ТОКСИЧНОСТ НА НЯКОИ БИОИНСЕКТИЦИДИ ВЪРХУ ЕКТОПАРАЗИТОИДА *BRACON HEBETOR* SAY (HYMENOPTERA: BRACONIDAE) ПРИ ЛАБОРАТОРНИ УСЛОВИЯ
TOXICITY OF SOME BIOINSECTICIDES ON THE ECTOPARASITOID *BRACON HEBETOR* SAY (HYMENOPTERA: BRACONIDAE) UNDER LABORATORY CONDITIONS

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Резюме

При лабораторни условия е проучена токсичността на три биоинсектицида - Дипел ВП, НимАзал Т/С и Трейсър 480 ЕК, разрешени за употреба при биологично производство в България, върху ектопаразитоида *Bracon hebetor* Say (Hymenoptera: Braconidae). Паразитоидите са хранени с капчици от 20% меден разтвор, смесен в съотношение 1:1 с тестваните биоинсектициди в регистрираните за употреба концентрации: НимАзал Т/С - 0,3%, Трейсър 480 ЕК - 0,03%, и Дипел ВП - 0,15% (третиране "*per os"*). Резултатите показват, че Трейсър 480 ЕК има токсично действие върху възрастните индивиди на паразитоида и още на първия ден след третирането смъртността е 100%. Биоинсектицидите НимАзал Т/С и Дипел ВП не са токсични и не влияят върху преживяването на паразитоида, както и върху копулирането, плодовитостта и способността на женските индивиди да паразитират гостоприемника си. Поколението на третираните с биопрепаратите Дипел ВП и НимАзал Т/С ектопаразитоиди преживява успешно върху гостоприемник – гъсеници на *Phthorimaea operculella* (Zeller).

Abstract

The toxicity of three bioinsecticides – *Dipel DF*, *NeemAzal T/S* and *Tracer 480 SC*, allowed for applying in the organic farming in Bulgaria, on the ectoparasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) was studied under laboratory conditions. The adult parasitoids were fed with drops of 20% honey solution mixed with tested bioinsecticides in concentrations registered for application: *NeemAzal T/S* – 0.3%, *Tracer 480 SC* – 0.03% and *Dipel DF* – 0.15% in ratio 1:1 ("*per os"* treatment). The results showed that *Tracer 480 SC* had a toxic effect on the adults and on the first day after the treatment 100% mortality was observed. The bioinsecticides *NeemAzal T/S* and *Dipel DF* were not toxic and did not affect the survival of the parasitoid as well as the copulation, fertility and ability of the females to parasitize their host. The generation of treated parasitoids survived successfully on the host – *Phthorimaea operculella* (Zeller) larvae.

Ключови думи: биоинсектициди, NeemAzal T/C, Tracer 480 SC, Dipel DF, *Bracon hebetor.*
Key words: bioinsecticides, NeemAzal T/S, Tracer 480 SC, Dipel DF, *Bracon hebetor.*

INTRODUCTION

With the development of the organic agriculture in our country alternatives to chemical insecticides has been considering. The most common is the biological control by application of bioagents and bioinsecticides which are selective and safe for the environment and human health (Stiener & Elliot, 1987; Stauffer & Rose, 1997; Miller & Uetz, 1998).

Bioinsecticides show efficacy to a large number of pests. The products allowed for use in the organic agriculture in Bulgaria are: NeemAzal T/S (azadirachtin), Tracer 480 SC (480 g/l. spinosad) and Dipel DF (*Bacillus thuringiensis* var. *kurstaki*).

Azadirachtin is the main active substance extracted from the seeds and leaves of the neem tree, *Azadirachta indica* A. Juss (Schumutterer,

1990; Ascher, 1993; Mordue & Blackwell, 1993). It acts as a repellent against phytophagous insects and also affects reproduction and development of pests preventing egg-laying of females and disrupts moulting of the larvae (Schumutterer, 1990; Mordue & Blackwell, 1993).

In our country this active substance is used as the registered botanical insect-acaricide NeemAzal T/S to control spider mites primarily on vegetables in greenhouses in a concentration of 0.3%. In the United States (Smith & Krischik, 2000) and in Turkey (Yardm et al., 2001) it was estimated as a selective to the ladybirds and to the most common entomo- and acarophagous species. Under laboratory conditions Tillman (2008) found that *Trichopoda pennipes* (F.), an endoparazitoid of adults, larvae and nymphs of *Nezara viridula* (L.), survived after direct treatment and food treatment with azadirachtin.

Spinosad is a fermentation product derived from the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao. It belongs to the group of natural substances for pest control. The commercial product with that active substance registered in Bulgaria is Tracer 480 SC against *Neodiprion sertifer* Geoffr. at concentration of 0.03%. The Company Dow AgroScience LTD stated in the list of crops and pests a dose of 30 ml/100 l water - a registered formulation against the potato moth *Phthorimaea operculella* (Zeller). The product Tracer 480 SC has a very quick initiating effect occurs as the knock-down effect. It is a contact and stomach insecticide and has translaminar properties. It is used for the pest control of the order Lepidoptera in cotton because of the high activity at low doses and its tolerance towards entomophagous insects (Nolting et al., 1997; Peterson et al., 1997). Although there are studies like those of Tillman (2008) who found that Spinosad was highly toxic to the endoparasitoid *T. pennipes*.

In organic farming bacterial preparations based on *Bacillus thuringiensis* are widely used. In the international farming they occupy about 90% of the market of biopesticides (Bhatnagar et al., 2004). *B. thuringiensis* var. *kurstaki* causes the death of more than 200 species of the order Lepidoptera and is registered to control many insects pests on agricultural, forest and ornamental crops. These biopesticides have been assessed as safe for the environment and human health (Otvos et al., 2005). In our country *B. thuringiensis* var. *kurstaki* is primarily used against the larvae of the pests at concentrations of 0.1-0.15%, and on this basis are registered the products - Dipel, Forrey.

Besides using bioinsecticides an important role in controlling the pests below the thresholds of economic harm is the application of bioagents (predators and parasitoids). The ectoparasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) is known as a wide polyphagous of more than 40 hosts (Balevski, 2000), including the potato tuber moth *P. operculella*.

The potato tuber moth is an economically important pest on potatoes in the country. It originates from Central and South America, and in the early 20th century invaded in Europe. In Bulgaria this pest was established for the first time in 1950 on potatoes in the area of Petrich (Stanev & Kaytazov, 1962). Since 2008 there has been an expansion in the distribution of the species in the country, spreading in the regions of Kyustendil, Pernik, Samokov, Ihtiman, Pazardzhik, Plovdiv, Smolyan, Burgas, Blagoevgrad, Kardzhali, Balchik and Dobrich (Vaneva-Gancheva & Grigorova, 2010; Subchev et al., 2013).

Regarding the ever-growing need of use of bioagents in the plant protection, the study of interaction and opportunities for co-utilization of the organic products and parasitoids is necessary and promising.

The aim of this study is to examine the longevity of the adults of *B. hebetor* and the ability of the females to parasitize the potato tuber moth larvae after "*per os"* treatment with bioinsecticides - NeemAzal T/S, Tracer 480 SC and Dipel DF.

MATERIALS AND METHODS

Bioinsecticides

The study was conducted under laboratory conditions in 2013 with adults of the ectoparasitoid *B. hebetor* treated "*per os"* with the following bioinsecticides at the registered concentrations:

NeemAzal T/S - containing 1% azadirachtin-A as an active ingredient in the form of an emulsifiable concentrate, at concentration of 0.3%.

Tracer 480 SC (480 g/l spinosad) - 0.03%

Dipel DF (16 000 IU per 1 mg product of *Bacillus thuringiensis* var. *kurstaki*) - 0.15%.

Bracon hebetor

The population of the parasitoid *B. hebetor* was reared in the insectarium of department "Entomology" at the Institute of Soil Science, Agrotechnologies and Plant Protection - Sofia. The rearing is carried out at a temperature of 26-30°C, 60-80% relative humidity and photoperiod of 18: 6 h (L: D) by the methodology of Balevski (1996).

Phthorimaea operculella

The population of potato tuber moth originated from the Institute of Tobacco and Tobacco Products - Markovo, kindly provided by the research assistant T. Vaneva-Gancheva. The *P. operculella* was grown in the insectarium at a temperature of 24-26°C, 60-80% relative humidity and photoperiod of 18: 6 h (L: D) by the methodology of Maharjan & Jung (2012).

Bioassays

The newly emerged females and males of *B. hebetor* in ratio 2:1 females: male were placed in isolators with a fourth instar larvae of the potato tuber moth and were fed by a brush with drops of 20% honey solution mixed with the respective concentrations of the tested bioinsecticides in ratio 1:1 ("*per os"* treatment). In the control parasitoids were fed only with drops of 20% honey solution. The treatments were conducted in 8 repetitions

The mortality of male and female parasitoids was daily reported. The ability of females to parasitize the larvae of the potato moth and the survival of the generation of the parasitoid was recorded.

Statistical analysis

The obtained data were statistically analyzed with the program StatSoft, ver. 10. The obtained results were determined for the parametere longevity. Confidential probability, $P \leq 0.05$ (Student's t-test) is accepted as criterion for significant difference between treatments and controls.

RESULTS AND DISCUSSION

Results showed that the parasitoids treated with Tracer 480 SC died on the following day after the treatment (Table 1). The results obtained are similar to those of Tillman (2008), who found that *T. pennipes*, an endoparasitoid on adults, larvae and nymphs of *N. viridula*, survived after direct and food treatment with azadirachtin whereas the product spinosad was highly toxic to this natural enemy.

The duration of life of the female and male parasitoids was not affected by the bioinsecticides NeemAzal T/S and Dipel DF. That confirms the assessment that biopesticide are safe for the environment and human health (Otvos et al., 2005).

A significant difference between the longevitiy of males and females was observed, which is a part of the biology of *B. hebetor* i.e. females live significantly longer than males (Balevski, 1995).

Among the newly emerged generation of *B. hebetor* a presence of males and females parasitoids was recorded, which proves that copulation between the treated adults had taken place. A high percentage of survival among the generation of the treated parasitoids was established. The number of the newly emerged adults in the control did not differ significantly from that in the two experimental variants. The percentage of emergence in the treatments with the bioinsecticides Dipel DF and NeemAzal T/S were respectively 62.5% and 75%. In the control emergence of 75% was observed which indicated that the products Dipel DF and NeemAzal T/S did not affect the survival of parasitoid offspring (Table 2).

Таблица 1. Продължителност на живот на женските и мъжките индивиди на *Bracon hebetor* Say директно третирани *(per os)* с биоинсектицидите НимАзал Т/С, Трейсър 480 ЕК и Дипел ВП
Table 1. Longevity of *Bracon hebetor* Say females and males treated directly *(per os)* with the bioinsecticides NeemAzal T/S, Tracer 480 SC and Dipel DF

Bioinsecticides Биоинсектициди	Concentration (%) Концентрация (%)	Longevity (days) Продължителност на живот (дни)					
		Females (♀) Женски			Males (♂) Мъжки		
		min.	max.	mean ± s.e.	min.	max.	mean ± s.e.
NeemAzal T/S НимАзал Т/С	0.3	25	30	27,94 ± 1,77	8	9	8,63 ± 0,52
Tracer 480 SC Трейсър 480 ЕК	0.03	1	1	1	1	1	1
Dipel DF Дипел ВП	0.15	25	30	29,63 ± 2,99	8	9	8,50 ± 0,53
Control Контрола	-	25	35	30,31 ± 2,98	8	12	8,70 ± 0,52

Таблица 2. Брой и съотношение на имагиниралите женски и мъжки индивиди на *Bracon hebetor* Say в поколението, получено от третирани възрастни индивиди на паразитоида с биоинсектицидите Дипел ВП и НимАзал Т/С

Table 2. Number and ratio of the emerged *Bracon hebetor* Say females and males in generation derived from treated parasitoid adults with the bioinsecticides Dipel DF and NeemAzal T/S

Repetitions Повторения	Number and ratio of the emerged *Bracon hebetor* Say females and males Брой и съотношение на имагиниралите женски и мъжки индивиди на *Bracon hebetor* Say		
	Control Контрола	Dipel DF Дипел ВП	NeemAzal T/S НимАзал Т/С
1	3 ♀	-	-
2	-	-	1 ♀ + 2 ♂
3	-	2 ♀ +1 ♂	3 ♀ + 3 ♂
4	2 ♀+ 1 ♂	4 ♀ + 1 ♂	4 ♀ + 1 ♂
5	3 ♀ + 1 ♂	2 ♀ + 2 ♂	2 ♀ + 1 ♂
6	3 ♀ +3 ♂	3 ♀ + 2 ♂	2 ♀ + 2 ♂
7	2 ♀+ 2 ♂	4 ♀ + 2 ♂	3 ♀ + 3 ♂
8	3 ♀ + 2 ♂	-	-
Total number Общ брой	16 ♀ + 9 ♂	15 ♀ + 8 ♂	15 ♀ + 12 ♂

Our results showed that NeemAzal T/S does not have a detrimental effect on *B. hebetor* adults. Adverse effects of some biologically active plant extracts on beneficial organisms have been reported by many other workers. For instance azadirachtin and pyrethrum seriously affected development of *Venturia canescens* adults (Tunka et al., 2012). Very few adult parasitoids emerged from azadirachtin treated hosts at LC50 and LC25 values, indicating a strong detrimental effect on the parasitoid. Treating the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) with neem-seed extract resulted in moulting delays and deformities in its predator, *Perillus bioculatus* Fabricius (Hemiptera: Pentatomidae) (Hough-Goldstein & Keil, 1991). In addition, neem seed extract decreased the numbers of *Encarsia* spp. (Hymenoptera: Aphelinidae) and *Aleurodiphilius* spp. parasitoids of *Bemisia tabaci*, an important pest for which azadirachtin showed good efficacy (Price & Schuster, 1991).

In some studies combined the treatment with Dipel DF and NeemAzal T/S and the endoparasitoid *Trichogramma evanescens*. The integration between the bioinsecticdes and *T. evanescens* enhanced the control of *P. operculella* (Mandour et al., 2012).

In field applications of spinosad seem to be less detrimental to natural enemies than laboratory treatments. In the laboratory, spinosad was moderately toxic to adults of the predator *Geocoris punctipes* (Say) (Boyd and Boethel, 1998). In the studies of Tillman (2008) spinosad was highly toxic to the parasitoid *T. pennipes.* In the present study, that bioinsecticide had similar effect on *B. hebetor* adults.

Behavioral studies are also important for succesful biological control. In our study the NeemAzal T/S and Dipel DF does not effect on ovipositional behaiviour of the parasitoid females neither their fecundity. The generation derived from treated with NeemAzal T/S and Dipel DF parasitoid adults was not affected and survived successfully.

In summary, results suggest that NeemAzal T/S and Dipel DF would be safer to *B. hebetor* adults than Tracer 480 SC in organically-grown crops.

CONCLUSIONS

1. The bioinsecticide Tracer 480 SC causes 100% mortality of the females and males of the ectoparasitoid *B. hebetor*, while the Dipel DF and NeemAzal T/S products do not have a toxic effect.

2. The treatment "*per os"* of *B. hebetor* adults with Dipel DF and NeemAzal T/S does not affect copulation, fertility and ability to parasitism of *B. hebetor* females.

3. The generation derived from treated with the bioinsecticides Dipel DF and NeemAzal T/S *B. hebetor* adults survives successfully on the host - the potato tuber moth larvae *P. operculella*.

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