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ACTIVITY OF AZADIRACHTIN RELATING TO *PIERIS BRASSICAE* AND *APANTELES GLOMERATUS*

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Abstract. Azadirachtin is the most active substance separated from *Azadirachta indica*. It acts destructively on numerous species of insects and mites. The aim of the presented investigations was to determine the effect of azadirachtin on one of the most dangerous pests of cabbage plants, *Pieris brassicae,* and on its natural enemy, *Apanteles glomeratus*. The results of the field observations did not show any repellent effect of azadirachtin relating to laying their eggs cabbage butterflies. However, a significant effect of the azadirachtin solution on reduction the number of cabbage butterfly caterpillars (it was more distinctly marked in respect to young larva stages) as well as on limited feeding by caterpillars. No negative influence of azadirachtin on a parasite of *Pieris brassicae* caterpillars, *Apanteles glomeratus* was distinctly shown.

Key words: *Pieris brassicae*, *Apanteles glomeratus,* azadirachtin

I. INTRODUCTION

In recent years, we have been observing an increasing trend to use alternative methods of pest control based on replacing chemicals pesticides by natural compounds. Plants having in their contents allelopatic, bactericidal, or insecticidal compounds are a rich source of such substances. The substances are mainly the compounds belonging to the secondary plant metabolites. Many of the plants containing considerable amounts of active substances grow in tropical and subtropical countries, where they are exposed to the pests activity during the year (Boczek 1983).

Plants belonging to the family *Meliaceae* contain especially large amounts of such compounds. Such species, members of the family like: *Azadirachta indica* Juss., *Meliaceae* Vat., *Azadirachta exelsa* Jack, *Melia azaderach* L. and others, contain active substances which act like both pesticides and medicines (Malinowski and Woreta 1997). They consist of 3 or 4 main compounds: azadichratin, mellianthrol, salannin, and nimbin or nimbidin, as well as a few dozen of others found in inconsiderable amounts, but with a possible effect on the final result of the activity. These compounds belong mainly to the group of terpenes (limonoids).

Azadirachtin (tetraterpenoid) is the most active of them. It acts detrimental on many species of insects and mites. It is a antifeedant for different pests. It has a destructive influence on hormonal system, mainly on ecdysteroids. Disturbing insect neurosecretory system, it causes functional disorders of cardial and contiguous bodies (Schulz 1985). It disturbs the development of insects and reduces their fertility.

The aim of the presented experiments was to determine how azadirachtin acts on one of the most dangerous pests of the cabbage plants: cabbage butterfly and its natural enemy – *Apanteles glomeratus*.

II. MATERIAL AND METHODS

Azadirachtin was used in form of water solutions in the following concentrations: 1%, 0.1%, 0.01%, 0.001%, and 0.0001%. The concentrations were prepared on the base of 30% natural azadirachtin in technical concentrate (Rhom and Haas) with addition of an adhesion increasing agent (Sandowit).

The investigations were carried out under laboratory and field conditions. In the field, observations were made on indicated plots of size 5 × 5 m, which were succeeding experimental combinations. On each plot, the observations were carried out on 5 plants which were repetitions. Two field experiments were made. During the first one, cabbage was sprayed directly before the period of massive egg laying by cabbage butterflies. Observations of the process of egg laying on the plants were carried out after 2 and 6 days, and each time a number of eggs laid on the treated plants were noticed. The second experiment consisted in treating the plants with egg clusters of *Pieris brassicae* by using azadirachtin. An effect of the solution on their development was analysed by determining a number of died eggs and hatching larvae 2 and 4 days after spraying.

In the laboratory, caterpillars of cabbage butterfly collected from cabbage plants were the experimental material. The experiment was carried out on Petri dishes in 5 repetitions. A dish with 10 caterpillars was one repetition. Larvae of stage L2/L3 and L4/L5 were put on cabbage leaves previously plunged in solutions of azadirachtin and then dried. Observations of the eggs survival rate were carried out.

According to the same methodical assumptions, some observations concerning the effect of the azadirachtin solution on intensity of caterpillar feeding were also made. In order to do that, cabbage leaves previously weighed on the analytical balance were plunged in solutions of azadirachtin and then dried. The weighed larvae of stage L4/L5 were put on the cabbage leaves. After 48 hours the leaves with caterpillars were weighed once more. On the basis of the weight differences between masses of leaves and caterpillars, before and after the experiment, the following parameters were estimated: the mass of food eaten by caterpillars, the amount of food used for the increase of larval bodies, and the absolute deterrent index according to the formula: $I = (C - T) : (C + T) \times 100$, where: C – a weight of the eaten food in the control combination, T – a weight of the eaten food in the test combination (Kiełczewski et al. 1979).

An analysis of the influence of azadirachtin on the endoparasite of cabbage butterfly caterpillars, *A. glomeratus*, was also carried out. In order to do this, caterpillars of stage L4/L5 feed with the food containing azadirachtin were observed. For each experimental combination there was stated a percentage of caterpillars whose bodies left larvae of *A. glomeratus* and made cocoons around them. Dead caterpillars were subjected to dissec-

tion in order to determine the ones whose bodies still contained larvae of *A. glomeratus* (dying together with them).

The results were statistically worked out with the use of variance analysis with a single classification and Student's test. Data obtained in each combination were compared with the results obtained in the control tests.

III. RESULTS

1. The effect of azadirachtin on laying eggs by females of cabbage butterfly and on the egg development

After treating cabbage plants with azadirachtin, clusters of eggs laid on them were counted (Tab. 1). Laying eggs by butterflies was observed on all the plants treated with the preparation (except the ones with the highest concentration of azadirachtin), as well as in the control combinations. Significant differences between the number of egg clusters on plants in comparison with the test combinations were noticed only after applying 2 largest doses of the preparation,

After applying azadirachtin to the plants with cabbage butterfly egg clusters and in the combination of 1% of concentration, brown spots and then drying up of 100% of eggs were observed (the results differed substantially from those obtained from test combinations). In cases of using lower concentrations of the preparation, no dying eggs were observed after 2 days, however, after 4 days the number of the dead eggs was low (14–21%) both in those combinations and in the control ones. From the other eggs, larvae hatched.

2. Effect of azadirachtin on larvae of *Pieris brassicae*

During observations made in the laboratory, it was found that azadirachtin used in concentrations of 1% and 0.1% showed a toxic effect with respect to caterpillars of cab-

Table 1

The influence of the azadirachtin on the laying eggs by cabbage butterflies in the field conditions

Tests combination	Number of eggs laid		Mortality of eggs (%)	
	after 2 days	after 6 days	after 2 days	after 4 days
Azadirachtin 1%	0a	0a	100a	100q
Azadirachtin 0.1%	2a	3a	34.3b	41.1b
Azadirachtin 0.01%	4b	5ab	0.0c	24.0c
Azadirachtin 0.001%	3ab	5ab	0.0c	21.2c
Azadirachtin 0.0001%	5b	6b	0.0c	14.6c
Control + Sandowit	4ab	8b	0.0c	16.5c
Control dry	6b	9b	0.0c	19.4c

Means followed by the some letter do not differ witch 5% of significance

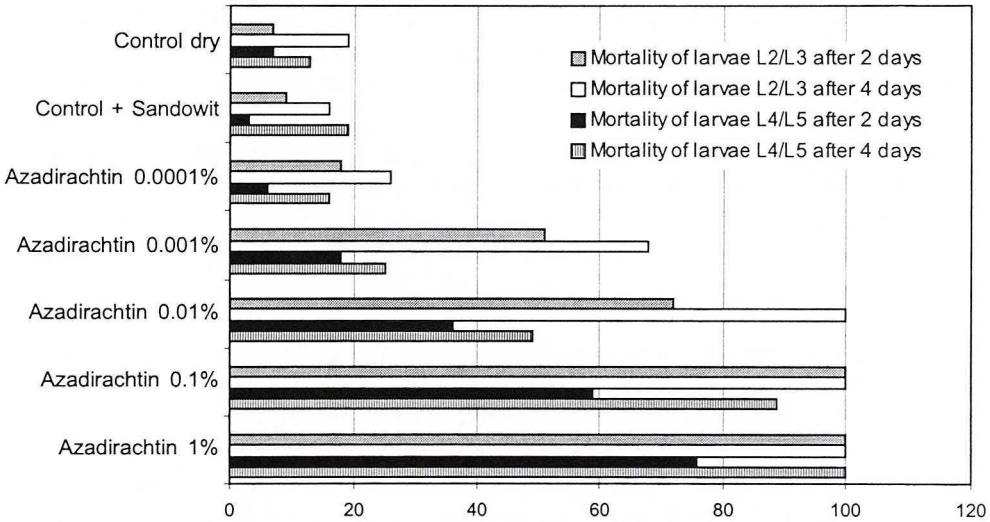

Fig. 1. Effectivenes of azadirachtin on *Pieris brassicae* larvae

bage butterfly causing 100% of mortality of larvae stage L2/L3 during 48 hours. However, after using the preparation in its lowest concentrations, the mortality of caterpillars showed no significant difference compared with the control combinations.

Lower mortality of larvae stage L4/L5 was observed. A total mortality was obtained after 4 days (after using the preparation in 1% of concentration). In the experimental combinations where azadirachtin was used in lower concentrations, the obtained data did not differ considerable from the control (Fig. 1).

3. The effect of azadirachtin on caterpillar feeding

A strong decrease of caterpillar feeding on cabbage leaves treated with azadirachtin (Tab. 2) was found. The calculated absolute deterrence index shows that caterpillars did not feed at all on leaves treated with the preparation or they slightly chewed leaves and

Table 2

Effect of azadirachtin on feeding of *Pieris brassicae* larvae

Tests combination	Absolute coefficient of deterrence	Body weight changes (in mg)	Food consumption per 1mg of body weight increase (in mg)
Azadirachtin 1%	84,1	−16.3	–
Azadirachtin 0.1%	76,0	− 2.8	–
Azadirachtin 0.01%	68,4	+17.1	47.2
Azadirachtin 0.001%	56,7	+29.4	39.4
Azadirachtin 0.0001%	48,6	+49.5	36.2
Control + Sandowit	–	+91.0	16.1
Control dry	–	+88.6	14.8

stopped feeding. No distinct differences between the mass of the food taken in individual experimental combinations were stated. It was confirmed by the data dealing with the body mass changes of the tested *P. brassicae* larvae. After using the preparation with the highest concentration, some decrease of its mass was observed. In the other experiment combinations small increases and larger food consuming per 1 mg of body mass increase were noticed, which would suggest a possibility of causing disturbances by azadirachtin in the insects metabolism.

4. Observations concerning the effect of the preparation on *Apanteles glomeratus*

The observations showed a small differentiation of the preparation on the endoparasite of cabage butterfly – *A. glomeratus*. A number of caterpillars, around which cocoons were found, were in range 56-78% with the reference to the controls. The lowest number of *A. glomeratus* larvae leaving the hostcaterpillars and making cocoons around them was noticed in combinations in which azadirachtin was used in the highest concentration. The zootomy of the dead caterpillars proved the presence of the dead larvae of *A. glomeratus*. In the other combinations larger numbers of endoparasite larvae survived. However, the obtained differences were insignificant (Fig. 2).

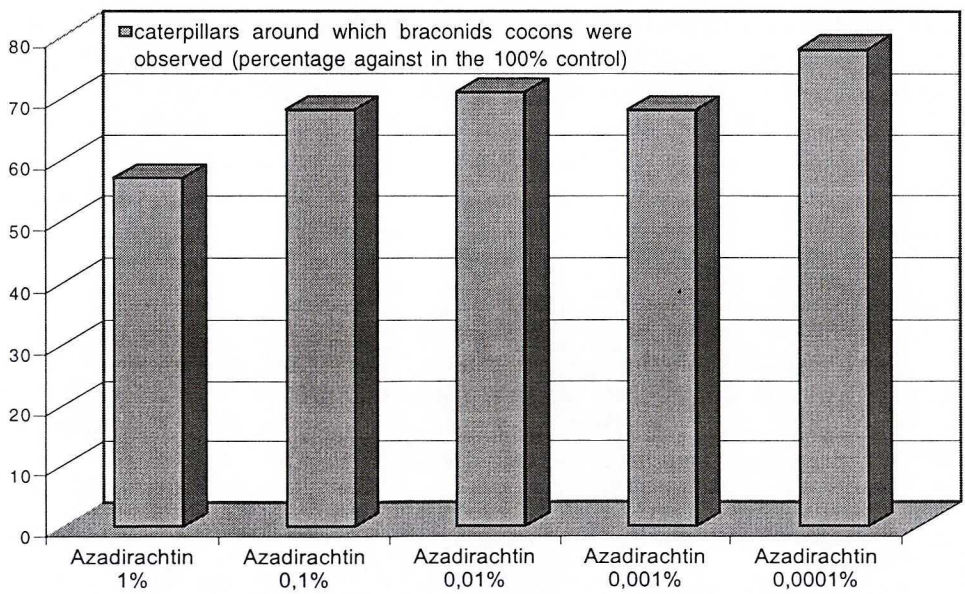

Fig. 2. Effect of the preparation on *Apanteles glomeratus*

IV. DISCUSSION

The results of the field observations concerning the process of laying eggs by cabbage butterflies on plants treated with azadirachtin did not show its deterrent effect. According to Ignatowicz (1995), females of the insects lay their eggs willingly on the plants

sprayed with pure azadirachtin. Repellent features have, however, extracts from leaves and seeds as well as powdered seeds and seed covers, and even powdered leaves of *Azadirachta indica*.

The observations on *P. brassicae* eggs mortality after treatment with azadirachtin showed that their total destruction was noticed only after applying the preparation with concentration of 1%. Saxena et al. (1980) stated that water and alcohol extracts from *Azadirachta* have hardly any influence on the eggs development and hatching of *Plutella xylostella Plutella xylostella* larvae (high concentrations of the extract caused only insignificant decrease of the hatched larvae number.)

An active effect of the preparation was stated in respect to cabbage butterfly caterpillars, which was marked by a strong reduction number. Zehnder and Warthen (1988), determining effectiveness of the extract of *A. indica* relating to caterpillars of *Spodoptera frugiperda, S. exempta, Helicoverpa zea, Ostrinia nubilalis, O. furnacalis, Sesamia calamistis, S. nonag – rioides*, found that those species may be controlled with the use of powdered preparations or seed extracts (positive effects were obtained, when procedures were made sufficiently early on younger stage of larvae – before their penetrating into the stems of plants). Adhikary (1985) observed antifeedant and restraining development features of azadirachtin regarding *Plutella maculipennis* and *Trichoplusia ni*. The results obtained by Jacobson et al. (1984) and Schmutterer (1985) indicate the reduction of the colorado potato-beetle (*Leptinotarsa decemlineata*) population beneath the economic harmfulness threshold. Powdered seeds of *A. indica* as well as their extracts are also used against stored product pests (Bhatnagar et al. 1990, Ignatowicz et al. 1994). With the use of preparations from *A. indica* practically all most dangerous pests of the conifers can be controlled (Malinowski and Woreta 1987; Schmutterer 1985).

A very active effect of azadirachtin on reduction of food taken by cabbage butterfly caterpillars was found. It confirms many data concerning strong antifeedant effect of azadirachtin relating to numerous pest species, e.g. *P. brassicae* (Kleeberg 1992; Osman 1993) *P. rapae* (Karelima et al. 1992, Kleeberg 1992), *Mamestra brassicae, Heliothis armigera* (Karelima et al. 1992), although Warten (1979) informs that this compound has a weaker influence on *P. brassicae* caterpillars.

No negative effect of azadirachtin on the endoparasite *A. glomeratus* was found. Reduction of population of this endoparasite after application of methanol extract from *A. indica* was observed by Osman and Bradley (1993). According to Schmutterer (1990), preparations containing extracts from seeds of *A. indica* are relatively safe for bees and natural enemies, e.g. for *Syrphidae, Cocconellidae.*

V. CONCLUSIONS

1. The results of field observations did not show any repellent effect of azadirachtin relating to laying eggs by cabbage butterflies.
2. Activity of the high concentrations of azadirachtin solution on caterpillars was found, which was expressed by a significant reduction of population, especially in relation to younger larvae stages.

3. Azadirachtin totally reduced feeding by *P. brassicae* caterpillars.
4. No negative effect of azadirachtin on the cabbage butterfly caterpillar endoparasite – *A. glomeratus* was observed.

VI. LITERATURE

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AKTYWNOŚĆ AZADIRACHTYNY W STOSUNKU DO BIELINKA KAPUSTNIKA I BARYŁKARZA BIELINIAKA

STRESZCZENIE

Azadirachtyna jest najbardziej aktywną substancją wyizolowaną z *Azadirachta indica*. Działa szkodliwie na liczne gatunki owadów i roztoczy. Celem przedstawionych doświadczeń było określenie jej wpływu na jednego z najgroźniejszych szkodników roślin kapustnych bielinka kapustnika (*Pieris brassicae*) oraz na jego wroga naturalnego baryłkarza bieliniaka (*Apanteles glomeratus*).

Wyniki przeprowadzonych obserwacji polowych nie wykazały repelentnego działania azadirachtyny w stosunku do składających jaja motyli bielinka. Stwierdzono natomiast znaczący wpływ preparatu na redukcję liczby gąsienic bielinka kapustnika (wyraźniej zaznacza się to w odniesieniu do młodszych stadiów larwalnych) oraz na hamowanie pobierania pokarmu przez gąsienice. Nie wykazano wyraźnie negatywnego wpływu azadirachtyny na pasożyta gąsienic bielinka – baryłkarza bieliniaka.