EFFICACY OF ENTOMOPATHOGENIC LOCAL STRAIN OF *BEAUVERIA BASSIANA* AGAINST THE LOCUST

Otgonjargal KHURELDAGVA[1], Ioana GROZEA[3], Codruta CHIS[3], Purevjargal GANBOLD[1] Battur BANZRAGCH[2]& Enkhbold NANJ[1],

[1]Institute of Plant Protection, Ulaanbaatar, Mongolia
[2]Mongolian University of Life Science, Ulaanbaatar, Mongolia
[3]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania

Corresponding author: Otgooo0529@yahoo.com

***Abstract**. Insects are considered organisms that can be controlled by the Beuveria bassiana fungus. The mode of action of the fungus is through contact, the parasite-host insect interaction being encouraged. From this interaction there is the death of the infected organism. In this case, the target (infected) organism is an insect of the order Orthoptera (locust), the genus Oedaleus (sp. Asiaticus). The purpose of the paper was to evaluate the effect of parasite spores on mortality and, implicitly, population reduction of the locusts. The working method consisted in determining the biological activity of the local Beuveria bassiana (G07) strain against locusts. Thus, it was shown that 100% bioactivity was present at 2.11 x 109 spores/ml in laboratory studies and showed 83.8% bioactivity against locusts from genus Angaracris at 3.5 * 10^{13} spores/ha, in the field experiment (pasture). So, it can be concluded that the Beauveria bassiana biological insecticide can be used at 3.5 * 10^{13} spores/ha against locusts in field conditions.*

***Key words**: insect, fungi, pasture, bio-insecticide.*

INTRODUCTION

It has been reported that there are more than 1000 species of microorganisms, which are pathogenic to harmful insects, most of which are fungi species (about 750). Other representative microorganisms are bacteria, protozoa and viruses. Two species of fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) are commonly used in the biological control of harmful insects. Over the last ten years more than 50 bio-products based on *Beuveria bassiana* have been produced internationally. Their applicability was addressed both to the external field of field, internal, greenhouse, but also to free pastures (ASGHAR AND PORT, 2013; GOETTEL ET AL, 1995; JARONSKI AND GOETTEL, 1991; MANEGUEDA, 1993).

Few attempts have been made at Mongolian level with regard to the use of bio-products in which *Beuveria bassiana* fungus is involved. Starting from this, we can say that research is particularly encouraged about the possibility of fungal isolation and, of course, its practical application. Many insects present in Mongolia (over 200) could be controlled with these bio-products and this have enormous significance as safe to both humans and animals, ecologically friendly and positive for agriculture and food safety (OTGONJARGAL ET AL, 2013; OTGONJARGAL ET AL 2016).

Therefore, to prove the bio insecticide of local strain *Beauveria bassiana* by laboratory methods and use this environment, human and animal friendly bio insecticide against the pests is very useful for the economy (BRADLEY ET AL, 1992).

MATERIAL AND METHODS

The fungal strain. A local strain of *Beauveria bassiana* (Go7) has been studied in the Microbiology Laboratory of the Mongolian Plant Protection Institute. It has been isolated from previously infected (dead) insect (locust) (Table 1). The researches were made in 2007 in a pasture space.

Table 1

The *Beuveria bassiana* strains and insect-host as factors involved in the experiment

The strains	The insect-host (species)	Order/Family of insect	Place of origin
Beuveria bassiana (Go7)	*Oedaleus asiaticus*	*Orthoptera/Acrididae*	Mongolia

About fungi culture. The method of isolation and cultivation of fungi consisted in using YPGA or Agar-Glucose-Peptone-Yeast in the following percentages (extracts): 1.5% - agar -2% glucose, 1% peptone and also extract of yeast in 1%). The all extracts were isolated in a specially room at a constant temperature of 25 degrees Celsius. The suspension containing the fungus conidia was subjected to a cooling process (Tween® 80/0.1 solution) for two weeks.

About insect tests.

Methods for testing the bioactivity of B. bassiana-G07 in the laboratory

In this way, the locusts of the genus Angaracris respectively (*A. barabensis*) were collected from the pastures near the Ulaanbaatar region.

Testing was done for 5 days and stored in xylonite containers (10:15:20 cm) containing fresh grass. There were 10 insects per container (a total of 15 containers). The following 5 variants were included in the analyze, such as 2.11 x 109 spores/(ml), 2.11 x 108 spores/ (ml), 2.11 x 107 spores/ (ml), 2.11 x 106 spores/ (ml) and finally the variant control with Tween® 80/0.1 solution (suspension).

Materials for testing were kept under controlled conditions, but only in natural light and at a temperature of 22 degrees (± 3). Every day, quantification, determination of insect mortality, and removal of dead insects (kept in the refrigerator for a period of two weeks) were made. Then was made observation on the death of locusts infected by *Beuveria bassiana* and also the insect samples were put in containers (Petri dishes) with filtering papers (wet) for 5-7 days.

The spores present on the body of the locusts identified as dead were transposed into the culture medium (mentioned above). This transfer was performed under sterile conditions. In this sense, the locust was sterilized with a light ethanol solution. By analogy, following the analysis of the identified spores, it was found that there was similarity between what was present on the insect's body and the *Beauveria bassiana* spores. For an accurate assessment of the mortality it was considered the Abbott formula to be effective:

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

P – bio-activity %;

Mo – mean of the mortality in treatment, %;

Mк – mean of the mortality in control, %

Methods for testing the bioactivity of B. bassiana-G07 in the field

The field experiment conducted in pasture of Argalant soum, Tuv region with 4 variants and 3 repetitions for 12 plateaus in 21 days. One plateau is 1 hectare, totally the experiments conducted in 12 hectares. To determine the distribution of the locust we using method of foot transect. The biological activity in the field experiments are calculated by Leskova formula.

$$E = 100 \cdot \left[1 - \frac{(Yo \cdot Xk)}{(Xo \cdot Yk)} \right]$$

E – bio-activity
Xo – total locust in experimental variants before the experiments
Xк – total locust in control before the experiments
Yo–survival locust in variants after the experiment
Yк – survival locust in control after the experiment

RESULTS AND DISCUSSIONS

Insect assays

Result of laboratory study. The results obtained by analyzing a number of 150 locusts belonging to the *Angaracris* genus (which were taken as samples from a pasture area) were aimed at highlighting the evaluation of the effectiveness of biological control of harmful locusts by using the *Beuveria bassiana* pathogen.

By detailing, the aspects related to the virulence of parasite identified of the selected insect pest have been analyzed (count of the survived and dead locusts in every day and compare the variants with the variant of control) (Table 2).

Table 2

Evaluation of the efficacy of selected strains of *Beauveria bassiana* (G07), against locust of genus *Angaracris*, in lab conditions

The fungal concentration (spores/ml)	Average values of locusts (active) quantified daily														Efficacy ** (%)
	1d *	2d *	3d *	4d *	5d *	6d *	7d *	8d *	9d *	10 d*	11 d*	12 d*	13 d*	14 d*	
Variant 1 $2.11 * 10^9$	10.0	9.33	9.04	9.04	6.32	3.48	0.29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Variant 2 $2.11 *10^8$	10.0	10.0	10.0	10.0	8.58	8.00	7.61	6.62	4.29	2.07	1.02	1.02	1.02	1.02	86.31
Variant 3 $2.11 * 10^7$	10.0	10.0	10.0	10.0	9.62	9.34	8.60	8.02	7.01	4.32	3.33	2.59	2.59	2.59	63.49
Variant $42.11 * 10^6$	10.0	10.0	10.0	10.0	10.0	9.61	9.33	9.01	8.30	7.00	5.30	4.62	4.02	4.02	45.21
Variant of Control	10.0	10.0	10.0	10.0	10.0	10.0	9.32	9.32	8.63	8.63	8.31	7.64	7.35	7.29	

** The day of quantification of active locusts, **percent of mortality of the locusts*

Applying the ANOVA test, there were significant differences between treatment and control groups ($p = 0.000$, $F = 5.876$).

Following the Post-Hoc analysis, significant differences were noted in the table below (table 2a).

Table 2a

Efficacy of treatment by ANOVA test

Groups	2.1 x 10^7	2.1 x 10^8	2.1 x 10^9	Control
2.1 x 10^6	0.88 P=0.46	2.00 P=0.09	4.24* P=0.00	-1.01 P=0.39
2.1 x 10^7		1.12 P=0.35	3.36* P=0.01	-1.89 P=0.11
2.1 x 10^8			2.24 P=0.06	-3.01* P=0.01
2.1 x 10^9				-2.24 P=0.06

By the table description we can see that the native strain of the *Beuveria bassiana* pathogen (Go7) expresses virulent evidence on the locusts analyzed and quantified later. It can be ascertained that the isolated strain pathogen had the effect to the maximum or even maximum recorded in variant 1 with a high concentration of spores (2.1 * 109 / ml). Good results were also obtained in variant 2 with conidia / ml concentration, 2.1 * 109 conidia / (ml), where the percentage was over 80% and in the other variants (V3 and V4) the results were good, concentrations were lower. Thus, it can be concluded that although there were different values, no significant difference was observed in the variants analyzed.

The results obtained at the maximum concentration of 2.11 * 109 spores/ (ml) showed a 100% value of bioactivity after 8 days after infection. After 10 days of infection with spores at a slightly lower concentration of 2.11 * 108 spores/ (ml) the bioactivity level reached 86.31% (Graphic 1).

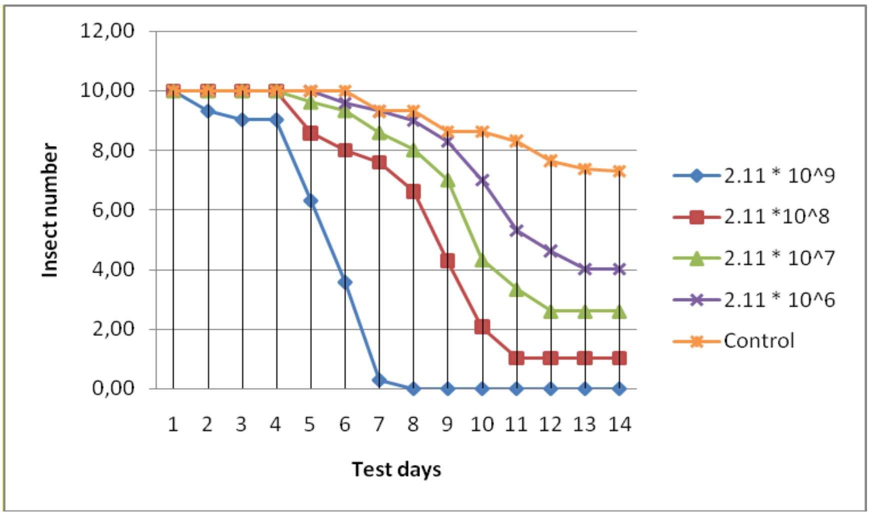

FIGURE 1 Mortality rate among infected locusts

This result is somewhat consistent with the researchers like as ASGHAR AND PORT who in 2013 mentioned that at a high concentration of spores, the mortality rate is especially high on the eighth day and the seventh day respectively. By comparison with *Beuveria bassiana*, other fungi caused mortality to peak values only on the tenth day of ingestion and on the eleventh day in the case of contact interaction. It can be said that making an analogy with the existing international demands, there is a confirmation of these at the level of the test country, Mongolia.

Other researches by ROBERT MANEGUEDA OUEDRAOGO in 1993 revealed that the use of the *Beauveria bassiana* strain to combat *Oedaleus senegalensis* species showed a bio-activity of approximately 70%, lower in the beginning and with a gradual slow start, starting on the sixth day. It can be said that, through our research, five days after the use of the bio-preparation, the occurrence of bioactivity and implicitly the death of infected insects was observed.

Result of field study. For 21 days, field research on the bioactivity of *Beuveria bassiana* fungi against grasshopper was carried out continuously. These have been extended to 12 hectares of pastures in 12 hectares. The division of the experimental lots was done mainly in 4 variants of 3 repetitions (picture 1).

Picture 1. Time spraying with *Beuveria bassiana* bio-insecticide (G07), in under observation pasture	Picture 2. Aspect of locust dead in field after spraying	Picture 3. Dead insects with germinated spores, in the laboratory room (wet room)

In the field studies were present a lot of species of locusts, which were identified directly in the field. Among these it can be mentioned follows: *Angaracris barabensis*, *Arcyptera* (*Pararcyptera*) *microptera*, *Bryodemella tuberculatum*, *Bryodema luctuosaluctuosa*, *Stenobothras carbonaras*, *Bryodemella tuberculata diluta*, *Arcyptera meridionalis*, *Dasyhippus barbipes* and *Myrmeleotettix palpalis.*

In order to express the bio-activity of fungus in control of locusts it was determine the total number of locusts in different periods (days) respectively 7, 14 and 21 days. Using foot transect methods at each section and compare the number with the control, the quantified of locust were made readings after and before the experiment (Table 3).

From the table 3 it can see that the biological activity of variant of concentration 2.5/3,0 x 10^{13} spores/ha was about 72%, and the bioactivity of variant of 3,5 x 10^{13} spores/ha was 83,8% (Pictures 2, 3).

Table 3

The bio-activity of *B.bassiana (Go7)* against the locust

Variant Concentrations of *Beuveria bassiana-Go7*	No of Rehearsals	The insect number				The bio-activity (%)
		Before treatment	After treatment			
			7^{th} day	14^{th} day	21^{th} day	
Variant Control	3	286	196	108	128	-
Variant 1 2.5×10^{13} spores/ha	3	305.55	149.88	42,11	40.33	72.55
Variant 2 3.0×10^{13} spores/ha	3	310	133.11	40,11	41.66	72
Variant 3 3.5×10^{13} spores/ha	3	320.67	92.67	33.88	24.88	83.86

CONCLUSIONS

The result in order to determining the biological activity of *Beuveria bassiana (G07)* strain against locust showed 100% activity at 2.1 x10^9 spores/ml in the laboratory experiment, and at the field studies it was showed an bioactivity of 83,8% against locusts (at 3.5 x 10^{13} spores/ha).

The bio-insecticide based on *Beauveria bassiana* can be used at 3.5 x10^{13} spores/ha against locusts in pasture, because very good efficacy.

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