Effect of Two NeemAzal™ Formulations on Honeybees under Semi-Field Conditions

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Abstract

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The effects of NeemAzal™ formulations: NeemAzal™ T/S (1% azadirachtin) and NeemAzal™ granules (1% azadirachtin) on honeybees, *Apis mellifera* L., were studied under semi-field conditions. Three plots at 15 m^2 each were sown with spring rape seeds *Brassica napus* cultivar Likolly (*Brassicaceae*/*Cruciferae*). In the first plot NeemAzal granules were added with the seeds during sowing. The second plot was sprayed with NeemAzal T/S during full flowering; Greemax™ was used as a wetting agent. The third one was sprayed with water only during full flowering as a control. For each treatment one tunnel tent (3 × 5 × 2 m) was used during the flowering period. Small bee colonies were exposed to the treated plants for 7 days. Evaluation was carried out by comparing the results in the treatments to the control and, furthermore, by comparing the pre- and post- application. The mortality in the tunnels and the flight activity were checked before, as well as after the treatment. The development of the bee brood was evaluated by using transparent acetate sheets to mark single cells in brood combs with their contents on different assessment dates. The time schedule of the assessment dates was chosen in order to check the bee brood at different expected stages during the development. The development of the bee brood was evaluated by calculation of brood termination rates in percentage and brood indices. The results show that residues of NeemAzal granules did not adversely affect bee mortality, foraging activity or brood development. By contrast, it was noticed that NeemAzal T/S caused some reduction in foraging activity and brood development.

Keywords: *Apis mellifera*; NeemAzal™; Greemax™; mortality; foraging activity; brood development

Chemical control of pests results in environmental pollution and serious side effects to humans, domestic animals and also to natural enemies. This situation dictates the need for safe and less expensive materials for pest control such as the use of certain plant extracts and components against insect pests.

Neem, *Azadirachta indica* A. Juss (*Meliaceae*), a biopesticidal tree grown widely in Africa and Asia, is used for controlling agricultural pests. Many chemical compounds in the neem tree such as azadirachtin, salanin, meliantriol, nimbidin and thionimone have been purified and tested for their ability to control

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diseases and pests of crops. Although these natural non-toxic, non-polluting pesticides are hard on aphids, white fly, mealy bugs and other pest insects, they are soft on honeybees, butterflies and other beneficial insects (Liu 1995a, b).

Azadirachtin is the major component responsible for neem's insect repellency, feeding deterrence, oviposition deterrence, disruption of growth and development, and suppression of reproduction (Schmutterer 1990; Coats 1994). Unlike other natural insecticides, such as rotenone and nicotine, azadirachtin is relatively non-toxic to mammals. Neither oral doses of 2000 mg/kg nor intraperitoneal injections of 1000 mg/kg induced demonstrable effects on rats (Coats 1994). Negative effects occur in humans only at high doses (Lai *et al*. 1990). Tests on non-target arthropods and fish have indicated excellent selectivity. Residue analyses show that azadirachtin is short-lived in the environment, especially in sunlight (Schmutterer 1990). However, azadirachtin was found to have spermicidal effects in many tested mammalian systems (Upadhyay *et al*. 1993; Garg *et al*. 1994), a potential side effect that might affect drones in a honeybee colony.

The effects of neem on honeybees and other beneficial insects are dose-dependent. At higher doses neem may not be completely safe to honeybees. However, it is known that neem pesticides degrade very fast, often within a few weeks. Unlike pyrethroid pesticides, neem residues are not expected to accumulate in the bee hives nor to have long-term effects on honeybees (Liu 1995a, b). Azadirachtin has also been reported to have little effect on forager honeybees (Schmutterer & Holst 1987; Naumann *et al*. 1994), worker bees (Melathopoulos *et al*. 2001a, b), and brood in the combs (Rembold & Czoppelt 1981; Naumann & Isman 1996). Toxicity and effects of residues of neem extract on the Asian honeybee, *Apis cerana* F. and the small honeybee, *Apis florea* F., have been investigated (Boonthal 1994).

The aim of this study is to examine the effect of two NeemAzal formulations applied on spring rape (*Brassica napus* L.). on honey bees *Apis mellifera* L. under semi-field conditions.

MATERIALS AND METHODS

Based on the recent OEPP/ EPPO No. 170 Guideline 2001, the "Arbeitsgemeinschaft Bienenschutz 2003" developed a new test method in the semi-field to evaluate side-effects of plant protection products on honeybees. Using this method a semi-field trial was carried out at the Bee Research Institute in Libčice nad Vltavou near Prague during 2004.

Test design. Three plots each (15 m²) were sown with seeds of spring rape *Brassica napus* cultivar Likolly on 1st April, 2004. For each treatment one tunnel tent (3 × 5 × 2 m) was used during the flowering stage of rape. The exposure period in the tents lasted for approximately 3 days before the treatment and for further 7 day after the application. During the exposure period polyethylene sheets were placed on the ground between the rows in the tents.

The application of NeemAzal. Two formulations of NeemAzal – NeemAzal granules (1% azadirachtin) and NeemAzal T/S (1% azadirachtin) were used. On the first plot, NeemAzal granules were added to the seeds during sowing at the rate of 77.0 g/15 m² (twice the recommended field rate). The second one was sprayed with NeemAzal T/S during the full flowering stage at the rate of 1.5 ml/15m² with a hand-held sprayer (1 l capacity). The application rate was 1 l of the product in 500 l water per hectare. Greemax was used as a wetting agent. The third plot was sprayed with water only during full flowering, as a control.

Test colonies. Honey bee nuclei were produced at the same time with sister queens, with each nucleus consisting of 2 brood combs, 1 food comb, and approximately 3000 worker bees. The nuclei (the "hives") were introduced into the tents 2 days before the planned application.

Evaluation of mortality. Mortality in the tunnel was monitored by daily counts of dead bees collected from the polyethylene sheets placed on the ground between the rows. After the exposure period in the tents the mortality in front of the hives was recorded for further 2 weeks outside the tents. The number of dead bees recorded during assessments was separated into numbers of dead adults and pupae. The assessment was carried out early in the morning to avoid the loss of dead adult and pupae due to e.g. the cleaning behaviour of the worker bees, and to predators such as wasps or birds.

Evaluation of flight activity. At each assessment time the number of bees foraging on the flowering rape in the tunnel was counted. The observations of the flight activity were carried out according to the scheme shown in Table 1.

Development of the bee brood. The assessment of the development of the bee brood in individual marked brood cells was carried out by using acetate

Table 1. Evaluation of flight activity (number of bees/cage)

Time of the test	Evaluation of flight activity
Three days before spraying	Twice a day during flight activity of the bees
Day of spraying	Shortly before spraying 3 times in the first hour after spraying 2 h after spraying 4 h after spraying 6 h after spraying
The folowing day after spraying	Twice during flight activity of the bees
During exposure period in tents	Once a day during flight activity of the bees

sheets. At the assessment before the application (Brood Area Fixing Day = BFD) a brood comb was taken out of each colony to mark areas with at least 100 cells containing eggs. The exact location of each cell and its content was marked on the acetate sheet. The sheet was attached with needles to the wooden frame and its position on the frame was marked. This allowed placing the sheet exactly in the same position on each of the following observation dates (Schur *et al*. 2003). The application in the tents was performed 2 days after BFD. Table 2 shows the time schedule of the brood assessment dates.

Brood index. The assessed contents of single cells in the brood combs were sorted out into six categories for further calculations:

Category 0 – termination of development,
Category 1 – egg stage,
Category 2 – young larvae (L1–L2),
Category 3 – old larvae (L3–L5),
Category 4 – pupal stage (capped cells),
Category 5 – empty after the hatch.

The values of all cells in each treatment, assayed on the same date, were summed up and divided by the number of observed cells in order to obtain the brood-index (Schur *et al*. 2003).

Brood termination rate. For the calculations of the brood termination rate in the percentage of the observed cells, the data were divided into 2 categories:

1 – Bee brood in the cell reached the expected brood stage at the different assessment dates and was empty, or containing egg, after the emergence of the adult bee on BFD+22 was evaluated as a successful development.
2 – If at one of the assessment dates the expected brood stage was not reached or food was stored in the cell during BFD +5 to +16, it was evaluated as termination of the bee brood development.

Afterwards, one mean value was calculated per colony and treatment (Schur *et al*. 2003).

RESULTS AND DISCUSSION

Effect of two NeemAzal formulations on honeybees mortality under semi-field conditions

The daily mortality values of honeybee workers in a tent test with spring rape treated with two NeemAzal formulations are reported in Table 3. The data showed that after spraying with NeemAzal

Table 2. Assessment of the development of the bee brood

Assessment date	Determined brood stage in marked cells
Brood area fixing day	egg
Assessment date	expected brood stage in marked cells
+ 5 days (± 1 day) after BFD	young to old larvae
+ 10 days (± 1 day) after BFD	capped cells (pupae)
+ 16 days (± 1 day) after BFD	capped cells shortly before emergence
+ 22 days (± 1 day) after BFD	empty cells or eggs/young larvae containing cells

T/S the number of dead bees increased and this increasing continued till 4 days after the application. The number of dead bees returned to normal on the 5th day after spraying. Honeybee mortality was affected less by NeemAzal granules than by NeemAzal T/S. The control and the NeemAzal granules treatments showed approximately the same number of dead adults during the exposure period in the tents.

The average number of dead bees per day after the application is obtained and divided by the average of dead bees per day before the application (Table 3). If only natural mortality occurs, the number of dead bees per day does not change very much and the index Q_M is close to 1. If the test substance induces an increased mortality, then the index exceeds 1. When the index applied to the control and both indices are compared by a simple mathematical division we come to a clearing index I_M for mortality. This illustrates the deviation of the test substance from the untreated control (Schmidt *et al.* 2003).

$$Q_M = \frac{\text{average number of dead bees per day after application (5 days)}}{\text{average number of dead bees per day before application}}$$

$I_M = Q_M/K_M$ (K_M like $Q_{M,}$ but for control)

The total numbers of dead adult bees and dead pupae before and after the treatment are summarised in Table 4. In case of NeemAzal T/S treatment, an increased number of dead adults and pupae was noticed. Mortality of pupae occurred in the NeemAzal T/S treatment around 2 weeks after the spraying (Figure 1). The control and the NeemAzal granules treatments showed approximately the same number of dead adults and pupae.

Our results are in agreement with those of Schur *et al.* (2003), who found that in all trials of the active fenoxycarb substance (Insegar 25 WG), which is known as an IGR, an increased number of dead pupae was noticed. The pupal mortality occurred approximately 14 days after the application and was at a different level during the trial.

Table 3. Effect of two NeemAzal formulations on the mortality of honey bee workers under semi-field conditions

Day	No. of dead bees/cage		
	Untreated	NeemAzal T/S	NeemAzal granules
–3	4	3	5
–2	3	4	3
–1	3	5	5
0	4	3	4
+ 2 h	0	2	0
+ 1	4	9	3
+ 2	5	7	5
+ 3	3	8	4
+ 4	4	9	4
+ 5	2	6	5
+ 6	4	5	2
+ 7	3	5	2
+ 8	2	4	0
+ 9	2	3	3
Total of 5 days after spraying	18	41	21
After spraying/day	3.0	6.8	3.5
Total before spraying	14	15	17
Before spraying/day	3.5	3.75	4.25
Index after/before	0.86	1.8	0.82
Clearing index substance/untreated		2.09	0.95

Table 4. Mortality during the experimental phase of the trials in the treatments

Treatment	Σ dead bees during the observation period before spraying		Σ dead bees during the observation period after spraying	
	adult bee	pupae	adult bee	pupae
Control	14	0	52	4
NeemAzal T/S	15	3	95	14
NeemAzal granules	17	1	48	5

Also, Mann and Dhaliwal (2001) evaluated the safety of NeemAzal (azadirachtin, at 10 000 ppm) to *A. mellifera* foragers at different dosages (1% at 200, 400 and 800 ppm). The data showed that NeemAzal used at the highest dosage was safe to honeybees, with 7.58% mortality in direct toxicity tests and 0.74% mortality when the bees were caged in cotton field after spraying. However, in the foliage bioassay, it caused 17.19% mortality of foragers. Thapa and Wongsiri (1997) found that there were no significant differences in mortality between the control treatment and both azadirachtin-A (Neemix) and azadirachtin-B (Advantage).

The safety of neem products to honeybees under the field conditions has also been reported by several authors, including Abrol and Kumar (2000a), Mann and Dhaliwal (2001), Kumar and Babu (1996), Abrol and Andorta (2000) and Allam *et al*. (2003).

Effect of two NeemAzal formulations on honeybees foraging activity under semi-field conditions

Foraging activity of honeybee workers in a tent test with spring rape treated with two NeemAzal formulations is reported in Table 5. Data showed that after spraying with NeemAzal T/S the number of bees foraging on the flowers decreased and this decreasing continued till 2 days after the application. Honeybee visits returned to normal on the 3rd day after spraying. Honeybee foraging was affected less by NeemAzal granules than by NeemAzal T/S.

We assessed the bees activity several times before spraying and calculated an average from all assessments of the days before the spraying. The assessments of the several days after application have been used for the calculation of the average of the activity after application. We have divided

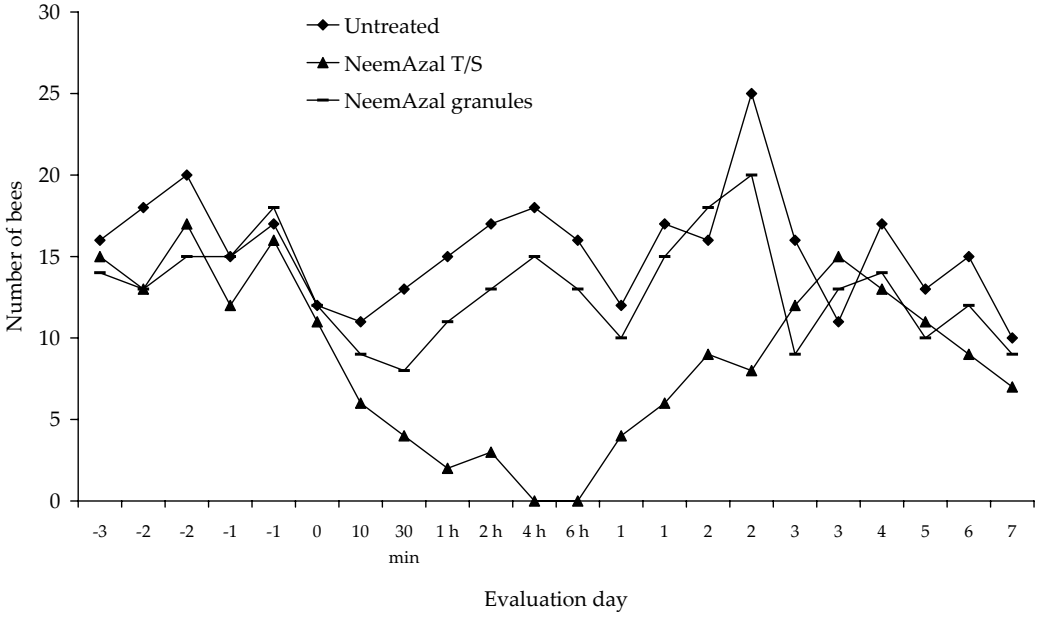

Figure 1. Number of dead pupae counted in front of the beehive of two NeemAzal formulations during the observation period

Table 5. Effect of two NeemAzal formulations on the foraging activity of honeybee workers in a tent test

Day	No. of foraging bees/cage		
	untreated	NeemAzal T/S	NeemAzal granules
–3	16	15	14
–2	18	13	13
–2	20	17	15
–1	15	12	15
–1	17	16	18
0	12	11	12
10 min	11	6	9
30 min	13	4	8
1 h	15	2	11
2 h	17	3	13
4 h	18	0	15
6 h	16	0	13
+ 1	12	4	10
+ 1	17	6	15
+ 2	16	9	18
+ 2	25	8	20
+ 3	16	12	9
+ 3	11	15	13
+ 4	17	13	14
+ 5	13	11	10
+ 6	15	9	12
+ 7	10	7	9
Total of 3 days after spraying	187	69	154
After spraying/day (12 evaluation)	15.58	5.75	12.83
Total before spraying	98	84	87
Before spraying/day (6 evaluation)	16.33	14.0	14.5
Index after/before	0.95	0.41	0.88
Clearing index substance/untreated		0.43	0.93

the average after spraying by the average before spraying.

If the index is close to 1, then the spray product does not affect the foraging activity. If the index is 0.5 or even less, avoidance of the treated crop by the bees can be expected. It is necessary to compare the index of the test substance with the index of the untreated control by a simple division. This is again a clearing index I_f. It indicates by which factor the test substance differs from the control. Schmidt *et al*. (2003) proposed as a tolerance limit the range between 0.5 and 2, which they consider as the normal reaction of the bees. If the clearing index calculates beyond this range, it suggests a possible effect.

$$Q_f = \frac{\text{average number of bees per square and per evaluation after application (3 days)}}{\text{average number of bees per square and per evaluation before application}}$$

$I_f = Q_f / K_f$ (K_f like Q_f but for control)

Our results are in agreement with the results of Sithanantham *et al.* (1997) who found that the day after plots of *Vigna unguiculata* were sprayed with 5, 10 or 20% neem seed kernel extract, fewer honey bees visited the flowers than those on the control plots. Honey bee visits returned to normal on days 3–5, except on the plots sprayed with the highest dose. Furthermore, Thapa and Wongsiri (1997) found that foraging activity of bees declined for 1–1.5 h immediately after the application of azadirachtin-A and azadirachtin-B. Malaipan *et al.* (1992), found that the numbers of honeybees (*Apis mellifera*) present on flowers of pummelos in open plots and in cages without any insecticide applications, were twice as high as for those with spray applications of neem extract.

On the other hand, Sontakke and Dash (1996), discovered that neem products did not have a significant effect on the foraging rate of honeybees (*Apis mellifera*) on mustard flowers under field conditions. Similarly, Naumann *et al.* (1994) reported that field applications of azadirachtin at 150 ppm on canola did not repel foraging honeybees. However, they commented that formulated azadirachtin at 0.1 ppm in sugar syrup changed the bees' preference to untreated syrup in a feeding-dish choice bioassay.

Azadirachtin has also been reported to have little effect on forager honeybees by several other authors (Schmutterer & Holst 1987; Naumann *et al.* 1994).

Effect of two NeemAzal formulations on the development of bee brood under semi-field conditions

Development of the bee brood in a tent test with spring rape treated with two NeemAzal formulations is reported in Table 6. The control and NeemAzal granules treatments showed increasing brood indices from BFD to BFD +16 but, by contrast, NeemAzal T/S treatment showed decreasing brood indices from BFD to BFD +16. Especially in the NeemAzal T/S treatment, the expected brood index on the assessment dates following the treatment was not reached. In the NeemAzal T/S treatment a low effect with a decreased brood-index was observed during the entire test periods. However, in the NeemAzal granules treatment no effect was observed.

The termination rates were 38, 31 and 65% in the control, NeemAzal granules and NeemAzal T/S treatments, respectively.

Our results are in accordance with those of Schur *et al.* (2003) who found that in trials of the fenoxycarb active substance (Insegar 25 WG), which is known as an IGR, the termination rate of brood ranged from 94% to 100%. In the control treatment a wide range in the termination-rate was observed in the trials (8–43%). They suggested that the increased brood termination in the control treatment in single trials could be explained by weather conditions.

Azadirachtin is known to affect insects primarily in their immature stages and has been reported to disturb larval and prepupal development, cause higher larval mortality, and reduce weight gain (Sharma *et al.* 1980; Rembold & Czoppelt 1981).

In the tests carried out by Peng *et al.* (2000), worker larvae were more sensitive to azadirachtin than adult worker bees, exhibiting an LC_{50} of 180.92 ng/ml to purified azadirachtin and 100.13 ng/ml to formulated azadirachtin. More than 90% of treated, normal-appearing, white prepupae and pupae showed precocious and abnormal pigmentation on their mouthparts and other appendages.

Naumann and Isman (1996) found that topical application of 0.5 µl azadirachtin at between

Table 6. Brood development (brood-index, brood termination rate in %) in the colonies of the treatment

Treatment	Brood indices during the observation					Brood termination rate in %
	BFD	BFD +5 (± 1 day)	BFD +10 (± 1 day)	BFD +16 (± 1 day)	BFD +22 (± 1 day)	
Expected indices	1	2–3	4	4	5 or 1–2	
Control	1.00	2.25	2.55	3.11	2.75	38
NeemAzal granules	1.00	2.06	3.14	3.50	3.35	31
NeemAzal T/S	1.00	1.98	1.75	1.60	2.05	65

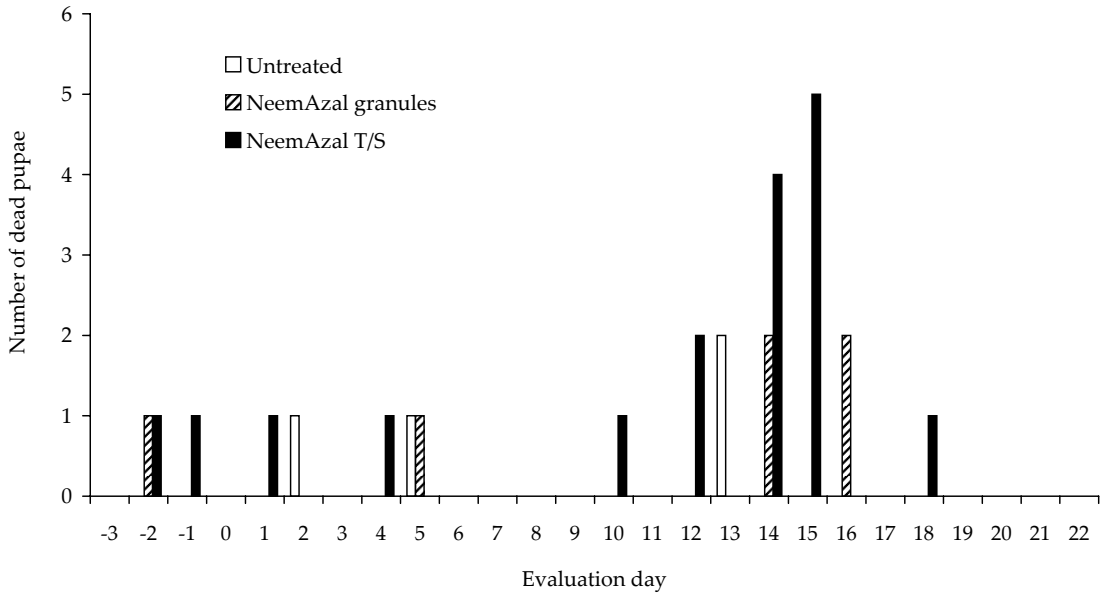

Figure 2. Effect of two NeemAzal formulations on the honeybee foraging activity during full flowering of spring rape in a tent test

100 and 500 ppm concentrations to fourth instar larvae (in the range of field application for phytophagous insect pest control) did not affect adult bees' lifespan.

Abrol and Kumar (2000b), tested the toxicity of neem oil (35 EC) in the laboratory by applying solutions of different concentrations (0.075–0.03%) to cells containing eggs and young larvae. The contents of most of the treated cells were removed by workers, and many of the remaining larvae were neglected and died by starvation. Any that survived were small and deformed.

CONCLUSION

In conclusion, our research shows that NeemAzal granules residues did not adversely affect bee mortality, foraging activity or brood development. It means that NeemAzal granules were safely used on spring rape without causing undue risk to bees. On the other hand, it was observed that NeemAzal T/S caused some reduction in foraging activity and brood development. NeemAzal T/S can be safely applied to spring rape in flower during periods of low or no honeybee activity.

The experiments should be considered as a pilot trial and the results as tentative, since due to insufficient resources available, no replication could be carried out during the trial. Consequently, no statistical analysis of the research data is possible, however, the results provide a basis for more experimental work, which would verify, or otherwise, the current data.

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References

Abrol D.P., Andorta R.S. (2000): Contact and oral toxicity of some pesticides to honeybee, *Apis mellifera* L. Indian Bee Journal, **62**: 29–43.

Abrol D.P., Kumar R. (2000a): Impact of pesticides on the brood rearing activity and foragers of honeybee, *Apis mellifera* L. visiting *Brassica campestris* L var. Toria. Indian Bee Journal, **62**: 19–27.

Abrol D.P., Kumar R. (2000b): Toxicity of neem (*Azadirachta indica* A. Juss) insecticide to immature stages of honeybee, *Apis mellifera* L. Indian Bee Journal, **62**: 47–53.

Allam S.F.M., Hassan M.F., Risk M.A., Zaki A.U., Papierok B. (2003): Utilization of essential oils and chemical substances alone or in combination against varroa mite (*Varroa destructor*), a parasite of honeybees. Bulletin OILB SROP, **26**: 273–278.

Boonthal C. (1994): Toxicity and residual effects of *Azadirachta indica* var. Siamensis extracts and cyhalothrin on *Apis florea* and *Apis cerana*. [MSc. Thesis.]

Interdepartment of Enviromental Sciences, Chulalongkorn University, Bangkok, Thailand.

Coats J.R. (1994): Risks from natural versus synthetic insecticides. Annual Review of Entomology, **39**: 489–515.

Garg S., Doncel G., Chabra S., Upadhyay S.N., Talwar G.P. (1994): Synergistic spermicidal activity of neem seed extract, reetha samponins, and quinine hydrochloride. Contraception, **50**: 185–190.

Kumar S.P., Babu P.C.S. (1996): Evaluation of some neem formulations for safety to certain beneficial insects. Pest Management and Economic Zoology, **4**: 19–24.

Lai S.M., Lim K.W., Cheng H.K. (1990): Margosa oil poisoning as a cause of toxic encephalopathy. Singapore Medical Journal, **31**: 463–465.

Liu T.P. (1995a): A possible control of chalkbrood and nosema diseases of the honeybee with neem. American Bee Journal, **135**: 195–198.

Liu T.P. (1995b): Controlling tracheal mites in colonies of honeybee with neem (Margosa-O) and flumethrin (Bayvarol). American Bee Journal, **135**: 562–566.

Malaipan S., Kongpitak P., Sujitratanan S. (1992): Comparative study on the effect of insecticides and neem extracts to the thrips and honey bee pollinators of pomelo cultivar Tongdee. Kasetsart Journal, Natural Sciences, **26**: 146–150.

Mann G.S., Dhaliwal G.S. (2001): Safety of azadirachtin to honeybee, *Apis mellifera* Linnaeus, foragers. Pesticide Research Journal, **13**: 118–121.

Melathpoulos A.P., Winston M.L., Whittington R., Higo H., Le Doux M. (2000a): Field evaluation of neem and canola oil for the selective control of the honeybee (Hymenoptera: Apidae) mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae). Journal of Economic Entomology, **93**: 559–567.

Melathpoulos A.P., Winston M.L., Whittington R., Smith T., Lindperg C., Mukai A., Moore M. (2000b): Comparative laboratory toxicity of neem pesticides to honeybee (Hymenoptera: Apidae), their mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) and brood pathogens Paenibacillus larvae and *Ascosphaera apis*. Journal of Economic Entomology, **93**: 199–209.

Naumann K., Currie R.W., Isman M.B. (1994): Evaluation of the repellent effects of neem pesticides on foraging honeybees and other pollinators. Canadian Entomologist, **126**: 225–230.

Naumann K., Isman M.B. (1996): Toxicity of neem (*Azadirachta indica* A. Juss) insecticide to larval honey bees. American Bee Journal, **136**: 518–520.

OEPP/EPPO (2001): Guideline for the efficacy evaluation of plant protection products – Side effects on honeybees. In: Belzunces L.P., Pelissier C., Lewis G.B. (eds): Proceedings of the 7th International Symposium on the Hazards of Pesticides to Bees. Sept. 7–9, 1999. Avignon, France, Les Colloques de I'INRA, **98**: 279–286.

Peng C.Y.S., Trinh S., Lopez T. E., Mussen E.C., Hung A., Chuang R. (2000): The effects of azadirachtin on the parasitic mite, *Varroa jacobsoni* and its host honey bee (*Apis mellifera*). Journal of Apicultural Research, **39**: 159–168.

Rembold H., Czoppelt C. (1981): Prüfung pflanzlicher Insektenwachs-tumshemmer aus *Azadirachta indica* im Aufzuchttest von Bienen Larven (*Apis mellifera*, Hym., Apidae). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, **3**: 196–198.

Schmidt H., Brasse D., Künast C., Mühlen W., von der Ohe W., Tornier I., Wallner K. (2003): Introduction of indices for the evaluation of tent tests and field tests with honeybees. Bulletin of Insectology, **56**: 111–117.

Schmutterer H. (1990): Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology, **35**: 271–297.

Schmutterer H., Holst H. (1987): On the effect of enriched and formulated neem seed kernel extracts AZTVR-K on the honeybee. Zeitschrift für Angewandte Entomologie, **103**: 208–213.

Schur A., Tornier I., Brasse D., Mühlen W., Von Der Ohe W., Wallner K., Wehling M. (2003): Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions. Bulletin of Insectology, **56**: 91–96.

Sharma G.K., Czoppelt C., Rembold H. (1980): Further evidence of growth disruption by neem seed fractions. Journal of Applied Entomology, **90**: 439–444.

Sithanantham S., Otieno Z.N., Nyarko K.A. (1997): Preliminary observations on effects of neem sprays on some on target arthropods in cowpea ecosystems. In: Fourth International Conference on Pests in Agriculture. Montpellier 6–8 January 1997, 2*:* 643–648. International Center of Insect Physiology and Ecology, Bow 30772, Nairobi, Kenya.

Sontakke B., Dash A.N. (1996): Studies on the effects of insecticides and neem formulation against major pests of mustard and their safety to honey bees. Indian Journal of Environment and Toxicology, **6**: 87–88.

Thapa R., Wongsiri S. (1997): Toxicity azadirachtin derivatives and synthetic pyrethroids on oilseed rape to *Apis cerana* (Hymenoptera: Apidae). In: Rodcharoen J., Wongsiri S., Mulla M.S. (eds): Proceedings of the 1st International Symposium on Biopesticides, Phitsanulok, Thailand, October 27–31, 1996.

Upadhyay S.N., Dhawan S., Garg S., Talwar G.P. (1993): Anti-fertility effects of neem (*Azadirachta indica*) oil in male rats by single intra-vas administration: an alternate approach to vasectomy. Journal of Andrology, **14**: 275– 281.

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Abstrakt

Shawki M. A.-A., Táborský V., Kamler F., Kazda J. (2005): **Působení dvou formulací NeemAzal^{TM} na včelu medonosnou v technickém izolátu maloparcelkového pokusu**. Plant Protect. Sci., **41**: 63–72.

Bylo sledováno působení dvou formulací NeemAzal^{TM} T/S (1% azadirachtin) a NeemAzal^{TM} granules (1 % azadirachtinu) na včelu medonosnou (*Apis mellifera* L.) v maloparcelkovém pokusu. Velikost každé pokusné varianty s jarní odrůdou řepky Likolly (*Brassica napus* L.) byla 15 m^2. V první pokusné variantě byla aplikována granulovaná formulace v době setí, druhou variantou byla neošetřená kontrola a ve třetí pokusné variantě byla aplikována emulzní formulace v kombinaci s koloidním aktivátorem Greemax^{TM} v období plného kvetení. Každá pokusná varianta byla zakryta izolační sítí 3 × 5 × 2 m. Do každé pokusné varianty byl umístěn malý pokusný úl s 3000 včelích dělnic v období plného květu na dobu 7 dnů. Odděleně v každém tunelovém izolátoru byla hodnocena mortalita a letová aktivita včel, u varianty postřikem před aplikací a po ní. Současně se sledoval vliv ošetření na vývoj plodu v plástech během jeho vývoje. Hodnocení vývoje plodu je vyjádřeno dobou jeho vývoje v procentech a indexací plodu. Získané výsledky ukázaly, že rezidua granulované formulace azadirachtinu nemají žádný vliv na mortalitu, chování a vývoj plodu v porovnání s neošetřenou kontrolou. U varianty aplikace postřikem byla u včel zaznamenána nižší schopnost aktivity vyhledávat potravu a stejně tak u vývoje plodu.

Klíčová slova: *Apis mellifera*; NeemAzal^{TM} T/S; Greemax^{TM}; mortalita; vyhledávání potravy; vývoj plodu

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