Pathogenicity of Natural Preparations of the Entomopathogenic Fungus *Beauveria bassiana* (Bals) Vuill on the Imported Cabbage Worm *Pieris rapae* L. (Lepidoptera: Pieridae)[1]

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ABSTRACT

The fungus *B. bassiana* was isolated from naturally infected larvae of leopard moth *Zeuzera pyrina* L. The isolate designated as strain LARC/FB 192 was found pathogenic to the imported cabbage worm *Pieris rapae* L.

Pathogenicity of this natural isolate on the larvae of *P. rapae* was evaluated. The time required to express its action was found to be related to the form used. *Beauveria bassiana* mycosis of *P. rapae* larvae 10 days after applying the fungus orally or topically as a dry powder and in a liquid form led to mortality rates of 89.15%, 83.38% and 91.63%, respectively. Applying the pathogen in a liquid form (SPS) appeared to be a better choice as judged by the significant differences in the average percent mortality rates at any given time interval.

Key-words; *Beauvaria bassiana,* strain name, pathogenicity, *Pieris rapae* L.

INTRODUCTION

The imported cabbage worm *Pieris rapae* L., is one of the major pests of cabbage and cauliflower in Libya causing severe damage to the heads or resulting in no heads being formed at all.

In Libya, insecticides have been used against this insect, but frequently the dosages and number of applications have been excessive. The effects on natural enemies and the environment led to direct thinking in the importance of directing insecticide research towards minimum effective rates, either by use of selective chemicals alone, and in combination with microbial pathogens or use of microbial pathogens alone to protect natural enemies of pest species.

The use of *Beauveria bassiana* (Bals.) Vuill., have proven to be effective against many pest species in North America (3, 4, 5, 11, 12, 14, 15, 16), in France (1, 6, 7, 8) in Libya (2), in China and the Soviet Union (9, 10, 13, 17). Development of a microbial

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control technique in cabbage and cauliflower requires the selection of highly active strains and testing them on these plants. The aim of the present work is to test *B. bassiana* preparations on the imported cabbage worm larvae.

MATERIALS AND METHODS

The isolate of *B. bassiana* used throughout these tests was originally obtained from a cadaver of the *Zeuzera pyrina* L. collected in January, 1991 from infested pomegranate tree branches around Tripoli area. Larvae were collected in the dried mummified form and immediately put into petri dishes containing wet filter paper and maintained at room temperature. Isolations were made on PDA plate culture incubated at 30°C. The fungus was characterised at the Agriculture Research Centre Lab. Tripoli, Libya as *Beauveria bassiana* (Bal.) Vuill and given the designation LARC/ FB 192. Subculturing was made on Sabouroud dextrose agar (SAD) supplemented with 2% yeast extract (W/V) in Erlenmeyer flasks and incubated at 30 °C and agitated on a mechanical shaker for 9 hr and incubated at the same temperature. After 20 days 100 ml from the media were filtered through a filter paper to obtain conidial spore suspension and incubated at 5 °C for bioassays.

Pieris rapae larvae employed in the bioassays were collected at the suburb of Abousetta in the nmorth east of tripoli. The insects were quarantined for 4 days prior to use in the bioassays. Larvae were kept in ice cream boxes 22.5 × 15 × 14cm, fed cauliflower leaves and maintained at room temperature (26 ± 2 °C). The larvae exhibiting reduced vigor were excluded from bioassays.

In the first experiment, both the upper and the lower surfaces of 2-3 cauliflower leaves per box were dusted with 4.2 × 10 spore/ml scraped from the solid prepared media of the *B. bassiana* fungus, one flask to each treatment; 15 larvae *P.* rapae were placed (oral feeding) on the leaves in each ice cream box perforated with a needle to allow gaseous exchange and maintained at 26 ± 2 C; six replicates (four treatments and two controls) were used. After 72 hr, treated leaves were replaced with fresh untreated leaves. The feeding started every other day by using untreated new fresh leaves; the moisture was controlled using water saturated cotton balls. Mortality was recorded after 3,7 and 10 days. A dose mortality line and LC 50 value were calculated for treated and untreated larvae.

The experiment was repeated using the same concentrations and number of treatments with the dry spore (DSP) method of inoculation. The pathogenicity of the fungus isolate was evaluated using 15 larvae of *P. rapae* per treatment. The larvae were placed on dry spore (DSP) of 20 days old SAD in Erleymeyer flask and shaken for 2-3 min. until spores were seen adhering to the larvae cuticle. In the third bioassay experiment, 100 ml of the conidial suspension of *B. bassiana* were treated with 2 drops of Tween 20. One ml of this spore suspension (SPS) containing 4.2×10^7 spore/ml was placed on the inner surface of a sterile plastic petri dish. Into each dish 15 larvae were introduced and left in contact with the inoculum for 2-3 min while control larvae were treated with distilled water. The larvae were then placed in clean perforated ice cream boxes. The moisture maintained using water-saturated cotton balls. Mortality was recorded in both experiments after 3,7 and 10 days.

RESULTS AND DISCUSSION

The results obtained (Table 1) clearly indicate the potentiality of the *B. bassiana* (Bals) strain LARC/FB 292 against the cabbage worm *P. rapae* L.

Table 1 – Effect of ***B. bassiana*** on the imported cabbageworm ***Pieris rapae*** as determined by the percent mortality of the treated larvae.

Form		Replicate	Percent mortality		
			3 days	7 days	10 days
Oral	Treated	1	53.3	53.3	80.0
		2	60.0	60.0	90.3
		3	60.0	93.3	93.3
		4	40.0	73.3	90.0
	Untreated	1	0.0	14.0	14.0
		2	0.0	17.0	17.0
		3	0.0	14.0	14.0
		4	0.0	14.0	14.0
Topical: DSP	Treated	1	80.0	80.0	91.6
		2	60.0	73.3	80.0
		3	53.3	80.0	83.3
		4	53.3	73.3	78.6
	Untreated	1	0.0	18.0	18.0
		2	0.0	18.4	18.4
		3	0.0	18.0	18.0
		4	0.0	18.4	18.4
SPS	Treated	1	73.3	93.3	93.3
		2	73.3	86.6	93.3
		3	86.6	86.6	86.6
		4	73.3	93.3	93.3
	Untreated	1	13.3	13.3	13.3
		2	20.0	20.0	20.0
		3	13.3	13.3	13.3
		4	20.0	20.0	20.0

Data analysed following three factors completely randomized design showed highly significant differences between total time and the form used with a calculated coefficient of variation of 14.53%. A calculated percent mortality ranging from 40 to 86% within 3 days post treatment was obtained. The susceptability of the larvae populations over time towards mycosis increased rapidly to a range from 80 to 93% within 10 days. Larvae dying in oral treatments increased rapidly after 7 days while mortality continued to increase slightly in (DSP) and to a lesser degree in (SPS).

Mycosis was observed on mummified larvae, hard larvae; deformed pupae and in some instances pupae developed melanized patches or deformed adult butterflies as compared with the untreated (Fig. 1) thus justifying the possible use of the fungus as a bioinsecticide for controlling the predation of these pests.

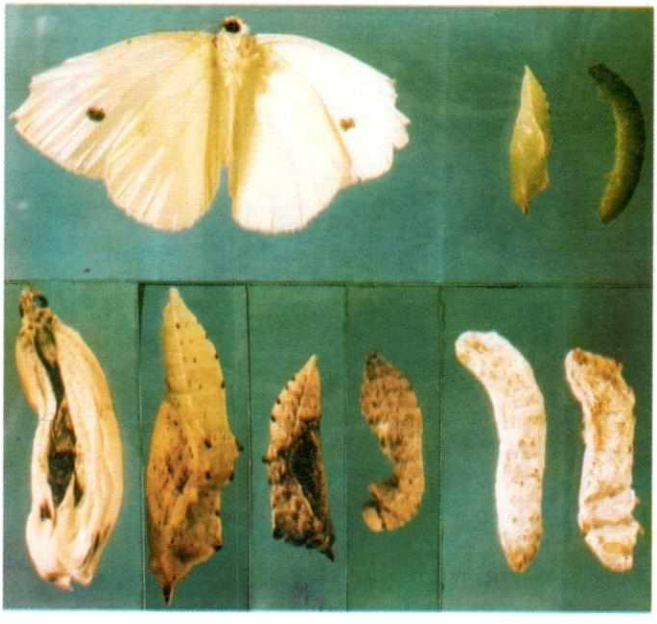

Fig. 1: Above (From right to left) Untreated *P. rapae* L. larva, pupa and adult butterfly. Lower (right to left) mummified, hard larva, deformed, melanized pupa and deformed adult treated with *B. bassiana* (Bals.) spores.

From ANOVA analysis (Table 2) there are highly significant differences in interaction between form x treatment and time where the calculated average percent mortality showed an LSD- value of 9.268 at ($=0.05$) between the means as a result of the interaction of the three factors. Applying the pathogen topically in (DSP) or (SPS) form gave higher mortality rates at any given time interval compared with the treatment in which the pathogen was fed orally using pretreated cauliflower leaves. This reflects the significance of using of the pathogen topically and particularly in the form of spore-suspension (SPS) which might be related to the establishment of a moist

surrounding on the insect cuticle which facilitates the germination of spores and hence the higher proliferation of the pathogen.

Table 2 – Average percent mortality of ***P. rapae*** larvae treated with ***B. bassiana.***

Mode	Treat[b]	Average percent mortality [a]		
		3 days	7 days	10 days
Oral	Treated	53.33	69.97	89.15
	Untreated	0.0	15.50	15.50
Topical: DSP	Treated	61.65	76.65	83.38
	Untreated	0.0	18.20	18.20
SPS	Treated	76.63	89.95	91.63
	Untreated	16.65	16.65	16.65

LSD Value = 9.268@ (= 0.05)

a = Average of four replicate, 15 larvae/replicate

b = Concentration 4.2×10^7 spore/ml.

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اختبار تحضيرات طبيعية من الكائن الممرض: فطر البوفيريا باسيانا *Beauveria bassiana* (Bals) vuill على يرقات حشرة أبو دقيق الكرنب *Pieris rapae* L. *(Lepidoptera: pieridae)*

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مستخلص

تم عزل وتشخيص الفطر بوفيريا باسيانا *Beauveria bassiana* (Bals) Vuill من يرقة حشرة حفار الساق *Zeuzera pyrina* L. مصابة طبيعياً وأعطى اسم السلالة LARC/FB 192، وعند إجراء اختبار الأمراضية لهذا الفطر على يرقات أبو دقيق الكرنب *Pieris rapae* L. وجد ممرضاً ليرقات هذه الحشرة، كما لوحظ أن الزمن اللازم للإمراضية يعتمد على الطريقة التي استخدم بها، إذ وجد باليرقات المعاملة بالفطر بتركيز 4.2×10^7 بوغ/مل عن طريق الفم (تغذية مع الأوراق) ومباشرة على جسم الحشرة في شكل مسحوق جاف وسائل على التوالي، أن نسبة الموت هي بمعدل 89.15٪، 83.38٪، 91.63٪ خلال 10,7 أيام. كما وُجد بالتحليل الإحصائي من خلال الفروقات المعنوية في معدل متوسط النسبة المئوية خلال أي فترة زمنية أن استخدام الفطر في شكل سائل يعتبر من أحسن الطرق.