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Evaluation of *Beauveria bassiana* (986) as a Biological Control of *Alphitobius diaperinus* in Poultry Bed of Wood Shavings

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ABSTRACT

***Background*:** *Alphitobius diaperinus*, lesser mealworm, represents one of the most important pests in the poultry farming industry worldwide. This insect serves as a mechanical host of pathogenic micro-organisms to birds and causes injuries in their digestive tract affecting the feed conversion. Both larvae and adult insects grow in the avian bed, over the soil of sheds and especially near the feeders, where there is a greater availability of food and water. *Alphitobius diaperinus* control, around the world and especially in Brazil, is based exclusively on chemical agents which generates resistant populations. The biological control by entomopathogenic fungi has been shown to be feasible and satisfactory when tested in vitro testing in the recent years. However it still lacks tests to evaluate these agents in environments that simulate field conditions. So, the aim of this experiment was to evaluate a *Beauveria bassiana* (986) as a way of biological control of *A. diaperinus* in poultry bed of wood shavings.

***Material, Methods & Results*:** *Alphitobius diaperinus* were collected from a poultry shed located in Garibaldi in Rio Grande do Sul and sent to the Laboratório Central de Diagnósticos em Patologias Aviárias (LCDPA), Universidade Federal de Santa Maria (UFSM). Then they were cultivated in plastic boxes on the poultry bed and fed as the recommended protocol. The *Beauveria bassiana* (986), isolated from ticks, was purchased from the Department of Entomology of ESALQ/USP and cultivated on Potato Dextrose Agar medium (PDA) at 27ºC, 80% RH for 4 days before the experiment. The conidia suspension was obtained through scraping cultivation with aqueous solution of Triton X-100 (0.01%) in the concentration of 3,83 x 10^8 mL^{-1}. Six plastic boxes were used, containing 8 cm of soil and 10 cm of sterile wood shavings in order to allocate the beetles. In each box, 400 insects (100 larvae and 300 adults) were divided into two groups - G1: 3 boxes treated with 27 mL of suspension conidia (3,83 x 10^8 mL^{-1}) and G2: 3 boxes treated with aqueous solution of triton x-100 0.01% - assisted by 7 days in an average temperature of 32.5 C and 72.4% RH and, after this period, the mortality of both larvae and adults was evaluated. There was no mortality of insects in any stage of the development.

***Discussion*:** Non-occurrence of deaths in the larval stage in this test can be explained by the fact that the larvae have the habit to dig galleries on the ground and they usually remain there to maintain a more propitious temperature to their development, preventing contact with the suspension. It was not assessed a longer time of incubation, because seven days are considered to be the average that approximates the most close to the period of time used in the system *"All in All out"* in the Brazilian poultry farming, period which would be used to apply the fungal solution in poultry sheds. In this experiment, the temperature did not exceed 34ºC, and even so this factor may have affected the growth of fungus in the containers. Another possibility would be a lower virulence of isolated 986 front *A. diaperinus*, since this isolated was obtained from ticks. In this experiment, the *Beauveria bassiana* isolated 986 was not effective for biological control of *Alphitobius diaperinus* in simulated ambient of poultry bed. So, factors as soil, wood shavings, high temperature and incubation time would interfere in the effectiveness.

Keywords: *Beauveria bassiana*, entomopathogenic fungi, *Alphitobius diaperinus*, lesser mealworm, poultry, biological control.

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INTRODUCTION

Alphitobius diaperinus represents one of the most important pests found in poultry industry. This insect serves as mechanical host of pathogenic microorganisms to birds and causes injuries in their digestive tract of then affecting feed conversion [6,7,11]. Both larvae and adult insects grow in the avian bed and the soil of sheds, especially near the feeders where there is greater availability of food and water. Due to its great ability to survive in this environment, populations are able to perpetuate in the period between batch creations, re-infesting the shed and increasing its population gradually [16].

The proliferation of these insects is controlled by chemical insecticides, organophosphates and pyrethroids, with little residual effect, offering risk to poultry health and managers. A study made with populations of A. diaperinus from the state of Paraná demonstrates that there are already resistant insects to the insecticides the most commonly used in Brazil [3].

Alternatively the biological control using entomopathogenic fungi seems to be feasible, since these are harmless to birds and humans [1]. In the literature, some works are found, in which the *Beauveria bassiana* efficiency was evidenced controlling this pest [2,5,10]. In Brazil, the published data related to this subject are restricted to in vitro tests on selection of isolates of B. bassiana and Metarhizium anisopliae, and showing the insect susceptibility in relation to this and variability in virulence of the isolates [4,14,17].

Beauveria bassiana is one of the most promising agents for biological control of arthropods, because it persists in the host population, leads to high mortality rates in larvae and adults, and is easily sprayed [1]. However, the action of entomopathogenic fungi is slow and the majority needs appropriate conditions of temperature and humidity to maintain its viability and pathogenicity. There are not enough studies that evaluate the potential of these fungi in environments that simulate the existing substrate in poultry sheds. For this purpose, this work aimed to evaluate an isolated *Beauveria bassiana* (986) as biological control of *Alphitobius diaperinus*, in the presence of wood shavings.

MATERIALS AND METHODS

The insects were collected from a poultry shed located in Garibaldi (Rio Grande do Sul, Brazil) and sent to the Laboratório Central de Diagnósticos em Patologias Aviárias (LCDPA), Universidade Federal de Santa Maria (UFSM) and grown according to the recommended protocol by Rice & Lambkin [13]. The *Beauveria bassiana* 986 was isolated from ticks and was purchased from the Department of Entomology of ESALQ/USP and cultivated on Potato Dextrose Agar medium (PDA) at 27ºC, 80% RH for 4 days before the experiment. The conidia suspension was obtained through scraping cultivation with aqueous solution of Triton X-100 (0.01%) in the concentration of 3,83 x 10^8 mL^{-1}.

For the test, six plastic containers containing 8 cm of soil were used, as a basis, and 10 cm of sterile wood shavings, simulating a traditional poultry bed environment. In each container, 100 larvae and 300 adult *Alphitobius diaperinus* were allocated. Then containers were divided into two groups: Group 1 (G1) sprayed with 27 mL of fungal inoculum and Group 2 (G2) sprayed with sterile aqueous solution of Triton X-100 (0.01%). Three receptacles were tested in each group. The groups were kept at an average temperature of 32.5ºC and 72.4% RH for seven days and after this period the insect mortality has been verified as the method used by Renault [12].

RESULTS

It was not observed mortality of larvae or adult insects after the seven days of the experiment.

DISCUSSION

Some hypotheses can be raised to explain this result. Da Silva *et al*. [6] did bioassays with *Beauveria bassiana* (986) and *Alphitobius diaperinus* and observed that there was no adult insect mortality in any tested concentrations. On the other hand, there was a mortality rate of 54% among larvae of stages I, II and III, 22.5% in stage IV, V, VI and 9.5% in stage VII and VIII. These indexes were achieved using an inoculum with 3.4 x 10^8 mL^{-1} conidia and it was considered the best condition to cause mortality of insects, justifying the use of 3,83 x 10^8 mL^{-1} conidia in this experiment. Nevertheless, the non-occurrence of deaths in the larval stage of this test can be explained by the fact that the larvae have the habit to dig galleries on the ground and there remain to maintain a temperature more propitious to their development, preventing thus contact with the suspension. This was a significant difference of this

experiment given that the work carried out submit the insects to immersion in solution containing different concentrations of conidia, for times ranging from 5 to 10 min, increasing their contact with the inoculum. In this work, it was noted that a larval mortality that ranged between 77% and 95% at 26ºC and between 2% and 68% to 32ºC, and the adult mortality ranged between 0% and 82% to 26ºC and between 0% and 26% to 32ºC [1].

Another possibility would be a lower virulence of isolated 986 front A. diaperinus, since isolated was obtained from ticks. Geden *et al*.[10] used two B. bassiana, one isolated from lesser mealworm's larvae in natural infection (WV) and other isolated from flies (NC), obtaining a larger insect mortality (100% in larvae) with isolated WV comparing with NC in 10 mL^{-2} conidia suspension.

Other studies have noted that the concentrations of 10^5 and 10^6 conidia mL^{-1} caused low mortality in larvae and adult insects until the 5th day of inoculation in Petri dishes, however on the 10th day after inoculation, the adult mortality ranged from 0 to 25%. For larvae the concentration 10^5 conidia/mL achieved also low mortality levels, however significant differences occurred ranging from 1.7% to 36.7% [15]. In this experiment was not assessed a longer time of incubation, because seven days are considered to be the average that is most close to time used in the system *"All in All out"* in the Brazilian poultry farming, period which would be used to apply the fungal solution in poultry sheds.

Another factor to consider is the temperature used in this experiment. This was based on the average earned in poultry sheds that range from 36,5ºC and 38ºC in the summer. However, in this experiment, the temperature did not exceed 34ºC, and even so this factor may have affected the growth of fungus in the containers, given the optimum temperature for its development is near of 27ºC [2].

It is important to remember that currently, the method of controlling this pest is based in insecticides, especially from the oganophosphates and pyrethroids family, e there are several reports around the world about the development of resistant populations, including in Brazil [3]. So the need for search new alternatives to control this pest is very important, mainly the ones that could not affect the bird's health and do not develop resistant populations.

Other proposals such as the use of phosphine gas [9] and entomopathogenic fungi have been raised [17] as a method of control, but although they have showed encouraging results, they are still far from being used due to some difficult problems with the applying method in the shed, such as the use of phosphine gas, and efficiency of fungi in a so adverse environment to its development.

In this experiment, the *Beauveria bassiana* isolated 986 was not effective for biological control of *Alphitobius diaperinus* in simulated environment of poultry bed. Factors as soil, wood shavings, high temperature and incubation time would interfere in the effectiveness.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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