STUDY RESULTS ON EXPERIMENTING BIOPRODUCT OF *M.ANISOPLIAE G09/22* LOCAL STRAIN AGAINST ECLIPOPHLEPS BOGDANOVI SERGTARB

B.Oyuntogtokh*, D.Dulamjav, M.Otgonzaya, D.Nasanjargal, M.Byambasuren

Plant Protection Research Institute, MULS, Mongolia

*-Corresponding author, E-mail: oyuntogtokh@plantprotection.mn

ABSTRACT

It is being main aim to reduce the usage of chemical substances for plant protection, in Mongolian agricultural sector, by isolating and of course determining local strain that can fit in condition of our country specifically to control harmful pests that are having negative impact on the environment especially in the forest and pasture plants. Thus, research work concentrated on isolating bioproduct based on local strain of entomopathogenic fungi Metarhizium anisopliae and to determine its biological activity. Bioproduct of Ma.Mn-G09/22 local strain have been experimented with 3 variants with 3 repetitions and comparing it with control in Munh-Khairhan sum of Tuv aimag. Our study involved open field and vivarium experiment which both sh
owing similar results, providing local strain G0922 of Metarhizium anisopliae fungi can be effective against harmful grasshopper Eclipophleps bogdanovi sergtarb under field conditions.

KEY WORDS: *Metarhizium anisopliae* G09/22 local strain, suspension, spore/ml, grasshopper.

INTRODUCTION

In order to increase the crop yield of grassland and agriculture, to produce safe and healthy food supply as well as to prevent and control insect pests, plant diseases and weeds, optimal methods and technology need to be developed and introduced.

In Mongolia, beginning from 20th century specifically at the end of 1960s chloride compound insecticide 12% Hexachlor have been used as a control method for grasshoppers that are harmful to the grassland. Then from the end of 1990s Pyrethroid compound insecticides 5% Decis and 20% Sumicide have started to take place (Insect laboratory, PPRI, 2007).

Out of 7000 species of grasshoppers belonging to *Acrididae* family, *Orthoptera* order, 137 species of grasshoppers (7 species of nymph) are distributed in Mongolia. So forth around 30 species of saboteur grasshoppers to pasture crop yield, are increasing year to year in our natural field (Chuluunjav, 2003).

Without polluting the environment, protecting grassland and forest from insect pest and harmful organisms in accordance with increasing population is becoming primary objective.

Entomopathogenic microorganisms are necessary for keeping the population of pests at certain point with its antagonist characterization.

In recent years, globally refusing to use chemicals to control pests instead interested in isolating microorganisms with low toxicity, less residues in environment and specific to certain pests and its usage have been increased continuously.

Researchers have found about 750 species of entomopathogenic fungi included in 85 genuses of 6 orders worldwide (Byambasuren, 2012).

In terms of diversity they rival the plant pathogens, while there are comparatively few (about 40) fungal pathogens of warm-blooded animals (Rippon, 1988).

Insect-pathogenic fungi such as *Metarhizium anisopliae* have been extensively studied as key regulatory factors in insect populations and as agents of biocontrol (Inglis *et al.*, 2001). Based on various studies done on entomopathogenic fungi *Metarhizium anisopliae* is proven to be effective against harmful insect pests. In this study we are experimenting newly isolated local strain of *Metarhizium anisopliae* called G0922. This local strain has been isolated from naturally infected locusts found in various locations of Mongolian provinces. Hence, feature of G0922 strain can be differentiated by yellowish green to green colonies formed when grown on PDA. Under microscopic analysis conidial spores can be seen in chains. (Byambasuren, 2012).

MATERIALS AND METHODS

0,05% of Tween 80 solution, dry mass of *Ma.Mn-G09/22*, and glycerin oil formulations prepared to improve the efficacy of spores against locusts.

Suspension preparation. 9 ml of 0,05% Tween 80 solution is added to each of the 4 purified test tubes. Then 1 ml of *Ma.Mn-G09/22* pure culture is added to first tube and mixed thoroughly. According to Koch's dilution method 1ml of previously mixed solution is transferred to next tube until the last tube become 10ml.

Counting number of spores contained in 1ml of suspension. Total number of spores in diluted suspension calculated using counting slide.

Following formula is used:

$$C = \frac{K \times N}{V}$$

C – number of microorganisms in 1g or 1ml of culture

K – dilution grade

N – Mean number of cell counted in 1 square of counting slide

V – volume of 1 square in counting slide /1:4000000 $см^3$/ [1,2,6,8]

Vivarium experiment. Grasshoppers picked up with hoop net and transferred into 2m x 2m plateau and sealed.

Table 1

Number of insects in each vivarium

№		Variants	Repetition	Number of grasshoppers
1	*M.anisopliae* 10^7	I	1	78
		II	2	114
		III	3	106
2	*M.anisopliae* 10^8	I	1	132
		II	2	88
		III	3	63
3	*M.anisopliae* 10^9	I	1	103
		II	2	84
		III	3	108
4	Control			128

Open field experiment. Randomly selecting 200 by 20 m of 4 square fields and mark its 4 ends.

Table 2

Insect density

№			Number of grasshopper	Field size
1	50 sweeping	*M.anisopliae* 10^7	239	200x20
2		*M.anisopliae* 10^8	101	200x20
3		*M.anisopliae* 10^9	134	200x20

Spraying suspension. Before spraying suspension 20 liter loads of manual sprayer, safety clothing with mask and rubber gloves needed. In vivarium and open field 3 variants have been sprayed using AutoMax sprayer to crop plants.

Technical results based on control method can be calculated by following formula:

$$E = \frac{(A - B)}{A} \times 100$$

E- technical result of control method

A- Mean number of insects before control

B- mean number of remaining insects after control

RESULTS

Study results on experimenting bio product *M.anisopliae*G09/22 local strain against pests in the field

Table 3

Vivarium experiment (3 days after experiment)

№	Dose (spore/ml)	Number of pests (Before using product)	Number of pests (After using product)	Technical result (%)
1	Control	128	125	-
2	*M.anisopliae*10^7	78	5	93,9%
		114	11	
		106	2	
3	*M.anisopliae*10^8	132	38	77,1%
		88	25	
		63	2	
4	*M.anisopliae*10^9	103	7	91%
		84	4	
		108	15	

Table 4

Open field experiment

21 days after experiment				
1	Control	380	369	-
2	*M.anisopliae*10^7	239	17	92,8 %
3	*M.anisopliae*10^8	101	8	92 %
4	*M.anisopliae*10^9	134	13	90,2 %

Mongolian main agricultural sector is livestock. Thus degradation of grassland resulted by haymaking in consecutive year in turn creating favorable environment for omnivorous insects and increasing their reproduction and distribution. In 2010, according to field experiment done by M.Byambasuren (Ph.D.) $2,8*10^8$ spore/ml dosage of *Ma.Mn-G09/22* local strain have resulted in 86,6% death in vivarium experiment. In 2013, $2,62*10^4$-10^7 spore/ml dosage of *Ma.Mn-G09/22* local strain was 80-100% effective when experimented on *(Chothippus allbomarginatus) and (Chothippus allbomarginatus)* in laboratory condition.

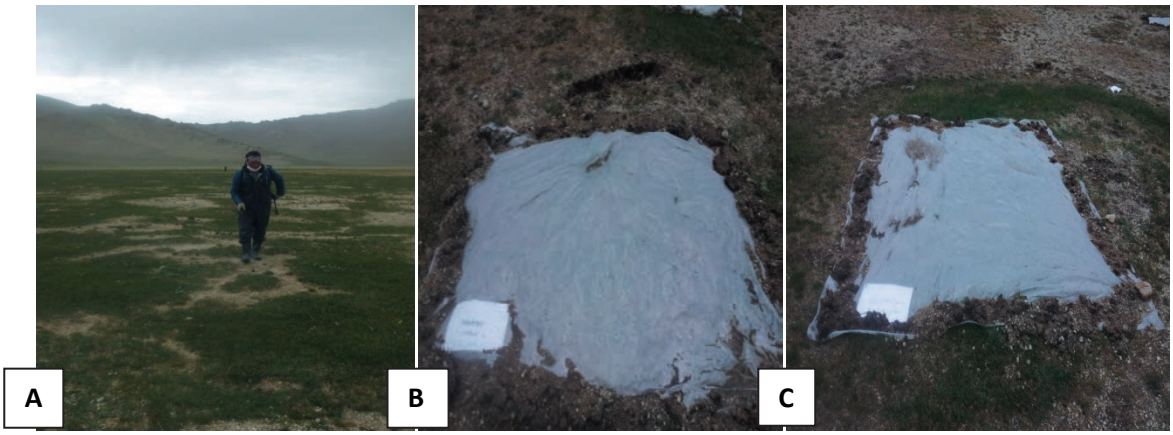

Figure 1. Process of spraying bio product of *M.anisopliae G09/22* local strain in open field. A, manual sprayer of 20 liters load. B, ULVA sprayer with low volume. B, 1st variant of *M.anisopliae G09/22* local strain bio product sprayed vivarium. C, 2st variant of *M.anisopliae G09/22* local strain bio product sprayed vivarium.

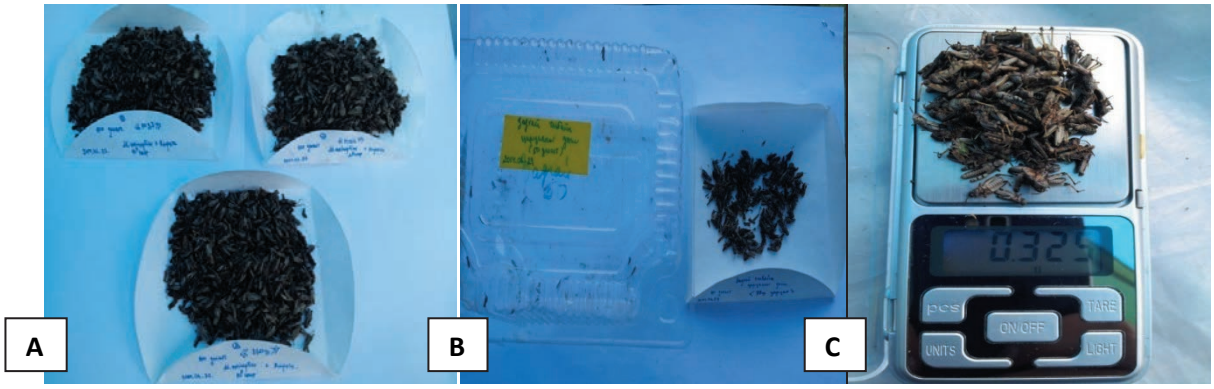

Figure 2. Sample of grasshoppers killed by *M.anisopliae G09/22* local strain infection. A, determining density of grasshopper collected from open field. B, Dead grasshoppers after treated with fungus bioproduct. C, weighing grasshoppers in one paper bag

Figure 3. Growth of *M.anisopliae G09/22* fungi on outer surface of grasshopper. A, fungi growth within 2-3 days on surface of grasshopper placed on Petri dish covered with wet filter paper. B, Magnified one of the grasshoppers placed in Petri dish. C, Grasshoppers after treated with *M.anisopliae G09/22* local strain.

CONCLUSION

Ma.Mn-G09/22 local strain bio product experimented in vivarium was 91-93,9% effective while in open field experiment 90,2-92,8% effective on *Eclipophleps bogdanovi Serg.Tarb*.

Therefore, entomopathogenic *Ma.Mn-G09/22* local strain can be used and produced to control harmful grasshopper in grassland and it can replace chemical insecticides since it is microbiological method.

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