Beauveria bassiana (Bals.) Vuillemin. Pathogenicity to grass-hoppers (Orthoptera: Acrididae). Lethal-time as related to the form and concentration used.

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ABSTRACT

The entomopathogenic fungus *Beauveria bassiana* (Bals.), Vuillemin, strain LARC/FB-192, isolated from naturally infected larvae of *Zeuzera pyrina* was found pathogenic to grasshoppers. The time required for the pathogen to express its action, as determined from the observed percent mortality, appeared to be related to the form and the concentration used.

High mortality rates of 100% resulted within 7 and 14 days when insects were treated with the dry-spore form (DSP) in either of the two concentrations. Treatment with the higher concentration (4.2×10^7spores/ml) of the spore-suspension form (SPS) led to mortality rates of 82.5 and 87.5% within 7 and 14 days, respectively.

The concentration of the pathogen, within the limits used in this experiment, showed a significant effect on the observed lethal-time (LT_{50}) when used as (SPS). The LT-50 was reduced from >7-days to <3-days as a result of increasing the concentation dose by a hundred-fold (from 3.8×10^5to4.2×10^7 spores/ml).

INTRODUCTION

Grasshoppers constitute a group of insect pests that are highly destructive to several crops throughout the world. Being capable of long distance flight (1) grasshoppers may cause devestating attacks and epizootics may eventually cover a vast area. The problem does not only concern the developing countries in Africa, but also extends to the northern hemisphere where great losses in wheat production in Canada, with an estimated value of 40 million as a result of grasshopper attacks were reported (2).

In arid and semi-arid zones, grasshopper outbreaks may cause serious disasters especially if agricultural production is practised through dry-farming system. Under such conditions crop stress due to lack of adequate moisture will intensify the damage caused by grasshoppers, particularly if hatching is synchronized with seedling emergence (5).

A variety of chemical insecticides have been used to control grasshopper populations, however biological control particularly the use of mycoinsecticides may

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offer the safest and the most economical alternative (1). Observations support the use of *Beauvaria bassiana* as a bioinsecticide against grasshoppers in semi-arid areas where a 100% mortality rate was achieved *in vitro,* 14-days post-treatment with a spore suspension of the entomopathogenic fungus (3).

B. bassiana, regardless of the mode of application (topical, injection, or oral), proved to be infectious to grasshoppers resulting in high mortality rate ranging between 82 and 100% (4). The application mode of the pathogen appeared to have an effect on the concentration of the infectious dose required to give high mortality rate and also on the longevity of time before the effect was observed (4).

The aim of this study was to test the possible use of *B. bassiana* in controlling grasshopper populations in Libya, with particular emphasis on the proper mode of application, and age of the culture in order to attain high mortality rates.

MATERIALS AND METHODS

Adult grasshoppers used in this study were collected by sweep sampling of an alfalfa field in Zawia (25-miles west of Tripoli). The insects were reared, before executing the experiment, in screen-cages at room temperature (26° ± 4° C) and on alfalfa and wheat-bran.

The entomopathogenic fungus to be tested for its mycoinsecticidal action was originally isolated from naturally infected larvae of *Zeuzera pyrina.* After being characterized in the laboratory as *Beauveria bassiana* (Bals.) Vuillemin, the fungus was designated as strain LARC/FB-192. To test its pathogenicity the fungus was grown in flasks on Sabouraud-dextrose agar (SDA) slants supplemented with 2% years-extract (w/v), and two forms of spore preparations were used: a dry-powder form (DSP) and a liquid-form (SPS). The surface growth in the culture flask was used as such for (DSP) form whereas the suspension (SPS) was made by adding a 100ml of sterilized Ringer's solution (1/4 strength) to the culture flask and the surface growth was swabbed with a prestirilized pipette. The mixture was then shaken mechanically for one hour and filtered into a sterilized vessel. Two drops of Tween-20 were added and the total number of spore/ml were counted using a hemocytometer. The suspension was then held in the refrigerator at 4° C until needed.

In the first experiment, forty adult grasshoppers were divided into four replicates (10 insects, each), and each group was dropped into a culture flask (DSP, 9-days old). The insects were left to move freely on the slant surface for 3 to 5 minutes to insure good contact with the fungal spores, after which they were set free into a screen cage and were provided with alfalfa and wheatbran. The moisture inside the cages was controlled using water saturated cotton-pads. A set of four raplicate controls (10 insects each) were also used.

Another set of forty adult grasshoppers were divided as before and each group of 10 insects was placed in a sterilized flask. One milliliter of the prepared (SPS), 9-days old, containing 3.8×10^5 conidia was pipetted into each falsk. The insects were manipulated by rotating the flasks manually for 3 to 5 minutes to get each one of them in touch with the suspension. The hoppers were then released into screen cages provided with the same amendments described above. The four control replicates were treated with 1 ml of Ringer's solution for the same increment of time.

The second experiment was carried out in the same manner with only one variation. The (DSP) culture was 20-days old and the (SPS) prepared after that period of growth showed a total spore count of 4.2×10^7 conidia/ml.

RESULTS AND DISCUSSION

Grasshoppers reponded positively to the entomopathogenic fungus *B. bassiana* strain LARC/FB-192, isolated from naturally infected larvae of *Z. pyrina*.

Pathogenicity tests, in vitro, indicated that the isolate is a potential mycoinsecticide when applied topically, causing mycosis of the treated insects with high mortality rates of 100% within 7 and 14 days when insects were treated with the dry-spore form (DSP) in either of the two concentrations. Treatment with the higher concentration (4.2×10^7 spores/ml) of the spore suspension (SPS) led to mortality rates of 82.5 and 87.5% within 7 and 14 days, respectively. Statistical analysis of the data presented in (Table 1), following three-factors complete-randomized design, showed highly significant differences between lethal-time and the form and concentration used with a calculated coefficient of variability of 17.79%.

Table 1 – Pathogenicity of ***B. bassiana*** to grasshoppers as determined by the percent mortality of the treated insects.

				% Mortality		
Form[a]	**Cond.**[b]	**Rep.**	**Days/**	3	7	14
SPS	1	1		0.0	30.0	70.0
		2		30.0	30.0	70.0
		3		20.0	70.0	90.0
		4		30.0	60.0	90.0
SPS	2	1		80.0	100.0	100.0
		2		50.0	80.0	80.0
		3		60.0	80.0	100.0
		4		60.0	70.0	70.0
Cont.	–	1		30.0	30.0	30.0
		2		20.0	30.0	30.0
		3		30.0	30.0	30.0
		4		20.0	30.0	30.0
DSP	1	1		20.0	100.0	100.0
		2		40.0	100.0	100.0
		3		10.0	100.0	100.0
		4		10.0	100.0	100.0
DSP	2	1		80.0	100.0	100.0
		2		50.0	100.0	100.0
		3		60.0	100.0	100.0
		4		80.0	100.0	100.0
Control	–	1		10.0	30.0	30.0
		2		30.0	30.0	30.0
		3		20.0	20.0	30.0
		4		30.0	30.0	30.0

Coefficient of variation: 17.79%.

[a] SPS = liquid-form, DSP = dry-powder, Cont. = non-treated control

[b] 1 : 3.8×10^5 spores/ml, 2 : 4.2×10^7 spores/ml

The calculated average percent mortality (Table 2) showed an LSD-value of 14.47 (at $\infty = 0.05$) between the means as a result of the interaction of the three factors suggesting that topical application of the fungus in the (DSP) form is more efficient in controlling grasshoppers at both concentrations used. The observed lethal-time required to give 50% control (LT_{50}) was between 3 and 7-days.

Table 2 – Average [a] percent mortality of grasshoppers.

Form	Conc.[b] \ Days	3	7	14
SPS	1	20.0	47.5	80.0
SPS	2	62.5	82.5	87.5
Cont.	–	25.0	30.0	30.0
DSP	1	20.0	100.0	100.0
DSP	2	77.5	100.0	100.0
Control	–	22.5	27.5	30.0

LSD-value at ($\infty = 0.05$) = 14.47

[a] Average of four replicates, 10 insects/replicate.

[b] Conc. 1 = 3.8×10^5 spores/ml, 2 = 4.2×10^7 spores/, 1.

Applying the pathogen in the (SPS) form seems to benefit from increasing the concentration dose, both in terms of producing a higher mortality rate at a given increment of time, and in reducing the observed (LT_{50}) from more-than 7-days, when used at a concentration of 3.8×10^5 spores/ml, to less-than 3-days at a concentration of 4.2×10^7 spores/ml.

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أمراضية الفطر *Beauveria bassiana* لحشرة النطاط (Orthoptera: Acrididae). زمن موت الحشرة وارتباطه بشكل وتركيز المستحضر الفطري المستخدم

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المستخلص

عزل الفطر الممرض للحشرات *Beauveria bassiana* محلياً من يرقة حشرة حفار ساق التفاح *Zeuzera* pyrina مصابة طبيعياً، ووجد أن الفطر ممرض أيضاً لحشرة النطاط. اتضح أن الزمن اللازم لإظهار فعالية الفطر الإمراضية محدداً من نسبة الموت المشاهدة مرتبط بشكل وتركيز المستحضر المستخدم.

معدلات الموت كانت عالية وبلغت 100٪ خلال 7 و14 يوماً عند معاملة الحشرات بالفطر في صورة أبواغ جافة في كل من التركيزين المستخدمين. المعاملة بالتركيز الأعلى (4.2×10^7 بوغ/ملل) في صورة معلق من الأبواغ أعطت معدلات بلغت 82.5 و87.5٪ خلال 7 و14 يوماً، على الترتيب.

تركيز الكائن الممرض في الحدود المستخدمة في هذه التجربة، أظهر تأثيراً معنوياً على زمن الموت المشاهد LT-50 عند استخدام الفطر في شكل معلق من الأبواغ، وذلك بخفضه من أكثر من 7 أيام إلى اقل من 3 أيام كنتيجة لزيادة التركيز مائة مرة (من 3.8×10^5 إلى 4.2×10^7 بوغ/ملل).