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EVALUATION OF EFFECT OF *METARHIZIUM ANISOPLIAE* ON  
REDUCTION OF NUMBERS OF *BLATTELLA GERMANICA* L.

OCENA WPŁYWU GRZYBA *METARHIZIUM ANISOPLIAE* NA REDUKCJĘ  
LICZEBNOŚCI *BLATTELLA GERMANICA* L.

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*Estimates were given of effectiveness of infestation of B. germanica L. by M. anisopliae strains originating from different regions of Poland. The investigated strains caused high mortality among experimental insects. Especially effective in reducing numbers of B. germanica were strains Browsk and Pruszyń, which even at a low density of spores caused high mortality of the test insects.*

INTRODUCTION

Various species of insecticidal fungi form known biological factors which play a substantial role in reducing harmful insects. These fungi due to their significance are the best investigated group. Most useful species were found in genera: *Beauveria*, *Metarhizium*, *Paecilomyces*, although they have been encountered in many other taxonomic groups such as: *Aschersonia*, *Aegerita*, *Fusarium*, *Hirsutella*, *Sorosprella* etc. Susceptibility of insects to various fungal species or strains is differentiated and it depends on many factors such as: humidity, temperature or even insects sex. Relationships between fungi and insects is multifold. Many species of fungi coexists with insects supplying them food. On dead insect tissues saprophytic fungi develop. A number of fungi are obligatory parasites of insects. They causes lethal disease known as mycoses. Finally fungi as microbioinsecticides were used as early as in the second half of 19 century.

Among biological factors used for controlling harmful insects some viruses, protozoans and fungi should be mentioned. Many of them were included in preparation proved to be useful biological means to control harmful insects. Up to know these preparations contain viruses which caused nuclear polyedroses, bacteria which caused bacterioses, eg *Bacillus thuringiensis* var. *kurstaki* – killing *Lepidoptera* larvae or *B. thuringiensis* var. *israelenis* – lethal to mosquitos and other insects. Other preparation

has been registered holding some protozoans, eg. *Nosema locustae* or fungi, eg. *Hirsutella thompsoni* lethal for mites living on citrus plants and *Lagenidium giganteum* – a parasite of mosquito larvae.

Preparations based on insecticidal fungi such *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, are being produced besides USA in such countries as former Czechoslovakia, Switzerland or China. In Poland on the basis conidial fungi: *B. bassiana* and *V. lecanii* an industrial production of biopreparation has been elaborated. Methods were patented (Patent No 288661, 1993, No 169335, 1996). *M. anisopliae* is a factor which caused so-called green muscardine disease name such on account of green coloration of hyphae.

The aim of these investigations was to determine pathogenic properties of chosen strains of *M. anisopliae* against *B. germanica* L. as well as to estimate their usefulness for controlling the numbers of these insects.

### MATERIALS AND METHODS

Experiment were carried out on cockroach, *B. germanica* L. with various strains of *M. anisopliae* (Metsch). Strains of *M. anisopliae* were taken from Department of Agroecology, Institute of Ecology, PAS. Five strains of *M. anisopliae* were investigated. Separate strains of *M. anisopliae* were introduced to the insects together with food mixture LSK used to feed laboratory animals. Each strain of fungi was added to food at 3 germ concentrations. Simultaneously water was supplied. Each glass container was supplemented with strip of filter paper accordion-pleated with width about 7 cm.

Experiments were conducted in weck jars at ambient temperatures on adults of both sexes of cockroach. The experimental insects originated from the culture run in the National Institute of Hygiene.

A total 1620 cockroaches of both sexes: 1350 nourished on food with admixture of fungus *M. anisopliae* with different germ density and 270 individuals used as the control were employed in the experiment.

### RESULTS

Mortality of cockroaches feeding on food contaminated with fungus *M. anisopliae* derived from different regions of Poland are presented in Table 1. The results thus obtained proved the high pathogenicity of these strains. During 30 days of experiment, at lowest density of fungus germs in food, mortality of females ranged 30 – 80% that of males: 59 – 85%. At the highest germ concentrations, on the other hand, mortality of cockroaches was higher: 58 – 90% in females, and 68 – 95% in males. After 50 days of experiment the mortality increased to 60 – 99% in females and 80 – 100% in males. In the control insects mortality was always low – 5%. Out of strains tested B strains of *M. anisopliae* from *Browsk* showed high pathogenicity. This strain even at the lowest density of spores showed high mortality in the two period of investigation (Tab. 1). Strain M of *M. anisopliae* deserved also attention. It showed high activity at all spore densities during the two experiments (Tab. I). At the lowest density of spores after 30 days of experiment it caused 80% mortality in females and 85% in males. At the highest spores densities the mortality of insects reached 90% in both sexes. After 50 days these values were still higher. It should be stressed that the densities of spores

of strain M used were more than 9 times higher as compared with those used for *Browsk* strain (Tab. I).

Table I. Mortality of *B. germanica* L. (%) fed with food contaminated with various strains of fungus *Metarhizium anisopliae*.

No.	Strain <i>Metarhizium anisopliae</i>	Spore density $10^6$ in food	% mortality after:				K (%)
			30 days		50 days		
			females	males	females	males	
1.	<i>M. anisopliae</i> B <sub>1</sub>	24	65	70	78	80	5
		48	60	75	83	90	
		72	63	78	99	99	
2.	<i>M. anisopliae</i> B <sub>2</sub>	70	30	58	68	80	
		140	55	70	73	80	
		210	58	68	78	83	
3.	<i>M. anisopliae</i> M	220	80	85	90	100	
		440	80	70	90	85	
		660	90	90	95	100	
4.	<i>M. anisopliae</i> ZP <sub>1</sub>	50	35	65	60	90	
		100	50	50	80	95	
		150	85	95	95	100	
5.	<i>M. anisopliae</i> ZP <sub>2</sub>	230	45	60	90	90	
		460	75	80	95	100	
		690	70	90	80	100	

- 1) B<sub>1</sub> - Browsk
- 2) B<sub>2</sub> - Brynek
- 3) M - Pruszyń
- 4) ZP<sub>1</sub> - Złoty Potok
- 5) ZP<sub>2</sub> - Złoty Potok
- K - control

Pathogenicity of the two strains of fungus derived from Złoty Potok (ZP<sub>1</sub>, ZP<sub>2</sub>) was diversified. Strain ZP<sub>1</sub> causes highest mortality only when the food was treated with the highest amount of its germs, whereas strain ZP<sub>2</sub> caused the highest mortality both when average and the highest dose of spores was added to the food.

After elapse of 50 days mortality of insects increased considerably (Tab. I).

#### DISCUSSION OF RESULTS

The effect of different densities of *M. anisopliae* on cockroaches is presented in Table 1. These data point to a high effectiveness of the fungus against the insect tested.

Among examined strains some differences were found in the level of pathogenicity against cockroaches. The differences became perceivable when the lower density of spores of these strains was added to the insect food. Strains from localities Brynek and Złoty Potok (ZP<sub>1</sub> and ZP<sub>2</sub>) caused lower mortality after 30 days of experiment

(30, 35 and 45%) as compared with strains derived from Browsk (B<sub>1</sub>), Pruszyn (M), these strains caused 65 and 80% mortality (Tab. I).

Males treated with the same strains as females turned to be more susceptible after 30 days of treatment. Their mortality amounted to 58, 65 and 60% of strains from Brynek (B<sub>2</sub>), Złoty Potok (ZP<sub>1</sub>, ZP<sub>2</sub>), respectively, and from Browsk (B<sub>1</sub>) and Pruszyn (M) such mortality amounted to 70 and 85%, respectively. The obtained results after 30 and 50 days of experiments prove that the strains B<sub>1</sub> (from Browsk) and M (from Pruszyn) are most useful for controlling the cockroaches of all strains investigated.

Experiments with entomopathogenic fungi and harmful insects have been conducted earlier. Under the laboratory condition the pathogenic properties of several strains of *Beauveria bassiana* and *Paecilomyces farinosus* against cockroaches (*B. germanica* L.) were investigated [9,10]. The findings suggest that properly chosen strain of *B. bassiana* can control the numbers of *B. germanica*. Trial was also made to evaluate the entomopathogenicity of *Paecilomyces farinosus* against the cockroaches [10]. The laboratory results point to the fact that pathogenicity against cockroaches is diversified depending on strains. Out of 10 strains of *P. farinosus* tested three caused high mortality among insects tested. Diversified pathogenicity found that mortality of test insects depended on strain, spore density and insect sex. This differentiation of pathogenicity of entomopathogenic fungi (*B. bassiana* and *P. farinosus*) against cockroaches was also corroborated by results of investigations carried out both strains of *M. anisopliae*. Pathogenicity of various species of fungi can be affected by different factors (eg. substratum type, temperature, humidity, etc). Bajan and Kmitowa [2] reported that entomopathogenic fungi can undergo various alteration leading to formation of groups, forms or strains etc., more or less aggressive and pathogenic. The authors draw attention to taking into account this phenomenon when examining the usefulness of species of entomopathogenic fungi for biological control. Our studies on *B. bassiana*, *P. farinosus* [9, 10] and *M. anisopliae* seem to confirm this opinion. Among strains of species just mentioned, these were some which even at a low density of spores yielded high mortality of the test insects, and on the contrary, high density of spores of some strain caused low mortality of insects tested under the some experimental conditions. This phenomenon can result from vulnerability or resistance of insects against the fungal strain applied. Bajan and Kmitowa [2] reported that the observed differences in *P. farinosus* strains such as growth rate, spore formation time, amount of spores produced it and their pathogenicity against various developmental stages of Colorado beetle can be manifestation of appearance of different forms or morpho-physiological races or different biotypes of *P. farinosus*.

Entomopathogenic *B. bassiana* is already used for production of biopreparation to protect forest against *Dendrolimus* sp. [5] or to control a pest, *Hylobius abietis*. It was found that during 8 years of storage in the form of powder, *B. bassiana* has lost only 50% of its activity [8]. The Polish preparation based on *B. bassiana* spores have maintained viability in 50% after 3 years of storage at a temperature of 4°C (Bajan et al. [3]). In our southern neighbours, similarly as in Poland technology was elaborated to produce biopreparation based on spores of *B. bassiana* and *V. lecanii* to control the Colorado beetle and orchard and greenhouse pests [3, 6, 9]. We have also information on producing preparation with the use of spores of *B. brongniartii* in Switzerland [1].

In the Great Britain [4] liquid substratum was used to produce biopreparation based on conidial spores of *M. flavoviride*.

It should be underlined that the entomopathogenic fungi are more and more often used to control the plant pests. The best solution should be using the already elaborated technology of production of biological preparation, which could be successfully competitive with chemical insecticidal substances against harmful insects [3].

#### CONCLUSIONS

The obtained results pertain to vulnerability of cockroaches (*B. germanica* L.) to various strains of *M. anisopliae*. They indicate that:

1. effectiveness of tested strains of *M. anisopliae* against cockroach is high.
2. most pathogenic to cockroaches were strains M and Browsk of *M. anisopliae*.
3. *M. anisopliae* brings about the highest mortality of cockroaches in comparison with *B. bassiana* and *P. farinosus* hitherto examined.

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##### Summary

Effectiveness of infestation *B. germanica* L. with strains of *M. anisopliae* originating from different regions of Poland. The tested strains have caused high mortality of the test insect. Especially effective in reduction were strains *Browsk* and *Pruszyn*, which even at relatively low spore density caused high mortality of examined insects.

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#### OCENA WPŁYWU GRZYBA *METARHIZIUM ANISOPLIAE* NA REDUKCJĘ LICZEBNOŚCI *BLATTELLA GERMANICA* L.

##### Streszczenie

Oceniono skuteczność porażania *B. germanica* L. przez szczepy grzyba *M. anisopliae* pochodzące z różnych okolic Polski. Testowane szczepy powodowały dużą śmiertelność wśród doświadczalnych owadów. Szczególnie skuteczne w redukcji liczebności badanych populacji prusaków były szczepy *Browsk* i *Pruszyn*, które nawet przy stosunkowo niskiej gęstości zarodników grzyba wywoływały wysoką śmiertelność wśród doświadczalnych owadów.

#### REFERENCES

1. Aregger E.: Conidia production of the fungus *Beauveria brongniartii* on barley and quality evolution during storage at 2°C. J. Invert. Pathol. 1992, 59, 2.
2. Bajan C., Kmitowa K.: The effect of the medium and temperature on the development of insect pathogenic fungi isolated from the Colorado beetle (*Leptinotarsa decemlineata* Say). *Ecologia*, Polska 1973, 21, 42.
3. Bajan C., Federko A., Kmitowa K., Mierzejewska E.: Polski owadobójczy preparat grzybowy na tle światowych osiągnięć w dziedzinie produkcji mikobioinsektycydów. *Post. Nauk Roln.*, 1994, 3, 13.

4. Jenkins W.E., Prior C.: Production of conidia by *Metarhizium flavoviride* in submerged culture. Program and Abstracts of XXV Annual Meeting of Society for Invertebrate Pathology, Heidelberg 1992, 164.
5. Riba G., Silvy C.: Combattre les revageurs des cultures, 1989, INRA, 230 pp.
6. Samsinakova A., Kalalova S.: Mass cultivation of entomophagous fungus *Verticillium lecanii*. Ceska Mykol., 1976, 30, 18.
7. Samsinakova A., Kalalova S.: Mass production of *Beauveria bassiana* for regulation of *Leptimotorsa dedemlineata* populations. J. Invert. Pathol. 1981, 38, 169.
8. Starcova H., Weiser J.: Shelf life of Boverol the formulated bioinsecticide with conidia of *Beauveria bassiana*. Program and Abstracts of XXV Annual Meeting of Society for Invertebrate Pathology, Heidelberg, 1992, 228.
9. Żukowski K., Bajan C.: Badania przydatności *Beauveria bassiana* do zwalczania prusaków (*Blattella germanica* L.). Roczn. PZH, 1996, 47, 333.
10. Żukowski K., Bajan C.: Badania laboratoryjne aktywności szczepów owadobójczego grzyba *Paecilomyces farinosus* w redukcji liczebności prusaków *Blattella germanica* L., Roczn. PZH, 1997, 48, 133.

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