifth instar larva was placed at the center of each leaf and allowed to feed for 24 h. Each treatment for each instar was replicated 25 times. A clear plastic area measuring meter (TXL-TAES, Weslaco, TX) was used to measure the leaf area removed or eaten by each *P. brassicae* larva. The proportion of leaf area eaten to the total leaf area was calculated.

2.4. Direct contact

In a further requirement, *P. brassicae* eggs and neonate larvae were used to determine the effect of direct contact of neem-based insecticides. For direct contact on eggs, <24- hours-old eggs, on cabbage leaves were sprayed with the neem-based insecticides, using a plastic handheld sprayer until runoff. Non-treated eggs were used as a control. After air-drying for 1 h, the leaves were placed in separate Petri dishes. Each of the 10 treatment replicates had 100 - 120 eggs. Hatched larvae were counted daily until all eggs eclosed or died. To determine the direct contact effect on larvae, one neonate larva was placed on a treated or control cabbage leaf in a Petri dish, and survival was determined after 3, 6 and 9 days. Each treatment and the non-treated control were replicated four times.

2.5. Statistical analysis

Cumulative oviposition data were analyzed using one-way ANOVA, two-by-two table test and Yates's corrected chi-square, and Kruskal–Wallis one-way ANOVA for the no-choice, and two- and six-choice assays, respectively. One-way ANOVA was used to

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