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Short research contribution

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INTEGRATED METHODS IN PEST CONTROL: EFFECT OF INSECTICIDES ON ENTOMOPATHOGENIC FUNGI (*BEAUVERIA BASSIANA* (Bals) Vuill., *B. BRONGNIARTII* (Sacc.)) AND NEMATODES (*HETERORHABDITIS MEGIDIS* Poinar, Jackson, Klein, *STEINERNEMA FELTIAE* Filipjev, *S. GLASERI* Steiner)

ABSTRACT: Sensitivity of two species of entomopathogenic fungi: *Beauveria bassiana* and *B. brongniartii* and of three species of entomopathogenic nematodes: *Steinernema glaseri*, *S. feltiae* and *Heterorhabditis megidis* to low doses of two insecticides carbosulfan and carbofuran applied to forest soil to control cockchafer's grubs was studied. Carbosulfan inhibited the growth of both fungi. Carbofuran stimulated the growth of *B. brongniartii*. Mortality of carbofuran treated nematodes was higher than mortality of those affected by carbosulfan. Both insecticides did not decrease the pathogenicity of fungi and nematodes towards *Galleria mellonella* L. larvae. The studies pointed to a possibility of using both insecticides in the Integrated Pest Management.

KEY WORDS: insecticides, entomopathogenic fungi, entomopathogenic nematodes, *Beauveria bassiana*, *Beauveria brongniartii*, *Steinernema glaseri*, *Steinernema feltiae*, *Heterorhabditis megidis*, IPM

Cockchafer's grubs (*Melolontha melolontha* L., Scarabaeidae) are one of the most dangerous pests damaging roots of trees and forests shrubs. Adult insects may also cause some damage shredding the leaves

and inflorescences of some trees but these losses are much smaller than those made by their grubs (Woreta 1994). Recently, maybug grubs caused a lot of damage to tuber crops and in strawberry and raspberry plantations. To avoid environmental pollution, the use of biological methods in plant pests control and a restricted chemical treatment is often recommended. Recent studies demonstrate, however, that the exclusive use of biological methods in grub control is ineffective (Zelger 1993, Vlug 1996). Bednarek *et al.* (2000) dealt with the possibility of using an integrated method to control *Melolontha melolontha* L. They studied also a possibility of increasing the effectiveness of entomopathogenic fungi and nematodes through their use in combination with low doses of insecticides. These doses being the less harmful to the environment, would result in diminishing natural defensive mechanisms in insects. The method is feasible when the chemical insecticide does not negatively affect the utilized organisms i. e. nematodes and fungi.

The experiments were aimed at studying the effect of insecticides used in Poland to control grubs on some parameters of

biological activity of various species of entomopathogenic fungi (*Beauveria* sp.) and nematodes (*Heterorhabditis* sp. and *Steinernema* sp.). Fungi of the genus *Beauveria* sp. are common in the soil habitat. The species most often found include: *B. bassiana* and *B. brongniartii*. *B. bassiana* is known as a pathogen of many insect species (Ferron 1985) while *B. brongniartii* – mainly as a pathogen of the cockchafer (*Melolontha melolontha*). (Zimmerman 1992, Keller *et al.* 1997, Keller and Eilenberg 2002). Nematodes of the families *Heterorhabditidae* and *Sterneinematidae* are also frequent in the soil habitat. They are represented e.g. by the species: *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, *Heterorhabditis bacteriophora* and *H. megidis*. They are the pathogens of many insect species (Hominic *et al.* 1996, Kooppenhöfer 2000). In the studies we used Polish isolates of fungi and nematodes which in earlier experiments were found to be most pathogenic towards the grubs of *Melolontha melolontha*. Swiss commercial preparation containing the spores and mycelium of *B. brongniartii* and the German strain of *H. megidis* were used for comparison.

Two carbamate insecticides were used: carbofuran (trade name Furadan 5 GR, FMC, USA containing 5% of the active substance) and carbosulfan (trade name Marshal SuSCon 10CG containing 10% of the active substance). Carbosulfan and carbofuran are the insecticides used to control cockchafer's grubs in forests (Woreta 1999, Stocki and Malinowski 2000). Both poison insects by contact. Carbofuran has additionally systemic properties and is taken up by plants through the root system. After using such an insecticide, insects may be also poisoned orally as a result of feeding on roots, which in turn took up the insecticide from soil (Malinowski – unpublished).

Two species of entomopathogenic fungi were used: *Beauveria brongniartii* (Sacc.) (a commercial strain, Co. Andermat, Switzerland) and *B. bassiana* (Bals) Vuill. BP97 (a strain isolated from the forest soil in southern Poland in 1997) and three species of entomopathogenic nematodes: *Heterorhabditis megidis* Poinar, Jackson, Klein HSH2 (a strain isolated by Dr R-U. Ehlers, Germany from *Melolontha melolontha*), *Steinernema glaseri* Steiner (a strain isolated from *M. melolontha* and *Amphimalon solstitialis* L.

by Dr M. Tomalak, Poland) and *S. feltiae* Filipjev OL97 (a strain isolated from the forest soil in northeastern Poland in 1997).

The influence of insecticides on the growth and pathogenicity of entomopathogenic fungi was studied. Growth of fungi was determined after 14 days of culture at 22°C by: (1) – measuring diameter of colonies growing on a solid Sabouraud medium (SDA) and by (2) – measuring dry weight of mycelium grown on a liquid Sabouraud medium (SDA). Diameter of a colony was measured every 2–3 days since inoculating the solid medium in 10 dishes with 3 replications as in the method of Lilly and Barnett (1959). Size of a colony is given as a mean value for 30 dishes. Dry weight of mycelium was estimated after separating it from a liquid medium (10 dishes in 3 replications) by filtration and after drying at 60°C for 24 hours as a mean for 30 dishes. Insecticides were added to the liquid and solid media in low doses equal to 1.5 mg l⁻¹ (ten times lower than that which is recommended in pest control). Medium without insecticide addition was adopted as a control. Insecticides were added to medium after autoclaving in aqueous solutions. Pathogenicity of the fungi was estimated upon the mortality of *Galleria mellonella* L. larvae (160 mg) sprayed with the water suspension of spores taken after 14-days culture from the surface of the colony of fungi grown on a solid medium with or without insecticides (control). Spore suspension of a density of 5 × 10⁵ ml⁻¹ was used to infect the larvae. Density of this suspension was calculated with the use of a Thoma hematocytometer (0.1 mm, 1/400 mm²). Insects were infected in Petri dishes lined with the filter paper (10 dishes with 10 larvae in each) and incubated at 22°C. In total, 100 larvae were infected in the experimental and in the control variant. Dead insects were counted during 21 days since infection (every 2–3 days since the 7th day) and removed to other dishes lined with the wet filter paper. Viability of spores from mycelia grown on the control and insecticide treated media was studied before the infection experiment. To do that, 0.1 ml of spore suspension of a density of 1 × 10⁶ was spread over the surface of microscopic slide with the Sabouraud medium, then placed in a wet chamber and incubated for 20 hours at a temperature of 22°C. Then 200 of spores (in 2 replica-

tions) were counted and the percentage of germinating spores was marked. After 20 hours of incubation over 90% of spores germinated, both from the control and insecticide treated medium.

To study the effect of insecticides on nematodes, infective juvenile (IJs) of each nematode species were introduced to Petri dishes (diameter 10 cm) filled with 10 ml of carbofuran or carbosulfan solution at a concentration of 1.5 mg l⁻¹ and incubated at 19.5°C. Five thousand IJs per dish in five replications were used in each variant. Three times during 7 days (every second day) 0.5 ml of solution was taken from every replication and dead and alive nematodes were counted under binocular (viability was checked by touching). This enabled determining the effect of insecticides on nematode mortality. Nematodes placed in distilled water served as a control. Then, the nematodes were sedimented to separate live from dead ones. Live nematodes were

rinsed in sterile water. Five hundred IJs were placed in every of 5 Petri dishes (diameter 10 cm) lined with filter paper. Then, 10 larvae of *G. mellonella* were introduced. Nematode invasion followed as a result of a free contact of nematode with the insect. *G. mellonella* larvae treated with the same dose of IJs kept in distilled water were used as a control. Dishes with insects were incubated at 26°C. Dead insects were counted every day during 5 days of incubation. Five replications with 10 insect larvae per dish were used in each experimental variant.

Results were processed with the multifactorial variance analysis at $P \leq 0.05$ after transforming the data. Mean values were differentiated with the Duncan's test.

The influence of insecticides on the growth of fungi was species-specific and depended on the insecticide (Table 1). Carbosulfan inhibited colony growth of both species of fungi. Colony growth of *B. bassiana* was inhibited stronger than was the

Table 1. Growth of *Beauveria bassiana* and *B. brongniartii* mycelium affected by insecticides (1.5 mg l⁻¹) after 14 days of incubation; mean values (\pm SE) (n = 30). Significant differences between insecticides and control (columns) are marked with different letters ($P \leq 0.05$).

Insecticide	Fungus mycelium			
	<i>Beauveria bassiana</i>		<i>Beauveria brongniartii</i>	
	Diameter of colony (cm)	Dry matter weight (g)	Diameter of colony (cm)	Dry matter weight (g)
carbosulfan	0.79a \pm 0.02	0.029a \pm 0.0	0.78a \pm 0.03	0.14a \pm 0.04
carbofuran	2.82b \pm 0.02	0.39b \pm 0.02	2.43c \pm 0.03	0.44c \pm 0.02
control	3.35c \pm 0.02	0.48c \pm 0.01	2.23b \pm 0.03	0.36b \pm 0.01

growth of *B. brongniartii*. Carbofuran, however, stimulated the growth of *B. brongniartii* colony but inhibited the growth of *B. bassiana*. In the case of nematodes, carbofuran affected *S. feltiae* on the 3rd and 5th day of incubation and *H. megidis* on the 3rd day of incubation (Table 2). Mortality in

these two insecticide treated nematode species was significantly higher than in the control. Mortality of *S. glaseri* in a solution with carbofuran was even smaller than in the control on the 1st and 3rd day of incubation. The second of the studied insecticides – carbosulfan – also affected nemato-

Table 2. Mortality (mean number of dead individuals per dish) of infective juveniles of *Stainernema glaseri*, *Stainernema feltiae* and *Heterorhabditis megidis* affected by insecticide (1.5 mg l⁻¹) after 1, 3 and 5 days of incubation; mean values (\pm SE). Significant differences between insecticides (columns) are marked with the different small letters.

Insecticide	<i>S. glaseri</i>			<i>S. feltiae</i>			<i>H. megidis</i>		
	1	3	5	1	3	5	1	3	5
carbosulfan	22.7b \pm 0.89	14.4b \pm 1.13	15.8a \pm 3.22	3.30a \pm 0.46	4.7b \pm 0.56	6.0a \pm 0.70	1.80a \pm 0.56	1.4a \pm 0.24	5.7a \pm 1.02
carbofuran	14.1a \pm 2.49	9.0a \pm 0.96	18.6a \pm 1.09	2.0a \pm 0.74	7.3c \pm 0.72	10.7b \pm 1.69	3.80a \pm 0.75	3.80c \pm 0.12	8.1a \pm 1.17
control	22.1b \pm 3.09	15.7b \pm 0.94	16.1a \pm 1.61	2.10a \pm 0.24	2.0a \pm 0.27	4.1a \pm 0.89	3.30a \pm 0.46	2.30b \pm 0.12	4.6a \pm 0.73

des of the species *S. feltiae* and *H. megidis* on the 3rd day of incubation. The mortality of *S. feltiae* was significantly higher and of *H. megidis* – significantly lower than in the control variant. This insecticide exerted, however, weaker effect on the studied nematode species than did carbofuran.

No significant differences were observed in *G. mellonella* larvae mortality 21 days after infection with spores of particular fungi species coming from the insecticide treated culture as compared to the control

variant (Table 3). In the case of *B. bassiana*, mortality of the larvae infected with spores from carbosulfan treated medium was higher than in the control until the 19th day of incubation. Higher larval mortality of *B. brongniartii* was observed only after 7 days since the infection with spores from media with the addition of carbosulfan and carbofuran (Table 3). No effect was found, however, of carbofuran on pathogenicity of *B. bassiana* species.

Table 3. Mortality (% of initial number of individuals) of the wax moth *Galleria mellonella* treated with *Beauveria bassiana* and *B. brongniartii* at the dose of 5×10^5 spores in ml. Fungi had grown previously for 14 days on the medium with carbofuran and carbosulfan insecticides (1.5 mg l^{-1}); numbers marked with different letters in columns are significantly different ($P \leq 0.05$).

Fungus species	Insecticide	Mortality of larvae on the indicated day of incubation (%)						
		7	10	12	14	17	19	21
<i>Beauveria bassiana</i>	carbosulfan	41b	60b	65b	73b	76b	83b	90a
	carbofuran	11a	34ab	39ab	51ab	57ab	64ab	70a
	control	10a	26a	31a	36a	46a	58a	70a
<i>Beauveria brongniartii</i>	carbosulfan	31b	46ab	48ab	61ab	71ab	78ab	84a
	carbofuran	34b	55b	56ab	67b	70ab	73ab	75a
	control	11a	34ab	44ab	50ab	64ab	76ab	88a

Mortality of the insects treated with nematodes, which were incubated with the insecticides did not significantly differ from the control variant irrespectively of the nematode species or insecticide (Table 4). Mortality of *G. mellonella* larvae in the variant with *S. feltiae* IJs incubated with carbofuran, was significantly higher than with nematodes of the same species incubated with carbosulfan on 4th and 5th days of incubation (Table 4). Only in the case of carbofuran treated nematodes of the *S. feltiae* species, significantly greater number of nematodes was observed in the body of larvae (9.8 ± 1.55) as compared to the control variant (4.4 ± 0.67).

As seen from available literature, no studies on the effect of the two carbamate insecticides on organisms like fungi and nematodes have been carried so far. Some authors described other insecticides, which may affect various parameters of biological activity of the entomopathogenic fungi (Bajan *et al.* 1995, Bajan *et al.* 1998). Stu-

dies of other carbamate herbicides showed that they did not influence nematode mortality but might cause a decrease of invasive properties of these organisms towards their host (Kamionek 1992).

As shown in our studies, both insecticides do not decrease pathogenicity of nematodes and entomopathogenic fungi though they may inhibit fungal growth. The mechanism of such a reaction of fungi is hard to explain at the current stage of studies. Our studies demonstrate that both carbosulfan and carbofuran may be recommended to be used in the forest practice in small doses within the Integrated Pest Management (IPM) procedures together with entomopathogenic fungi and nematodes to control *M. melolontha* grubs. It is possible that carbosulfan may stimulate pathogenic process in the case of using entomopathogenic fungi and result in a synergistic effect in the pest control. The combined use of small doses of chemical insecticides (carbosulfan and carbofuran) with pathogenic organisms

Table 4. Mortality of the wax moth (*Galleria mellonella*) larvae (% of initial number of individuals) treated with *Steinernema glaseri*, *S. feltiae* and *Heterorhabditis megidis* at the dose of 50 IJs per insect; nematodes were previously incubated for 5 days with carbofuran or carbosulfan insecticides (1.5 mg l⁻¹); numbers with the different letter in columns are significantly different ($P \leq 0.05$).

Nematode species	Insecticide	Mortality of larvae (%)				
		Days of incubation				
		1	2	3	4	5
<i>Steinernema glaseri</i>	carbosulfan	0	70a	85ab	95bc	95bc
	carbofuran	0	80a	90ab	90ab	90ab
	control	0	80a	90ab	95bc	95bc
<i>S. feltiae</i>	carbosulfan	0	70a	80a	80a	80a
	carbofuran	0	80a	90ab	95bc	95bc
	control	0	75a	85ab	90ab	90ab
<i>Heterorhabditis megidis</i>	carbosulfan	0	100b	100c	100c	100c
	carbofuran	0	95b	100c	100c	100c
	control	0	95b	95bc	95bc	95bc
No nematodes	carbosulfan	0	0	0	0	0
	carbofuran	0	0	0	0	0
	control	0	0	0	0	0

(like the nematodes *Steinernema glaseri*, *S. feltiae*, *Heterorhabditis megidis* or fungi *Beauveria bassiana*, *B. brongniartii*) would enable to decrease chemical environmental contamination and to increase the effectiveness of these organisms in pest control.

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