

# DIVERSE EFFECTS OF NEEMAZAL-T/S REVEALED BY PREIMAGINAL STAGES OF COLORADO POTATO BEETLES, *LEPTINOTARSA DECEMLINEATA* SAY

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## Abstract

*NeemAzal-T/S (1% Azadirachtin A, Trifolio-M) (NAZ) was tested against immature stages of Colorado potato beetles (CPB), *Leptinotarsa decemlineata* Say. In the eggs treated with 0,1% water dilution of NeemAzal-T/S the embryonic development was almost complete but 47% of the larvae were not able to hatch. From the eggs treated with 0,5% NeemAzal-T/S only 24% of the larvae hatched, the others perished inside the egg shell.*

*The topical application of larvae showed that the 0,1µl dose of 0,5% and 0,1% was mortal for all the 2-day-old larvae of the first instar. 4<sup>th</sup> instar larvae, topically treated with 1µl 0,1% and 0,5% NeemAzal-T/S showed a diversity of effects: direct toxic action, the prolonged development of larvae and failures in morphogenesis due to interfering in hormonal regulation.*

*Leaves treated with 1% water dilution of NeemAzal-T/S showed potent antifeedant properties on the 4<sup>th</sup> instar larvae which consumed only 12% of leaf surface.*

## Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, became a potato pest insect in Estonia during the last twenty years and the damages on potato plants increased every year. The mechanical control by gathering the beetles on the potato fields has since been the main acceptable method of control. However, this method is realisable only on small plots. Natural means are of primary interest for organic growers as control measures against CPB.

The demand for effective biological control of the CPB also exists because of acquired resistancies against synthetic insecticides of several types.

There exists a series of promising data for the practical use of Azadirachtins in control of CPB (Schmutterer, 1987; Zehnder and Warten, 1988; Otto, 1996). By these data the potent effects, revealed by the preparations made from neem oil, are due to their antifeedant properties and physiological modes of action (Schmutterer, 1984, 1987; Saxena, 1987; Kleeberg, 1997; Mordue (Luntz) 1998).

The aim of the present paper was to evaluate NeemAzal-T/S in laboratory tests against preimaginal stages of CPB.

## **Materials and methods**

The Colorado potato beetles (CPB), *Leptinotarsa decemlineata*, were collected in the vicinity of Tartu (Estonia). Further the laboratory rearing continued at 23-25°C in long day conditions (18:6) on the potato plants grown indoors.

We used 0.1%, 0.5% and 1% of water emulsions of NeemAzal-T/S (1% Azadirachtin A, Trifolio-M GmbH) (NAZ) for the treatments. All tests were made in five replications.

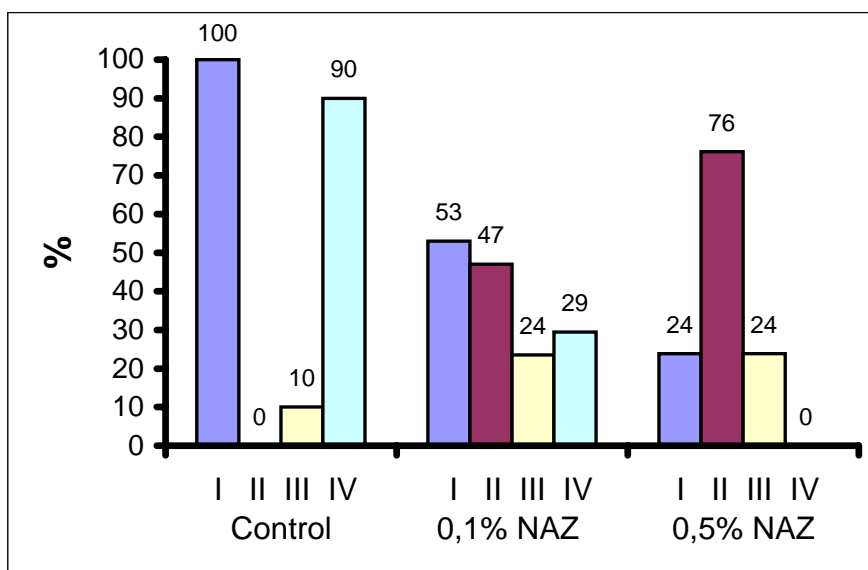
## **Results**

### ***Topical application on eggs***

In the control, i.e. untreated egg clutches, the larvae hatched jointly almost at the same time. In the eggs treated with 0.1% NeemAzal-T/S the embryonic development was almost ended but nearly half (47%) of the larvae were not able to hatch from the egg shell. From the eggs treated with 0.1% NeemAzal-T/S only 24% of the larvae hatched, the others perished inside the egg shell (Fig. 1). The hatched larvae did not eat the egg shells as it normally occurs. These egg shells were obviously not edible due to the repellent residue of NeemAzal-T/S.

The larvae hatched from a clutch showed an affected behaviour and dispersed on the plant instead of feeding on the first day on the leaves they hatched. The lower and higher concentrations resulted in 24% mortality during moulting into the second instar (Fig.1). The larval development of survived individuals lasted 5-6 days longer than normal.

In case of eggs treated with 0.5% NeemAzal-T/S 76% of larvae perished during the hatching period. The hatched larvae died during the first and second instar.



**Fig. 1:** The results of topical application of egg clutches of Colorado potato beetles with NeemAzal-T/S in 0.1% and 0.5% water emulsions. Columns: I- the percentage of eggs from which normal larvae hatched; II- part of the larvae perished during hatching; III- the percentage of hatched larvae perished during later development; IV- percentage of larvae developed into adults.

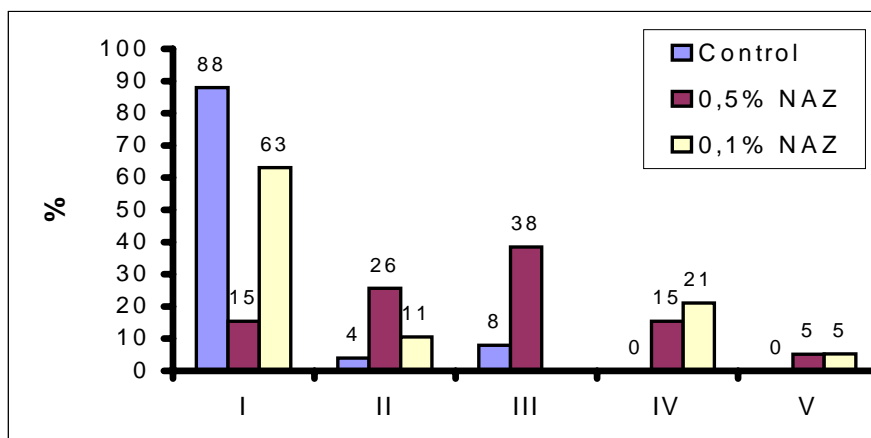
### **Topical application of larvae**

The 0,1 µl dose of 0,5% and 0,1% NeemAzal-T/S was mortal for all the 2-day old larvae of the 1<sup>st</sup> instar. Mortality of these larvae was regarded as a direct toxic action of NeemAzal-T/S.

The last 4<sup>th</sup> instar larvae, topically treated with 1 µl 0.1% and 0.5% NeemAzal-T/S, showed a diversity of effects. In Fig. 2 the percentage of individuals perished as larvae, prepupae, intermediates or as the adults with deformed elytra, is summarized. A part of the individuals (26%) died as larvae, but most of the individuals (38%) died in prepupal stage, if 0.5% NeemAzal-T/S was used. In case larvae were treated with a weaker concentration (0.1%) larval mortality was only 11%, but many adults (21%) emerged with deformed elytra. The treatments with both 0.5% and 0.1% NeemAzal-T/S resulted in 5% intermediates (Fig.2), and 15% and 63% of externally normal adults emerged, respectively.

### **Antifeedant effects**

The leaves treated with NeemAzal-T/S showed clear antifeedant properties. On the leaves submerged in 1% NeemAzal-T/S the 4<sup>th</sup> instar larvae consumed only 12% of the leaf surface while 0.5% NeemAzal-T/S resulted in consuming 30% of leaf surface. These lower and higher NeemAzal-T/S concentrations caused 13.3% and 27% larval mortality respectively. The survived larvae developed mostly into lethal intermediates. Only 6% (1% NeemAzal-T/S) and 16.6% (0.5% NeemAzal-T/S) externally normal adults emerged from the survived larvae.



**Fig. 2.** The results of topical application of NeemAzal-T/S 0,1% and 0,5% water dilutions of the 4<sup>th</sup> instar larvae of the Colorado potato beetles. Column groups: I- normal adults emerged; II- perished during larval development; III- perished at the prepupal stage; IV- adults with deformed elytra; V- intermediates

## Discussion

Our results about the treatments with NeemAzal-T/S show a diversity of biological effects. NeemAzal-T/S acts by direct toxicity on the first larval instar, the antifeedant mode of action appears in all larval instars, and in addition we suggest the morphogenetic action in several development stages.

The ability of azadirachtin to function at hormonal concentrations and to produce ecdysone-type effects in susceptible insects had led to the idea that it in fact acts as an ecdysteroid (reviewed by Addor, 1995). According to Schmutterer (1990) the Azadirachtins modify the morphogenetic programs of insects by interfering in hormonal regulation.

According to Mordue (1997) and Mordue and Blackwell (1993) the azadirachtins cause a blockade in the release of neurosecretorial hormones from the brain. The disruption of these morphogenetic hormones result in typical insect growth regulating effects i.e. slowing of growth, delayed moulting, moult abnormalities, while in adults it results in reduced fecundity and sterility (Mordue, 1998). For NeemAzal-T/S a diversity of biologic effects revealed by CBP was characteristic. Externally these effects were similar to those we obtained in tests with juvenoids or juvenile hormone synthetic analogues or juvenoids (Kuusik and Kogerman, 1978).

However, in our test the application of NeemAzal-T/S on eggs and larvae showed the typical effects known from treatments with juvenile hormone analogues i.e. the inability of larvae to hatch from egg shells and the mortality of larvae soon after the hatching (see Slama et al., 1974; Sehnal and Skuhavy, 1976). These effects may be caused by malfunctioning of internal organs due to morphogenetic failures. By Haasler (1984) the neem seed extract disturbed the balance of hormone titres, maintaining a high juvenile hormone titre during the metamorphosis in *Manduca sexta*, the tobacco hornworm.

It is clear that the control of CPB by Neem must be based on the summary of all the direct (i.e. antifeedancy) and indirect effects (via endocrine system ) of this natural insecticide. Our next step would be the field trials of NeemAzal-T/S against Estonian CPB populations.

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