

THE EFFECT OF NEEMAZAL-T/S ON THE MORTALITY OF MITE *TETRANYCHUS URTICAE* KOCH AND SOME INSECTS - *APHIS GOSSYPII* GLOV. AND *THRIPS TABACI* LIND.

DIE WIRKUNG VON NEEMAZAL-T/S AUF DIE STERBLICHKEIT DER MILBE *TETRANYCHUS URTICAE* KOCH UND EINIGER INSEKTEN - *APHIS GOSSYPII* GLOV. UND *THRIPS TABACI* LIND.

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Abstract

*Die Wirkung des Pflanzenschutzmittels NeemAzal-T/S (1% Azadirachtin) in den Konzentrationen 0,1% und 0,5% wurde im Labor auf Eiern, Erstlarven und diapausierenden Adulte der Milbe *T. urticae* festgestellt. Alle Milbenstadien wurden topikal mit 1 µl des Mittels behandelt. Bei der Blattlaus *A. gossypii* wurden die jungen Larven mit NeemAzal-T/S übergossen. Die Thripslarven und Adulten wurden topikal mit 1 µl des Mittels behandelt. Die Entwicklungsstörungen und Sterblichkeit aller Versuchsobjekte wurden während eine Woche ermittelt.*

Die Bearbeitung mit der höheren Konzentration (0,5%) des Mittels hat bei der Milbe hohe Sterblichkeit hervorgerufen. So verdarben 85,9% der Eier. In einigen Eiern hatte sich die Embryonalentwicklung fortgesetzt, die Larven konnten jedoch nicht ausschlüpfen. Im Vergleich zu den unbehandelten Eiern der Kontrolle schlüpften die Larven der am Leben gebliebenen Eier 1-2 Tage später. Durch die Behandlung mit dem 0,1%igen Mittel konnten sich 24,6% der Eier nicht weiterentwickeln; 15% der Larven starben ab. Mit dem 0,5% Mittel wurden 70,4% der Larven getötet. Von diapausierenden Adulten starben 19,4% respektive 33%.

Bei den Blattläusen wurde die Sterblichkeit am dritten Tag nach der Behandlung ausgewertet. Am siebenten Tag waren die geschädigten und toten Larven gelb gefärbt. Die Larvensterblichkeit 31,4% bzw. 74,1% korrelierten entsprechend zu den verwendeten Konzentrationen (0,1 und 0,5%).

Bei den Thripsen hat die Behandlung mit dem 0,1%igen Mittel 40,2% und mit dem 0,5%igen Mittel 89,9% der Individuen abgetötet. Die Sterblichkeit war schon am zweiten Tag nach der Behandlung festzustellen.

Bei allen Versuchstieren hat die Anwendung des höher konzentrierten Mittels schnellere und höhere Sterblichkeit verursacht.

1 Introduction

The neem tree, *Azadirachta indica*, is widely spread in tropical regions, has proved to be one of the most universal sources of natural pesticides for a great number of insect and mite pests. So far, the seed kernel extract of neem, known as Azadirachtin, has been most thoroughly tested, and it has been extracted in bigger quantities than other components of neem (Schmutterer et al. 1981; Schmutterer & Zebitz 1984; Ascher 1995). Azadirachtin has been tested for the control of mites, nematodes and the insects of different orders in different regions of the world (Jacobson 1987, Parmnal 1987). The wide spectrum of action of the preparation has been established. In numerous studies the repellent, antifeedant and deterrent properties of Azadirachtin, but also its effects on female fecundity and neuroendocrine system, are shown (Ruscoe 1972; Schmutterer et al. 1981; Saxena 1987; Schmutterer 1987; Addor 1995) Since Azadirachtin has often caused morphogenetic effects, it has been first of all characterised as the insect growth regulator (IGR). In literature low toxicity of Azadirachtin, especially in the case of moderate doses, is emphasised, but at the same time the fact that insects perished on the following day after the treatment is evident (Saxena et al. 1984; 1984a). From the point of view of plant protection, it is not very important through which mechanisms the action of the neem preparation is revealed if it leads to a visible decrease in the number of pests. From the practical aspect the direct quick killing effect is often essential. The aim of the present paper was to examine the toxic effect of a NeemAzal-T/S (1% Azadirachtin, produced by Trifolio-M GmbH) on three of the most widely spread pests in our greenhouses *Tetranychus urticae* (Acari, Tetranychidae), *Aphis gossypii* (Homoptera, Aphididae) and *Thrips tabaci* (Thysanoptera, Thripidae).

2 Materials and methods

The toxicity of 0.5% and 0.1% water emulsions of NeemAzal-T/S was studied at different developmental stages of two spotted spider mite (*T. urticae*), in the larvae of aphids *A. gossypii* and in the second instar larvae and the adults of tobacco thrips *T. tabaci*. The pests were gained from highly infested cucumber plants grown in a greenhouse. The experiments were carried out at the temperature of $24 \pm 1^\circ\text{C}$ and in natural light conditions. In the tests the methods of toxic studies described by Helle & Overmeer (1985) were used, according to which the efficiency of preparations is estimated by the direct mortality of the individuals. For test objects cucumber leaf sectors were used as nutrient substrates. Sectors were cut out of the leaves so that veins would remain on the edges of the cuttings to avoid the flowing of water on the leaf and its wetting. The leaf sectors were placed to float on water in petri dishes. The dishes were left uncovered to avoid too high air humidity. Floating on water, the leaf stays fresh and the pests have no possibility to leave it. Water was changed and the dishes were disinfected every day, in that way the leaves remained green until the end of the experiment. The method of topical application was mainly used for treatment of insects. A micro-syringe was exploited for dosing of NeemAzal-T/S water emulsion. All these operations were made under stereo-microscope at a magnification of 16x. All the experiments were made in five replications. The number of treated individuals (N) is marked in figures 1 and 2. For the statistic evaluation of differences in the results between tests variants Chi² test was used.

3 Results

Tetranychus urticae. To obtain eggs of a certain age, five adult females were placed on each leaf disc and left there for 24 hours. The deposited eggs were counted and each of them was treated with 1 μ l of the emulsion. From the moment of their treatment, the eggs were observed under a microscope in daily intervals during one week. From untreated control eggs larvae hatched on 4-5th day after oviposition and 14.5% of eggs perished.

Thrips tabaci. Tobacco thrips lays its eggs into plant tissue or between the petals and sepals of flowers which makes it difficult to study them. That is why only the postembryonic stages of development, second instar larvae and the adults of unknown ages were used in the experiments. To replace the larvae and the adults of thrips on new leaves, they had to be administered a mild narcosis. The cucumber leaves with thrips were submerged into water for a minute and then the wet individuals were placed on a clean and dry piece of leaf. The treatment was not started until the thrips had completely recovered and become active again. Each individual was treated dorsally with 1-2 μ l of emulsion. Mortality was determined on the 5th day of the experiment.

The death rate was 89.9% when 0.5% emulsion was used (Fig.2) . There was possible to identify death cases already on the 2nd or 3rd day because they did not move any more. In the treatment with 0.1% emulsion, 40.2% of the individuals died, but in the control the percentage of mortality was only 3.7%. In the 5th day of the observation the dead individuals had turned brown, haemolymph had spread out of their bodies and they had stuck to the leaf. The death rate comparison between the different treatments and their comparison with the control variant showed significant differences ($p < 0.01$).

4 Discussion

In the favourable conditions of greenhouse the generation of mites, aphids and thrips lasts less than two weeks. To save the infested plants, the pest-control is almost always necessary. Under the pressure of permanent pesticides' treatments the resistance to synthetical pesticides can develop in pests quickly. This is one, but not the only reason why the alternative means of plant protection should be searched for. The direct and indirect effect of Azadirachtin on mites has been studied by Mansour et al. (1987) and they have discovered that low concentrations are not dangerous to predators, but at the same time they reduce the fecundity of harmful mites. Consequently, Azadirachtin could be joined into the system of integrated pest control. In our tests with different developmental stages of two spotted mite, the most susceptible ones to Azadirachtin turned out to be eggs, while some of the eggs had completed their embryonic development, but their larvae died while hatching. Schauer & Schmutterer (1981) have also found a strong toxic action on the eggs of red spider mite treated with the extract of neem. In some cases no larvae hatched at all and in other cases larvae hatched but had no vitality, they did not feed and died. The authors consider starving to be one of the possible causes of their dying. The concentration of 0.5% proved to be toxic enough for larvae as well, but the larvae who survived, moulted two days later than in the control. The prolonged time between moulting has been explained with the reduction of ecdyson in haemolymph (Rembold et al 1987). Schmutterer (1984) does not exclude the possibility that there may be an indirect effect on moulting and metamorphosis were due to the reduced

consumption of food, larvae do not reach a weight necessary for inactivating *corpora allata*.

Schauer (1984) has thoroughly studied the action of neem extracts on aphids but using topically applied moderate doses, she did not manage to attain toxic effects. When the larvae of aphid fed on the leaves treated with Azadirachtin, they died during moulting. They could not free themselves from their old exuviae, although morphological changes were not perceived.

In the tests with 0.5% NeemAzal-T/S emulsion 85.5% of the eggs were killed. By the last day of the observation the colour of perished eggs had turned light brown and their shells were wrinkled. A part of the eggs had passed their embryonic development, but the larvae died, being unable to emerge from their shells.

In the treatment of eggs with 0.1% NeemAzal-T/S emulsion their death rate remained low, reaching only 24.6% and showing insignificant difference with the control ($p=0.9$). Nevertheless, there was a significant difference between compared concentrations ($p<0.01$).

The first instar larvae of the spider mite were used in the tests. They were placed on the parts of cucumber leaves with the help of a needle and treated topically with 1 μ l of emulsion. Mortality was determined on the 5th day after the treatment. Larvae were considered to be dead when they did not respond to touch and their colour faded. The toxic effect of NeemAzal-T/S on mite larvae depended on the concentration. The treatment with 0.5% and 0.1% emulsions killed 70.4% and 15.1% of larvae respectively. Statistically significant mortality was caused only by 0.5 % water emulsion of NeemAzal-T/S ($p<0,01$).

The red coloured pre-diapausing females as adults were used in the experiment. Each specimen was treated with 1-2 μ l of the emulsion. Mortality was determined on the 7th day. Treatment with 0.5% NeemAzal-T/S emulsion killed 33% of adults. The mortality of control adults and of sample treated with 0.1% emulsion was 5.9% and 19.4% respectively. There were no significant differences in the mortality rate between the control variant and the variants of different concentrations of NeemAzal-T/S . The low sensitivity of the pre-diapausing adults could be explained by the physiological changes which take place in the organism. In diapausing organisms the metabolism rate is low and therefore it could be less sensitive to unfavourable environmental factors.

Aphis gossypii. Two female individuals were placed on each leaf cutting and left there for 48 hours. Then the adults were removed and larvae were counted and treated with NeemAzal-T/S water emulsions. As the larvae of aphids are covered with a hard wax layer they were overflowed with the emulsion for 2 minutes. In the control, distilled water was used for submerging the larvae. When two minutes had passed, the rest of the liquid was removed using filter paper and a syringe, but a certain amount of the emulsion remained on the surface of the leaf. The estimation of mortality was made in daily intervals during one week. The ultimate death rate was estimated on the 7th day. During the first two days all the larvae were active when both 0.1% and 0.5% emulsions were used and there were no differences noticed as compared with the control. The leaves did not show any signs of phytotoxicity either but the concentrations of Azadirachtin that are higher than 2% may have phytotoxic effects on leaves. On the 3rd and 4th day it was difficult to establish whether the larvae were alive or dead, there were no external changes to be seen, but their reaction to the

touch was only slightly perceivable. On the 5th day the death among the larvae was identified as they did not respond to the touch anymore. The experiment was finished on the 7th day. At this time cuticle of larvae had already become wrinkled and turned brown. The control larvae had already moulted in the 7th day. The mortality of the larvae was in correlation with the concentration of the used emulsion. 0.50% and 0.1% emulsions resulted in 74.1% and 31.4% mortality respectively (Fig.2). In the control variant, only 7.8% of larvae perished, showing a significant difference from the treated samples. Toxicosis and rapid killing is only one effect of Azadirachtin on the aphid *A. gossypii*.

5 References

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