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Effects of biopesticide Neem EC on the Large White Butterfly, *Pieris brassicae* L. (Lepidoptera, Pieridae)

M. Grišakova, L. Metspalu, K. Jõgar, K. Hiiesaar, A. Kuusik and P. Põldma

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi St. 64, 51014 Tartu, Estonia; e-mail: luule.metspalu@emu.ee

Abstract. The effects of Neem EC (M/S RYM Exports – The Indian Neem Tree Company, 1% azadirachtin) were assessed on the Large White Butterfly, *Pieris brassicae* – a major pest of cruciferous plants. Duration of the larval stage, mortality of larvae and prepupae, and weight of pupae were studied. The time needed for completion of the larval stages by individuals fed on treated cabbage increased significantly, compared with the control: 16–37 days in the test variant, versus 11–18 days in the control. Neem EC also induced high mortality, caused by lethal failures of larval-larval and larval-pupal ecdysis, which were typical for insecticides possessing morphogenetic activity commonly referred to as IGR-activity. The mortality of larvae and prepupae in the test variant was significantly higher than in the control. Considerably fewer pupae were gained in the test variant than in the control variant. The pupae of larvae that had been feeding on the control were significantly heavier than those of the larvae feeding on the treated plants. The experiment revealed that Neem EC had both toxic and antifeedant/deterrent effects but also acted as a growth regulator for *P. brassicae* larvae.

Key words: biopesticide**,** Neem EC, large white butterfly, *Pieris brassicae larvae*

INTRODUCTION

During the past six decades, chemical preparations have dominated in pest control. This has brought about the pollution of the environment, danger to humans, and developing resistance against toxicants of over 500 species of insects and mites (Thomas, 1999), forcing scientists to develop new types of pesticides. Secondary plant compounds have been the subject of thorough investigation for the past 30 years. Extracts from the neem tree (*Azadirachta indica* Juss.) have emerged as an excellent alternative to synthetic insecticides for the management of insect pests. The compounds from neem have a number of properties useful for insect pest management. These include toxicity, repellence, feeding and oviposition deterrence, insect growth regulator activity, etc. (Schmutterer, 1990; Koul, 2004; Mordue (Luntz), 2004). 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) & 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 ecdyson-type effects in susceptible insects. (Ascher & Meisner 1989; Schmutterer, 1990; Mordue (Luntz) & Blackwell, 1993; Govindachari, et al., 2004). Different insect species showed varying degrees of sensitivity to various

neem extracts and components. For example, Naumann and Isman (1995) found that neem seed oil extract had little or no effect on three species of noctuid moths: *Trichoplusia ni, Peidroma saucia,* and *Spodoptera litura.* According to Turcani (2001), cabbage leaves sprayed with neem seed kernel suspension reduced the damage by *Pieris brassicae* larvae but failed to give any significant protection to castor plant leaves against the hairy caterpillar, *Amasacta albistriga*. Thus, it is important to study the effect of a certain preparation on a certain pest.

This research addresses the need for finding effective options for managing the cruciferous insect pest *P. brassicae* in Estonia in the face of the declining popularity of conventional chemical insecticides.

The aim of the current investigation was to study the effects of Neem EC, widely used in several countries around the world today (Prajapti, http://www.neemuses.com), on the larvae of *P. brassicae* via food.

MATERIAL AND METHODS

The egg clutches of the *P. brassicae* were collected from the fields near Tartu, Estonia and incubated in Petri dishes, in L12:D12 at 21 ± 2°C and r.h. 75 ± 10%. The larvae hatched from one and the same egg clutch were used for both control and test as one replication. Larvae were fed with fresh cabbage leaves and reared in 1 l. glass jars.

For our experiments, we used Neem EC obtained from India (M/S RYM Exports – The Indian Neem Tree Company). Neem EC belongs to the category of medium- to broad-spectrum pesticides and it functions by intervening at several stages of the life of an insect. The preparation of Neem EC (1% azadirachtin) was diluted with distilled water to 100 ppm. Leaf-dip experiments were carried out. Freshly cut leaves of cabbage were dipped in the solution (test) or distilled water (control) for 10 s, and dried for 30 minutes on filter paper sleeves. Newly moulted 3rd instar larvae were allowed to feed on neem-treated leaves for 48 h and then fed with untreated leaves. Six replicates of larvae were used (n = 250). The following criteria were studied: duration of the larval stage, mortality of larvae and prepupae and the number of living pupae. The results were checked every day until death or pupation. Pupae were weighed on the third day after pupation to 0.1 mg on an analytic balance.

Data were analysed by ANOVA, the means were compared by the Tukey HSD test, and *t* - test was applied, at a significance level of $P < 0.05$.

RESULTS

The results showed that the 3rd larval stage lengthened by a couple of days in the treated variant, compared with the control. In the test variant, larvae started to die beginning on the fourth day of the experiment, when moulting to the 4th growth stage began. Larvae of the control were already at the 4th growth stage by that time. During moulting, about half of the test variant larvae perished. They ate less than those of the control variant and the duration of growth stages extended in comparison with the control. Larvae perished every day in the test variant starting from the 4th growth stage, however, the death rate increased again during moulting into the 5th growth stage and at the prepupal stage.

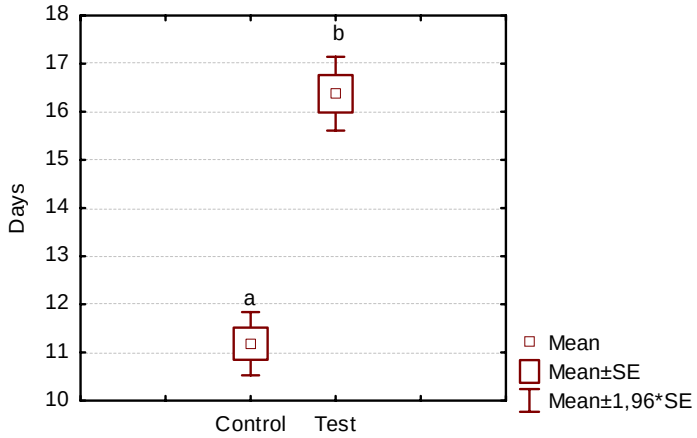

Fig. 1. Duration of the larval stage of *P. brassicae* after treatment with Neem EC. Mean values denoted with different letters are significantly different ($P < 0.05$, Tukey HSD test).

In the control variant, the mean time from the beginning of the experiment to the formation of pupae (Fig. 1) was 11.18 days, whereas in the treated variant it was 16.37 days (two sample *t*-test; t = -10.07 df = 74, $P = 0.000$). The majority (95%) of the test variant larvae and prepupae perished during the observation period, whereas in the control 12.1% of the individuals perished. The mortality of larvae and prepupae in the test variant was significantly higher than in the control variant (t-test, $P=0.000$). Substantially fewer pupae were gained in the test variant than in the control; the difference proved statistically significant (*t*-test, $P = 0.003$) (Fig. 2).

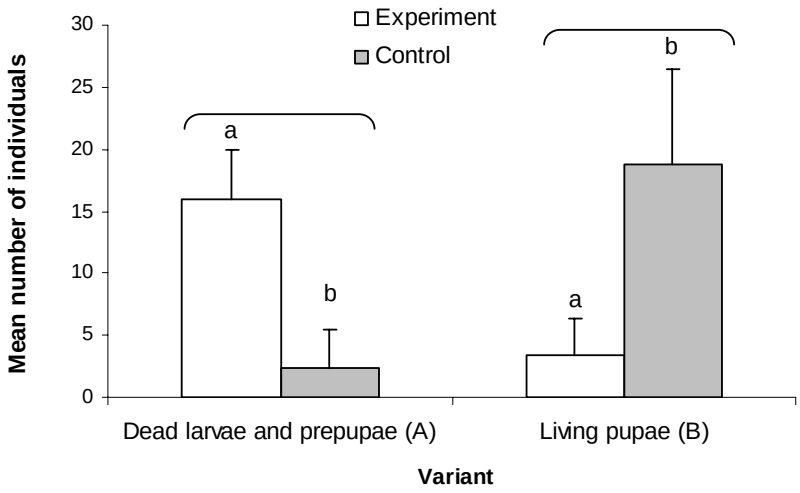

Fig. 2. Mean number of larvae and prepupae perished in the test period (A) and living pupae (B) of *P. brassicae* in the control and test variants. Standard deviations are graphically shown. Bars denoted with different letters (each horizontal bar) are significantly different ($P < 0.05$, *t*-test).

The body mass of newly formed pupae was recorded. Results showed that control pupae were significantly heavier than test pupae ($F_{1,22}$ = 62,32, P = 0.000, Fig. 3). The average weight was 276 ± 18.5 mg in the control and 203 ± 24.4 mg in the test. Body mass values ranged from 169–232 mg, test and 248–303 mg, control.

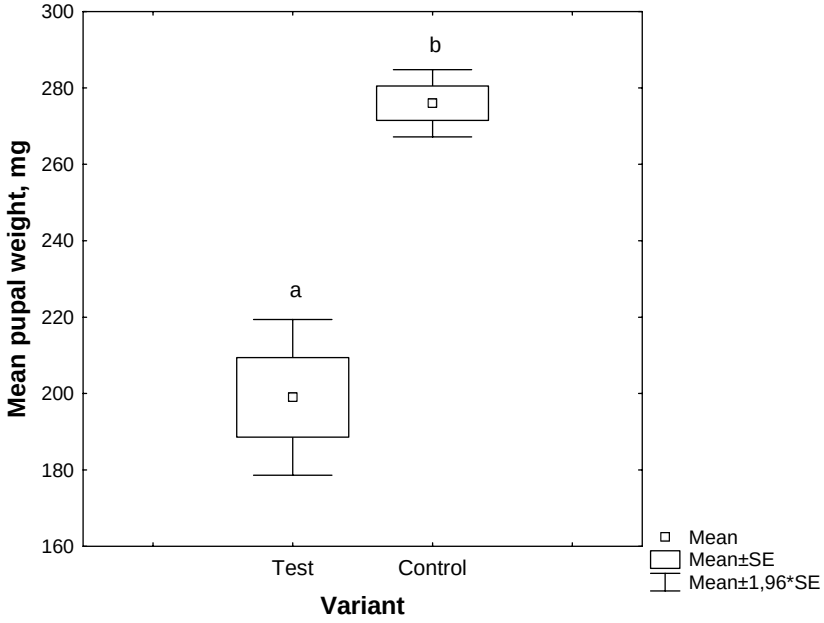

Fig. 3. Average body mass of *P. brassicae* pupae in the test and control variants. Different letters indicate statistically significant differences (P < 0.05, Tukey HSD test).

DISCUSSION

The results showed that Neem EC was effective against *P. brassicae*, significantly reducing the survival of larvae feeding on cabbage leaves treated with the extract. In our tests, the extract did not produce rapid mortality. No larvae perished within the first 3 days of the experiment. Alternatively to the direct toxic effect, the antifeedant action was proved. The neem tree contains over 140 different limonoids in its different tissues (Isman et al., 1996; Koul, 2004), many of which are biologically active against insects as antifeedants (Jacobson, 1987; Mordue (Luntz), 2004). Inhibition of feeding behaviour by azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells or both (Mordue (Luntz) & Blackwell, 1993). Schmutterer (1990) and Ascher et al. (1992) defined primary and secondary antifeedant effects of azadirachtin. The primary effects include the process of chemoreception by the organism (e.g. sensory organs on mouthparts that stimulate the organism to begin feeding) whereas the secondary processes are effects such as gut motility disorders. Our experiment showed that Neem EC caused a delay in the feeding of *P. brassicae* larvae. The larvae only began taking sample pieces from the treated food on the second day: the feeding rate was considerably smaller compared with the

control. Yoshida and Toscano (1994) found that the relative consumption rate of *Heliothis virescens* larvae treated with azadirachtin was 25% of the control. The mortality of larvae in our experiment began on the fourth day. Experiments by Luik and Viidalepp (2001) showed that when larvae of *P. brassicae* were fed with cabbage treated with Neem-Azal T/S (1% azdirachtin A), all larvae perished during 7 days, in the case of 0.1% water emulsion. A characteristic of our experiment was thatafter the larvae were transferred to untreated feed, their feeding there was also restrained. Larvae of the treated variant delayed both in growth and development in comparison with the control. Neem seed extract prolonged larval development, and induced larval mortality of *Bemisia tabaci* on cotton foliage (Coudriet et al., 1985). Many authors have found that neem has properties influencing the growth and development of insects. A pre-condition for moulting is inactivation of *corpora allata*. It can occur only when the insect has reached a certain minimum mass threshold of the growth stage. Metamorphosis requires synchrony of many hormones and other physiological changes in order to be successful. Schmutterer (1990) suggested that azadirachtin modifies the programme of insects by influencing the hormonal systems, especially that of ecdysone and juvenile hormone. It is thought that neem extract components block the release of neurosecretory substance from the brain, which controls the release of the hormones from their endocrine glands (Mordue (Luntz), Blackwell, 1993; Mordue (Luntz), et al., 1996; Mordue (Luntz), 2004).

In our experiment, larvae perished directly before moulting or in the process of moulting. Usually larvae were unable to get rid of the old integument. Also characteristic was the occurrence of defects in moulting into the 4th instar, the following 5th instar and during pupation. These observations, especially the nature of the moulting defects, suggest effects on the neuroendocrine system of the *P. brassicae*. From there it may be concluded that a juvenile hormone level higher than normal persisted in the organism, allowing the growth but inhibiting metamorphosis. It is also known that neem may act as an exogeneous juvenile hormone or its analogue (Schmutterer, 1987). However, it is possible that, as a result of intoxication, histopathological changes may occur in the *corpora cardiaca* of the insect, causing disorders in secretion of hormones. Azadirchatin has also shown direct detrimental and histopatological effects on most insect tissues, e.g., muscles, body fat , and gut epithelial cells (Mordue (Linz) & Blackwell, 1993).

A significant ($P < 0.05$) loss of pupal mass was also observed, presumably due to reduced feeding on neem-treated cabbage. Pupae of the test variant were not viable; they all died within three weeks following pupation, whereas there was no mortality among the control variant pupae. The experiment revealed that Neem EC had both toxic and antifeedant/deterrent effects but also acted as a growth regulator for *P.brassicae* larvae. The tests will be repeated to obtain more detailed information on the impact of Neem EC. The initial tests showed that Neem EC is capable of reducing the number of *P. brassicae* in gardens.

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