

# Effectiveness of *Beauveria bassiana* Bioinsecticide against the *Erannis jacobsoni diak*

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## ABSTRACT

We tested the bioactivity bioinsecticide of *Beauveria bassiana* –G07 /*Bassiana muscardin*/ against the Jacobson's spanworm /*Erannis jacobsoni Diak*/, which is the most destructive pest in the forest. The experiment was conducted 5 variants ( $1.5 \times 10^6$  spore/ml,  $1.5 \times 10^7$  spore/ml,  $1.5 \times 10^8$  spore/ml,  $1.5 \times 10^9$  spore/ml, control), 3 times on the larvae in the laboratory in 14 days. For the field experiment, we used 4 variants ( $1.5 \times 10^{13}$  spore/he,  $2.5 \times 10^{13}$  spore/he,  $3.5 \times 10^{13}$  spore/he, control), 3 times in 21 days. From the result of laboratory study the  $1.5 \times 10^9$  spore shows 91.9% bioactivity, and in the field study  $3.5 \times 10^{13}$  spore/he shows 80% bioactivity. Local strain *B. bassiana* – G07 and cultures of *B. Bassiana*, which were isolated from larvae, died during the laboratory experiment, were detected and it was identified as species *B. bassiana* by PCR.

**Keywords:** Jacobson's spanworm, bioinsecticide, bioactivity, *Bassiana muscardin*.

**Submitted :** December 18, 2020

**Published :** March 9, 2021

**ISSN:** 2684-1827

**DOI:** 10.24018/ejfood.2021.3.2.209

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## I. INTRODUCTION

According to the 2014 year – end report of Mongolian land database, agricultural land was 115008.6 thousand hectare or 73.53 per-cent and the forest land was about 14320.5 thousand hectare or 9.16 percent. In the forest, about 188630.9 hectares are damaged and 73.4 percent or 138635 hectares are infected with pest and diseases, and disease and pest control was made all about 32864.6 hectares in forest land [1].

Our country tends to use bioinsecticides against the forest pests. Although the biopesticides used in Mongolia are still 100 % import-ed from other countries and the usage of chemicals are not substituted yet. Therefore, to prove the bioinsecticide of local strain *Beauveria bassiana* by laboratory methods and use this environment, human and animal friendly bioinsecticide against the forest pests is very useful for the economy and food safety.

## II. MATERIALS AND METHODOLOGY

### A. Fungal Strain

Local strain of *B. bassiana* –G07 stored at the Microbiological laboratory in the Institute of Plant protection was investigated in this study. Strain of *B. bassiana*-G07 isolated from infected grasshopper collected in 2007 from pasture. *B. bassiana*-G07 was registered in the NCBI GenBank under MT083997.1

### B. Culturing the Fungus

From stocks, the culture was revived using the yeast extract peptone glucose agar (YPGA – with 2% glucose, 1% peptone, 1% yeast extract and 1.5% agar) slants. They were maintained in a chamber at 25 °C. The suspension of the conidia was

swilled (0.1% Tween-80) from 14 day old cultures for the experiments.

### C. Insect Assays

#### 1. Laboratory experiment

Collect the larvae of Jacobson's spanworm from the larch forest in Erdene soum, Tuv aimag /Coordination: 47077135, 107064451/ using the branch shaking methods, fed in plas-tic containers about 5-7 days in the laboratory and used for the experiments. Added 10 individuals in one container, totally 15 plastic containers were used for the experiment, spraying the bioinsecticides in it, and changed the fir needles in 2 days and counted the number of the dead and survived larvae and determined the bioactivity using Abbott formula.

$$P = \frac{Mo - Mk}{100 - Mk} \times 100$$

$P$  – bioactivity, %.

$Mo$  – mean of the mortality in variant, %.

$Mk$  – mean of the mortality in control, %.

#### 2. Field experiment

The field experiment conducted in larch forest of Erdene soum, Tuv aimag with 4 variants and 3 repetitions for 12 plateaus in 14 days. One plateau is 0, 25 he /50 m: 50 m/, the experiments were conducted in 3 hectares in total.

To determine the distribution of the pest, use short and 16 cm wood for shaking the tree and hit the tree 2-3 times. Before hitting the tree prepare  $10 \times 10$  m cotton or plastics depending on location of the tree crown. After shaking, count the pests coming down and identify with their species. The biological activity in the field experiments was calculated by Leskova formula. [2].

$$E = 100 \cdot \left[ 1 - \frac{(Y_o \cdot X_k)}{(X_o \cdot Y_k)} \right]$$

$E$  – bioactivity.

$X_o$  – total larvae in variants before the experiments.

$X_k$  – total larvae in control before the experiments.

$Y_o$  – survival larvae in variants after the experiment.

$Y_k$  – survival larvae in control after the experiment.

Dead larvae during the experiments were put in wet chamber and checked whether they are infected and died by the *B. bassiana*. Sterilized the outer part of the larvae using weak ethanol solution and took samples from the internal organ and incubated on YPGA medium /yeast 0,2%, peptone 1%, glucose 2%, agar 1,8%/. After the growth of the microorganism used eye or microscope /150-600/ to determine the colonies.

#### D. Molecule Biological Analysis

##### 1. DNA extraction

Culturing the fungus: Stock conidial suspension of fungus culture isolated from larvae was inoculation at 1% into YPG broth (2% glucose, 1% peptone, 1% yeast extract). The culture was incubated (25°C) for 4-5 days on a rotary shaker at 150-180 rpm and harvested by centrifugation.

Using Genomic DNA Purification Kit #K0512 (Fermentas) for DNA extraction.

##### 2. Primers

SCAR primers (Table 1) were synthesized by the Sigma.

TABLE 1: SEQUENCE OF PRIMERS FOR *B. BASSIANA*

№	Primers	Sequence (5'–3') <sup>d</sup>
1	OPA15 F <sub>441</sub>	TTC CGA ACC CGG TTA AGA GAS
	OPA15 R <sub>441</sub>	TTC CGA ACC CAT CAT CCT GC
2	OPB9 F <sub>677</sub>	TGG GGG ACT CGC AAA CAG
	OPB9 R <sub>677</sub>	TGG GGG ACT CAC TCC ACG

##### 3. PCR mixture and conditions

Total of 25 µl of mixture, containing 12.5 µl Dream tag (Fermentas, USA), 1 µl of each primer (forward and reverse primers), 3 µl template DNA, 7.5 µl ddH<sub>2</sub>O.

TABLE 2: EFFICACY OF *B. BASSIANA* AGAINST JACOBSON'S SPANWORM IN LABORATORY

№	Variants /spore/	Larvae in days								Mortality %	Bioactivity %
		0	2	4	6	8	10	12	14		
1.	1.5×10 <sup>6</sup>	10	9.66	8.66	7.33	5.0	4.0	3.33	2.33	76,6	71.9
2.	1.5×10 <sup>7</sup>	10	9.66	9.33	7.66	4.33	3.33	2.33	2.0	80.0	76
3.	1.5×10 <sup>8</sup>	10	9.33	8.0	4.66	3.0	2.66	1.66	1.33	86,6	83.9
4.	1.5×10 <sup>9</sup>	10	9.66	9.0	5.33	3.33	2.66	2.0	0.66	93.33	91.9
5.	Хяналт	10	10	9.33	9.0	8.66	8.66	8.33	8.33	16.66	-

$F > F_{crit}$

$S_x = \sqrt{s^2/n}$  7.85281266

$S_d = \sqrt{2 \cdot s^2/n}$  3.963032339

$HCP_{0.5} = T_{0.5} \cdot S_d$  8.837562116

#### B. Result of Field Experiment

The field study of “*Bassiana muscardin*” bioactivity against the larvae of Jacobson's spanworm with 4 variants in 12 fields were continued in Larch forest of Erdene soum, Tuv aimag /Coordination: 47077135, 107064451/ in 21 days.

The amplication profile was 2 min initial denaturation at 94 °C, 10 cycles of denaturation at 94 °C for 15 s, annealing at 63 °C for 30 s and elongation at 72 °C for 45 s; followed by 15 cycles of denaturation at 94 °C for 15 s, annealing at 63 °C for 30 s and elongation at 72 °C for 45 s, with an additional 5 s for each successive cycle; and a final elongation at 72 °C for 7 min. PCR products were visualized in 1% agarose gels stained with ethidium bromide.

### III. RESULTS

#### A. Result of Laboratory Experiment

The laboratory study was conducted on the larvae of Jacobson's spanworm with 5 variants /1.5×10<sup>6</sup> spore/ml, 1.5×10<sup>7</sup> spore/ml, 1.5×10<sup>8</sup> spore/ml, 1.5×10<sup>9</sup> spore/ml, control/ and 3 repetitions in plastic container for 14 days. During the experiments, counted the survived and dead pests every 2 days and compared the variants with the control. The result is shown in the Table 2 and in the Fig. 1.

From the result 1,5×10<sup>9</sup> spore has shown about 60 percent activity after 8 days and has 91.9 percent bioactivity after 14 days.

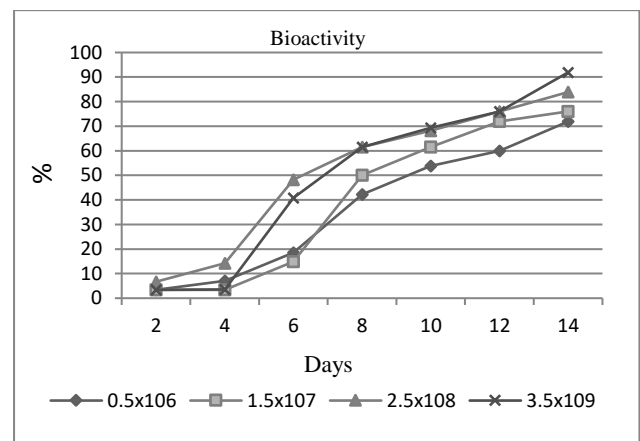


Fig. 1. Efficacy of *B. bassiana* against Jacobson's spanworm.

TABLE 3: EFFICACY OF BIOINSECTICIDE OF *B. BASSIANA* AGAINST JACOBSON'S SPANWORM IN FIELD

№	Variants	Larvae in one tree			Bioactivity %
		0	7	14	
1	Control	75.77	55.77	35	-
2	<i>Bassiana muscardin</i> 2.5×10 <sup>13</sup> spore/he	117.88	54.44	22.88	57.96
3	<i>Bassiana muscardin</i> 3.0×10 <sup>13</sup> spore/he	135.66	66.88	16.66	73.4
4	<i>Bassiana muscardin</i> 3.5×10 <sup>13</sup> spore/he	117.77	45.66	10.77	80.18

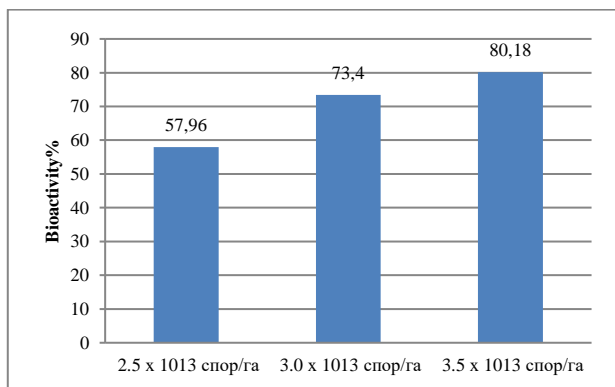


Fig. 2. Bioactivity of “Bassiana muscardin” bioinsecticide against Jacobson’s spanworm.

From the result, we can see the biological activity of  $3,0 \times 10^{13}$  spore/he was 73,4%, and the bioactivity of  $3,5 \times 10^{13}$  spore/he was with 80,18%.

According to the weather forecast during the experimental days, the ambient average temperature was 26,07-26,40 °C and the precipitation was 3,4-3,6 mm, it means the air humidity was insufficient and the average air temperature was convenient.



Fig. 3. Spreading Bassiana muscardin bioinsecticide.



Fig. 4. Larvae killed by *B. bassiana*.

Dead insect for test was placed in Petri dishes covered with wet filtering paper for fungal emergence. Dishes were incubated at  $25 \pm 0.5$  °C in incubator for 7 days.

After host death and utilization of all its internal nutrients, fungus emerged from insect body and produced aerial mycelia and conidia on it (Fig. 5).

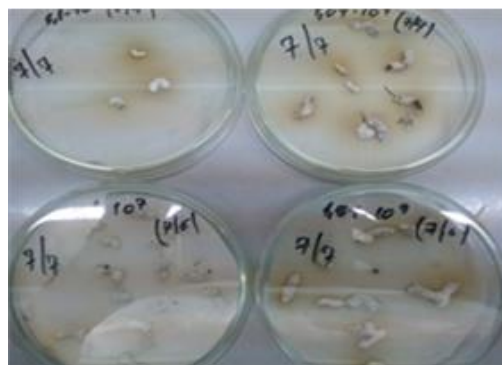


Fig. 5. Emergence of *B. bassiana* from dead larvae.

### C. Results of PCR

Using the above-described PCR protocol, PCR assays strains of *B. bassiana* /*B.b-G07*/, 3 isolated from infected insect cadavers (pending experiment), healthy insect showed that SCAR primers OPB9 F/R677 and OPA15 F/R441 was specific to of *B. bassiana* (Fig. 6).

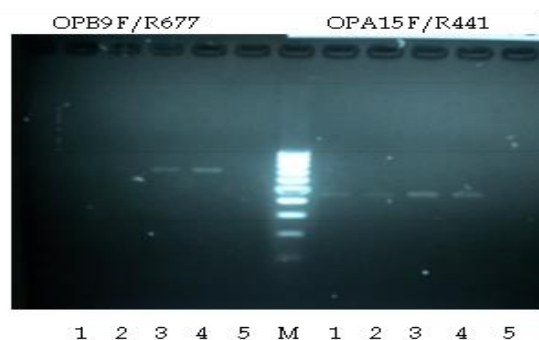


Fig. 6. PCR products of DNA samples detected with *B. bassiana* – specific 677bp gene fragment (SCAR primer OPB9 F/R677) and 441bp gene fragment (SCAR primer OPA15 F/R441). M- Standart marker (100bp); 1; 2; 3 cultures isolated from infected larvae cadavers, died during the laboratory experiment (*B.b-G07* treated); 4- local strain *B. bassiana-G07* 5. Healthy larvae.

PCR assays of genomic DNA from fungal cultures from infected larvae and strain all showed DNA fragments corresponding to the specific product for each SCAR primer.

## IV. DISCUSSION

*B. bassiana* bioinsecticide has never been used before and it is new work for our country. The *Beauveria bassiana* species are tested for usage against forest pests in worldwide. For example, at the Plant protection institute in Bulgaria, researcher S. Draganova et al. [5] studied the *B. bassiana* – 561b strain against in first larvae and second larvae of *Lymintria dispar* L /Gypsy moth/and the bioactivity of  $1 \times 10^8$  spore was 47,78% after 17 days. Nedvekyteet al. [6] researchers of Lietuva tested *Beauveria bassiana*-DPK02 strain and the  $1 \times 10^8$  spore has 100% bioactivity against *Bupalus piniaria* (L), forest pests in 12 days. Our study result has shown 83,9% bioactivity at  $1,5 \times 10^8$  spore against the larvae of Jacobson's spanworm and the bioactivity at  $1,5 \times 10^9$  spore was 91,9% and thus our result has shown the same results.

OPA-14 F/R445, OPB9 F/R677 and OPA15 F/R441 SCAR primers were highly sensitive, capable of detecting

100pg *B. bassiana* GHA genomic DNA, and thus could be used to detect varying levels of the fungus in the field [7].

## V. CONCLUSIONS

1. In the laboratory study the *Bassiana muscardin* bioinsecticide and from the result the bioactivity was 91.9% at  $1,5 \times 10^9$  spore against larvae of Jacobson's spanworm.
2. The bioactivity of *Bassiana muscardin* bioinsecticide was 80% at  $3,5 \times 10^{13}$  spore/he against larvae of Jacobson's spanworm in the field study.
3. The convenient dosage of the *Bassiana muscardin* bioinsecticide is  $3.5 \times 10^{13}$  spore/he.
4. Local strain *B. bassiana* –G07 and cultures of *B. bassiana*, which were isolated from larvae, died during the laboratory experiment, were detected and it was identified as species *B. bassiana* by PCR. SCAR primers OPB9 F/R677 and OPA15 F/R441 was specific to of *B. bassiana*.

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