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EVALUATION OF THE EFFECTIVENESS OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* (BALS. -CRIV.) VUILL. 1912 FOR THE MANAGEMENT OF *MELOLONTHA MELOLONTHA* (L.). (COLEOPTERA: SCARABAEIDAE) AND *AGRIOTES LINEATUS* (L.) (COLEOPTERA: ELATERIDAE)

Summary

The aim of this study was to find the optimal natural substrate for spore multiplication of entomopathogenic fungus Beauveria bassiana (Bals. -Criv.) Vuill. 1912 and to evaluate the effectiveness of B. bassiana for the management of larvae of cockchafer Melolontha melolontha (L.). (Coleoptera: Scarabaeidae) and click beetles Agriotes lineatus (L.) (Coleoptera: Elateridae). In laboratory conditions, among four types of grains, wheat was the most appropriate substrate for mass production of B. bassiana spores was wheat. The highest mortality of white grubs and wireworms was observed five weeks after inoculation B. bassiana spores in concentration 10^6 and was respectively 27.5% and 30%.
***Key words**: Beauveria bassiana, sporulation, white grubs, wireworms*

BADANIA NAD MOŻLIWOŚCIĄ WYKORZYSTANIA ENTOMOPATOGENICZNEGO GRZYBA *BEAUVERIA BASSIANA* (BALS. -CRIV.) VUILL. 1912 DO OGRANICZANIA POPULACJI CHRABĄSZCZA MAJOWEGO *MELOLONTHA MELOLONTHA* (L.). (COLEOPTERA: SCARABAEIDAE) I OSIEWNIKA ROLOWCA *AGRIOTES LINEATUS* (L.) (COLEOPTERA: ELATERIDAE)

Streszczenie

Celem badań było znalezienie optymalnego naturalnego substratu do namnażania entomopatogenicznego grzyba Beauveria bassiana (Bals. -Criv.) Vuill. 1912 oraz określenie przydatności tego grzyba do zwalczania larw chrabąszcza majowego Melolontha melolontha (L.). (Coleoptera: Scarabaeidae) i larw osiewnika rolowca Agriotes lineatus (L.) (Coleoptera: Elateridae). W badaniach laboratoryjnych, spośród czterech rodzajów zbóż, najlepszym substratem do produkcji zarodników grzyba B. bassiana były ziarna pszenicy. Największa śmiertelność pędraków i drutowców obserwowana była po 5 tygodniach od aplikacji zarodników grzyba w koncentracji 10^6 i wynosiła odpowiednio 27,5% i 30%.
***Słowa kluczowe**: Beauveria bassiana, produkcja zarodników, pędraki, drutowce*

1. Introduction

Larvae of the genus *Agriotes* (Coleoptera: Elateridae) and *Melolontha* (Coleoptera: Scarabeidae) are widespread agricultural pests affecting numerous crops throughout the world and causing damage of economic importance [12, 26]. Both, wireworms and white grubs feed below ground causing extensive and lethal damage to the roots. The extent of the root damage increases with the age of the larvae.

Use of biological control agents for insect species has increased the global attention during the last few decades. Among the effective biopesticides some bacteria (*Bacillus* spp.) [11, 22], fungi (*Isaria* spp., *Beauveria* spp., *Metarhizium* spp.) [1, 9, 21, 27] and nematodes (*Heterorhabditis* spp., *Steinernema* spp.) revealed a high efficacy [15]. Entomopathogenic fungi (EF) are a group of microorganisms naturally occurring in environment [2]. The potential of mycoinsecticides based on *Metarhizium anisopliae* (Metschn.) Sorokin 1883, *Isaria fumosorosea* Wize 1904, *Beauveria bassiana* (Bals.-Criv.) Vuill. 1912 and *Beauveria brongniarti* (Sacc.) Petch 1926 against various insect pests, including wireworms and grubs has been proved by many researchers [6, 12, 14, 18, 23]. Spores of EF come in contact with the cuticle of insects, germinate and grow directly into the inner body of their host. Inside the insect's body, fungus proliferates, producing toxins and eventually killing their host [2]. *B. bassiana*, despite the general mode of infection through the integuement, may infect insects per os, particularly insects with chewing mouthparts [7]. High potential of *B. bassiana* also results from the fact that this asexual fungus is easily cultured on media and produces large quantities of conidia [16]. Although commercial products based on this fungus are available, there is a need to test indigenous isolates that are better adapted to the conditions prevailing in Poland. In addition, it is necessary to indicate the solid substrate that determines optimal production of spores of *B. bassiana*.

The aim of this study was (i) to determine the optimal solid substrate for maximal sporulation of *B. bassiana* and (ii) to evaluate in laboratory conditions the effectiveness of EF *Beauveria bassiana* for the management of larvae of two common insect pests: *Melolontha melolontha* and *Agriotes lineatus*.

2. Material and methods

Laboratory experiments were conducted in the years 2015-2016. In the first year of research, four grains were tested for estimation of the sporulation of *B. bassiana*. After that the evaluation of effectiveness of two fungal spores concentration for the management of larvae of *M. melolontha* was done. In 2016 the study concerned the possibility of using the EF to control the population of wireworms of *A. lineatus*. Due to low efficacy in the first experiments with soil insect pest, only higher fungal spores concentration was used. Isolate of *B. bassiana* originated from resources of the Research Institute of Horticulture in Skierniewice. White grubs for the experiment were collected from infested organic orchard in Nowy Dwór, whereas wireworms were gathered from field localized in Zalesie. To exclude natural mortality, larvae were subject to monthly quarantine.

2.1. Spores production of *B. bassiana*

Sporulation of EF was assessed on four types of grains: rye, wheat, maize and rice. 50 g of each grain and 30 ml of distilled water were placed in 250 ml Erlenmeyer flasks and then autoclaved at 121°C for 20 minutes. Each flask was inoculated with three discs from a 2-week-old PDA (BTL) culture of *B .bassiana* and incubated at 25°C. For all tested grains three replicates were used. After three weeks, spores were removed from grains by adding 100 ml of distilled water with 0,05% Triton x-100 (BioShop) and shaken. The spores were counted using the Bürker chamber. The results were statistically analyzed using variance analysis (ANOVA). The significance of differences between means was evaluated using the multiple Duncan's test at a level α=0.05. The analysis was performed in STATISTICA v. 10 (Stat Soft Inc., 2011).

2.2. Pathogenecity tests

Larvicidal bioassay was performed with four replicates, each with 10 larvae of tested pests (*M. melolontha* or *A.lineatus*) for treatment. Spores of *B. bassiana* were cultured on wheat. After three weeks of incubation at 25°C, spores were harvested and the final concentration was adjusted to $1x10^4$ and $1x10^6$, respectively to the treatment. White grubs and wireworms were dipped in 10 ml of the fungal suspension containing spores for 20 seconds. Control larvae were dipped in distilled water. Larvae were placed in plastic cups filled with autoclaved soil originated from the same place where pests were collected. As food source, a slice of carrots (for white grubs) or potatoes (for wireworms) was placed in each cup. Cups were incubated at 25°C in the dark. The humidity was maintained by regular spraying with distilled water. Mortality was assessed at weekly intervals for five weeks post-inoculation. Dead larvae were incubated at 25°C for about 7 days to allow fungal outgrowth of the cadaver. Data were elaborated by regression method according to linear function (y=bx + a) for relation between number of dead larvae counted in successively dates of observation (y) and number days as passing from date of inoculation to successive dates of observation (x). Equation y=bx+a determined regression line, in which parameter "b" in linear relation indicated angle slope of line and means tempo of larvae mortality, and parameter "a" meant initial level of larvae population in day of treatment.

3. Results

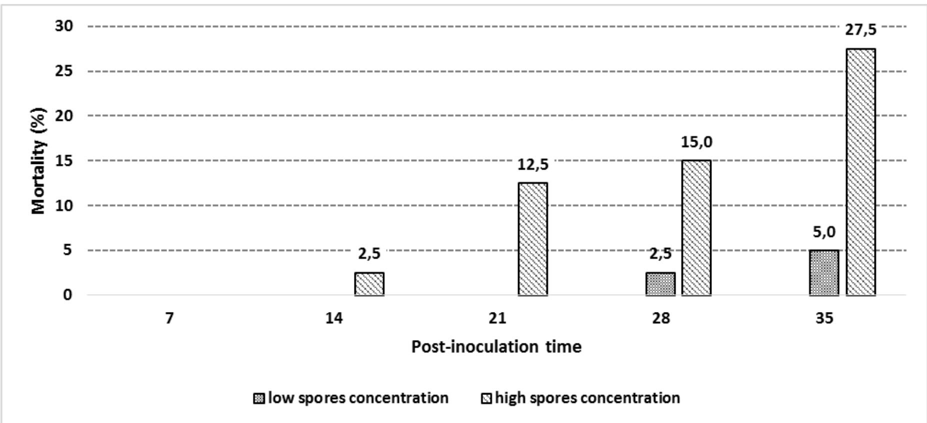
Three weeks after inoculation of four types of grains, spore production of *B. bassiana* was the highest on wheat (Table 1). Average spore yield obtained from 1 g of wheat was 18,52 $x10^5$. The differences between the rice, maize and rye were insignificant. The lowest yield production was recorded for rye (4,83 $x10^5$). Wheat was selected as a solid substrate to spore production of *B. bassiana* for the pathogenicity tests.

Table 1. Spore production of *B. bassiana* on different types of grains
Tabela 1. Namnażanie B. bassiana na różnych rodzajach zbóż

Grains	Average spore yield/1 g of grain ($x10^5$)
Rye	4,83 a
Maize	9,86 ab
Wheat	18,52 b
Rice	6,67 a

Source: own work / Źródło: opracowanie własne

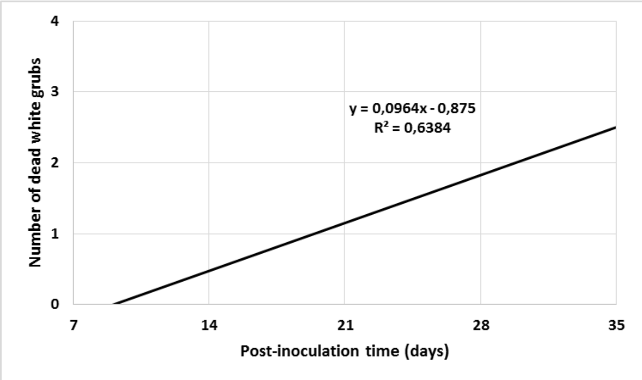
Experiment conducted with *M. melolontha* revealed differences in virulence among tested spore concentration of *B. bassiana*. Five weeks after inoculation of white grubs, mortality reached only 5% in case of lower spore concentration (10^4) and 27,5 % in case of higher spore concentration (10^6) (Figure 1). The percentage of mortality increased proportionally over time. Dead specimens overgrown with hyphae of *B. bassiana* were observed since the second week after inoculation (Figure 2).



Source: own work / Źródło: opracowanie własne

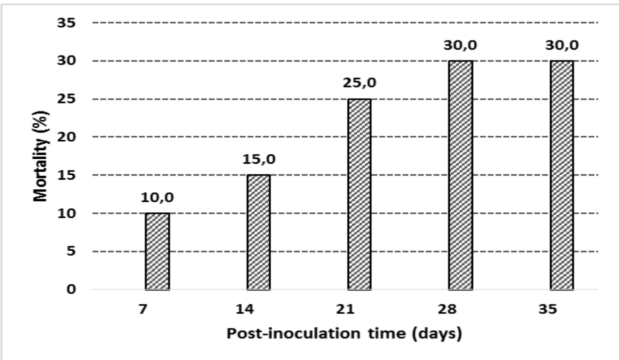
Fig. 1. Mortality of white grubs of *M. melolontha* after treatment with *B. bassiana* (low spores concentration-10^4, high spores concentration -10^6)
Rys. 1. Śmiertelność pędraków M. melolontha po aplikacji B. bassiana (niska koncentracja zarodników 10^4, wysoka koncentracja zarodników 10^6)

The results of the experiment with *A. lineatus* were similar to those obtained for *M. melolontha*. The highest mortality was observed four weeks after inoculation of wireworms and remained at the same level until the fifth week. Effectiveness of 30% was obtained in a shorter time than in the case of white grubs (Figure 3). The number of dead wireworms increased proportionally over time and the first dead wireworms overgrown with white fungal hyphae were recorded one week after treatment (Figure 4).



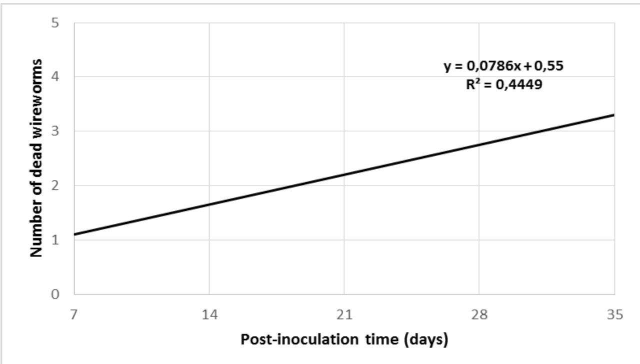
Source: own work / Źródło: opracowanie własne

Fig. 2. Relationship between number of dead white grubs and post-inoculation time of *B. bassiana*.
Rys. 2. Śmiertelność pędraków w zależności od czasu po aplikacji B. bassiana



Source: own work / Źródło: opracowanie własne

Fig. 3. Mortality of wireworms of *A. lineatus* after treatment with *B. bassiana* (spores concentration 10^6)
Rys. 3. Śmiertelność drutowców po aplikacji B. bassiana (koncentracja zarodników 10^6*)*



Source: own work / Źródło: opracowanie własne

Fig. 4. Relationship between number of dead wireworms and post-inoculation time of *B. bassiana*.
Rys. 4. Śmiertelność drutowców w zależności od czasu po aplikacji B. bassiana

4. Discussion

The success of the fungal control of insect pests depends on several factors. Not only isolation, characterization and pathogenicity of entomopathogenic fungus are important. The effective mass production of the microbial agents is also essential. EF are multiplied on various media, both liquid and solid [3, 17, 25]. The substrate selection depends on the fungal species, but also on its isolate. Karanja et al. [10] observed significant differences in the mean weight of spores of the isolates of *B. bassiana* and *M. anisopliae* depending on the type of substrate. Results of present study confirmed the previous observations of Sahayaraj and Namasivayam [20]. Testing the six grains as a solid substrate for multiplication of *B. bassiana*, they recorded significantly higher spore production on wheat. In contrast to our study, in their research rice was found to be the next best media, whereas we harvested from this substrate the lowest spore yield. The use of the wheat as a successful growing substrate for *B. bassiana* was also noticed by El Damir [5]. It seems, that wheat is suitable medium for other entomopathogenic fungi, as was note in respect to *M. anisopliae* [24].

B. bassiana is not well known agent from genus *Beauveria* to control *M. melolontha*. Up to know management of the crockchafer grubs was based on *B. brongniartii* (Sacc.) Petch. [4, 6, 13]. In Germany, where the climate conditions are similar to those in Poland, effectiveness after *B. brongniartii* treatment varied from 4% to 30% [28]. In our study pathogenicity of *B. bassiana* in respect to larvae of *M. melolontha* and *A. lineatus* conducted in laboratory conditions was about 30%. These results indicate that the use of *B. bassiana* against tested insects pests was equally effective as *B. brongniartii*. Previous studies on the control of insect pest indicated differentiated effectiveness of *B. bassiana* fungus. Safavi [19] recorded 100% mortality of *Tenebrio molitor* (Coleoptera: Tenebrionidae) 5 days after treatment, whereas Ansari et al. [1] noted inefficacy in control of *A. lineatus* after application of *B. bassiana*. Other researchers noticed mortality of Scarabaeidae caused by this fungus similar to presented results. Use of *B. bassiana* by Goble et al. [8] on *Schizonycha affinis* gave 34.2 % efficiency.

5. Conclusions

1. Wheat is the optimal solid substrate for maximal sporulation of *B. bassiana*.
2. The use of *B. bassiana* in control *M. melolontha* and *A. lineatus* resulted in about 30% mortality.
3. The effectiveness of *B. bassiana* was not immediate and the maximum efficacy of the treatment was reached after 5 weeks.

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