PATHOGENICITY OF THREE ISOLATES OF THE ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* TO CONTROL *Hyphantria cunea* (DRURY) (LEPIDOPTERA: ARCTIIDAE) LARVAE

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Abstract

The objective of this study was to determine the pathogenicity of *Beauveria bassiana* against *Hyphantri acunea*(Drury) larvae under the laboratory conditions. The *H. cunea* larvae were collected in Georgia and entomopathogenic fungi (PaF04, PaF09, PaF76) were isolated from *Pristiphora abietina* larvae in the previous study. Applications were performed by immersing the larvae in the conidial suspension(10^6 conidia/ml) for 30 s. All fungal isolates tested were pathogenic to the *H. cunea* larvae causing mortality between 90±5,77% and 96,6±3,33%. Mortality caused by *B. bassiana* was not significantly different between the isolates ($p<0,05$). LT_{50} values varied between 2,36±0,31 and 2,53±0,17 days. The present results suggested that *B. bassiana* has an excellent potential for biological control of *H. cunea* larvae. Additional field research is needed to determine effect of *B. bassiana* is at controlling *H. cunea* larvae in the field conditions.

Key words: *Hyphantria cunea*, *Beauveria bassiana*, Biological control, Entomopathogenic fungi.

Introduction

The fall webworm, *Hyphantria cunea* (Drury) is a pest polyphagous feed on the number of host plants exceeds 600 species from wide ranges of forest and fruit trees to several agricultural crops [1, 2, 3].

Native to North America, *H.cunea* spread of trees different areas in Europe and Asia [4]. The larvae of fall webworm are rapid defoliators and feed in huge nests. In severe infestations, larvae are able to completely defoliate host trees. Young larvae feed upon the upper and lower leaf surfaces, leaving the veins. Larger larvae feed on the whole leaves and build impressive silk webs that sometimes enclose entire branches [5].

There has been much research on biological control. The most researched microorganisms are *Bacillus thuringiensis*. Preparations of *B. thuringiensis* kurstaki were reported to be most effective [6, 7, 8, 9, 10]. Other pathogenic organisms are the fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* [6, 11, 12, 13].

Todorova *et al*. reported that pathogens are not associated with a specific host, source or country of origin [14]. In this study used isolates were highly pathogenic to *P. abietina* larvae in the previous study (unpublished data). Therefore, we determined the pathogenicity of this *B. bassiana* isolates against *H. cunea* larvae under the laboratory conditions.

Materials and Methods

Biological material

The *H. cunea* larvae were collected in Georgia and reared on mulberry (*Morus alba*) leaves in an incubator maintained at 25±1^{o}C and 60±5% relative humidity (RH) with 12:12 (L:D) photoperiod. Three isolates of the entomopathogenic fungus *B. bassiana* (PaF04, PaF09, PaF76) were isolated from *P. abietina* larvae in the previous study.

Conidial Suspension and Viability

The isolates cultured in Sabouraud dextrose agar (SDA) and kept for two weeks at 25^{o}C for fungal growth and conidial production [15]. Conidia were suspended in

sterile distilled water ,to which a surfactant (0.2 ml/l Tween 80) was added to reduce clumping of the conidia, and vortexed for 5 min to produce a homogenous suspension [16]. The spore concentration was determined using a haemacytometer and adjusted to 10^6 conidia/ml.

Conidial viability was tested in a SDA plates. Droplets were taken from suspension in sterile conditions and spread over the medium. Sterile microscope cover slips were placed on the plates followed by incubation in the dark at 25±2°C. After 24 h, percent germination was determined from 100 spore counts on each plate [17]. Only conidia with a germ tube as long as the conidium's width were considered to have germinated. Spore suspensions were sealed with parafilm and kept at 4°C in a refrigerator until use [18].

Bioassays

For the applications, freshly cut, 3 cm diameter mulberry leaf discs were placed upper surface down on water-saturated cotton in 9 cm diameter petri dishes. Inoculations were performed by immersing the larvae in the conidial suspension for 30 s. Controls consisted of larvae immersed in distilled water plus Tween 80. *H. cunea* larvae were transferred to these leaf discs. All petri dishes were covered with their tops which had two holes of 6mm diameter for proper ventilation. Petri dishes were placed in a growth chamber at 23±2°C and 65±5% RH with a 12:12 h photoperiod [19].

Mortality was recorded daily for a period of 5 days. Dead larvae were surface sterilized in 70% ethanol, dried and transferred to Petri dishes lined with moist filter paper for 10 days to observe mycosis. Mortality caused by fungi was confirmed by microscopic examination of spores on the surface of the larvae [20].

Statistical analysis

The experimental design was a randomized complete block with three replicates, and each replicate consisted of 10 larvae. Data from the experiment were

subjected to analysis of variance (ANOVA), and probit analysis was used to estimate the lethal time to 50% mortality (LT_{50}).

Results and Discussion

This study was determined the pathogenicity of *B.bassiana* against *H. cunea* larvae under the laboratory conditions. In viability tests, 92–100% of spores germinated and all fungal isolates tested were pathogenic to the *H. cunea* larvae causing mortality between 90±5,77% and 96,6±3,33%. Mortality caused by *B. bassiana* was not significantly different between the isolates ($p<0,05$). LT50 values varied between 2,36±0,31 and 2,53±0,17 days (Table 1). These results confirm the susceptibility of *B.bassiana* on *H. cunea* as reported by a few workers [11, 12, 13]. We have also demonstrated that the highly pathogenic isolates from other host were pathogenic to *H. cunea* in the laboratory. Shaw et al. reported that fungal isolates from non-acarine hosts were pathogenic to *Varroa destructor* (Acari: Mesostigmata), Dimbi et al. reported that the most virulent isolates towards adult fruit flies were recovered from soil [21, 22]. On the other hand, some workers indicate that strains of entomopathogenic fungi are more virulent to insect species from which they were isolated, or from closely related species [17, 23].

Table 1. Total mortality (% ± SE) of *H. cunea* after application of *B. bassiana* conidia suspension.

Fungal Isolates	Mortality (%)*	Lethal Time (day)*
Paf04	96,66±3,33a	2,36±0,31a
Paf76	96,66±3,33a	2,53±0,17a
Paf09	90±5,77a	2,36±0,18a

*P<0.05, within columns, means followed by different letters are significantly different.

The biological control of agricultural and forest-damaging insect pests has been often considered as a favorable alternative to the use of chemical insecticides. The use of biological control agents such as pathogens as part of an integrated pest management

(IPM) strategy could reduce the dependence on chemical control. Fungal entomopathogens have been used more frequently than other types of pathogens for biological control. The present results suggest that isolates PaF04, PaF09 and PaF76 have good potential as a biological control agent within an IPM programme.

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