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EFFECT OF TEMPERATURE ON EFFICACY OF *Beauveria bassiana* VUILLEMIN AND *Metarhizium anisopliae* VAR.ACRIDIUM GAMS AND ROZSPAL AGAINST THE DESERT LOCUST, *Schistocerca gregaria* (FORSKAL).

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ABSTRACT

The influence of temperature on efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against the desert locust, *Schistocerca gregaria* (Forskal), were investigated in the laboratory. Fifth nymphal instar was inoculated by 1.5 $\times 10^3$ spores/nymph by topical application using micropipette under the pronotum, at 50% RH. *B.bassiana* caused mortality for insects at temperature between 22-31°C, and 25°C was the optimum that caused more rapid death and the LT_{50} was 5.3 days. In contrast *M. anisopliae* has wide range of temperature that can caused death until 37°C, but 28-31°C was the optimum temperature and the LT_{50} was 5 days. At this optimums temperature, four doses (1.5×10^2, 1.5×10^3, 1.5×10^4, and 1.5×10^5 spore/nymph) were used against 4th and 5th nymphal instars of the desert locust. For 4th nymphal instar, It' was obvious that *M. anisopliae* caused rapid mortality among infected 4th instar nymph comparing with *B. basiana* at all the tested doses except incase of dose1.5×10^2 spore /nymph, where treated nymph with *B. bassiana* died faster than those treated with *M. anisopliae*. While for the 5th nymphal instar, It' was clear that *M. anisopliae* caused significant rapid mortality comparing with *B. bassiana* in case of dose1.5×10^2spore/nymph. While *B. bassiana* caused significant rapid mortality than *M. anisopliae* in case dose1.5×10^4.

Keywords: Beauveria bassiana; Metarhizium anisopliae var.acridium; Schistocerca gregaria; Entomopathogenic; Temperature.

INTRODUCTION

The desert locust, *Schistocerca gregaria* (Forskal), is an economically pest in semi and hot arid areas. The deleterious effects of chemical pesticides used to suppress outbreaks of this pest have prompted development of alternative control methods such as microbial control (Prior and street, 1997). Fungal pathogens in the class Deuteromycotina, which can be grown easily in mass culture and which penetrate directly through the host cuticle, were consider to be the most promising agents (Prior and Greathed, 1989). *Metarhizium anisopliae* and *Beauveria bassiana* are the most widely encountered pathogens of acridids in Africa that consider as a microbial insecticide against the desert locust (Lomer *et al.*, 1997; Prior and street, 1997).

Two of the most important environmental factors affecting the ability of an entomopathogenic fungus to infect and overcome its host are humidity

and temperature (Hall and Papierok, 1982; Benz, 1987 and Ferron *et al.*, 1991) .In acridids, the thermal constraints are not only the results of ambient conditions, but also achieved thermal host thermoregulation (Boorstein and Ewald, 1987; Carruthers *et al.*, 1992 and Inglis et al., 1996). In addition, acridids elevate their body temperature higher than ambient by habitat selection, orientation to solar radiation, or both (Chappell and Whitman, 1990; Heinrich, 1993). The objectives of this study were to determine effects temperature on efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* var.acridium against fifth nymphal instars of the desert locust.

MATERIALS AND METHODS

1- Tested insects:

Fourth and fifth nymphal instars of the desert locust were used. The individuals were taken from stock culture maintained for several generations. Insects were reared in the laboratory according to the technique of (Hunter-Jones, 1961).

2-The entomopathogenic fungi:

The entomopathogenic fungi, *M.anisopliae* and *B. bassiana* were used. The spores of *M.anisopliae var. acridium* isolate IMI330189, were used kindly provided by (Biological Control Products), South Africa. But *B. bassiana* was provided by nematodes lab in Cairo University.

The entomopathogenic fungi were grown on oat –mealdodin agar (ODA) medium selectively allows the growth of *M. anisopliae* and *B. bassiana*, while inhibiting the growth of the other fungi and bacteria (Beilharaz and Parberry, 1982; Chase *et al.*, 1986). Conidial suspensions were prepared by pouring approximately 5ml of vegetable oil onto the culture and scraping the fungus away from the agar. This suspension was placed in a sonicator for one minutes to break up the conidial chain and poured through a 90µm sieve to obtain a conidial suspension free from large mycelia particles .Conidial counts were made using a haemactometer.

3-Effect of temperature on efficacy of entomopathogenic fungi:

Fifth nymphal instars of 1-2 days old were inoculated by 1.5×10^3 spores/nymph by topical application using micropipette under the pronotum according to prior *et al.*, (1995). Each7 inoculated nymphs were kept in an opened plastic cylinder (diameter 8 cm and length 25 cm) at both ends which covered with a sheet of cloth for ventilation. Fifth temperatures were assessed, 25, 28, 31, 34, and 37°C degrees, for *M.anisopliae* while 21, 25, 28, 31 and 34°C degrees, for *B. bassiana*. Following inoculation, were placed in incubators. Three replications were used for every treatment and compared with control. The control was treated with 10µl of sterile plant oil and placed in the same temperature of treatment. The insects were fed on Egyptian clover (*Trifolium alexandrium*). Cadavers were examined for presence of sporulating layer of entomopathogenic. Mortality percentages were calculated after 1- day incubation period after treatment to dead all

inoculated nymphs. Mortality percentages were corrected by (Schneider-Orelli). Every Nymph died during a bioassay was kept in Petri dishes alone and Incubated at 25C. There were recorded percentages of entomopathogenic which grow on surface body of .Nymphs. Percentages mortalities data were subjected to probit analyses according to Finney, (1971), to calculate time mortality responses LT_{50}, LT_{90}.

4- Bioassay of the entomopathogenic fungus against nymphal instars of the desert locust:

Forth and fifth nymphal instars of 1-2 days old were used .The insects were treated by using the same above methods. Four doses were applied for every pathogen. Doses were 1.5×10^2, 1.5×10^3, 1.5×10^4, and 1.5×10^5 spore/nymph. Treated nymphs were kept at optimum temperature for every fungus. Percentages mortalities were subjected to probit analyses according to Finney, (1971) to calculate, LT_{50}, LT_{90} values and its regression lines.

RESULTS AND DISCUSSION

1– Effect of temperatures on efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against fifth nymphal instars of the desert locust, *S.gergaria*.

1-1- *Beauveria bassiana*:

Data presented in figure (1) showed, the effect of different temperatures on the efficacy of *B.bassiana* against the fifth nymphal instar of the desert locust, *S.gregaria*. It's obvious that temperature significantly affected the efficacy of *B. bassiana* against the fifth nymphal instar of the desert locust. Disease development was more rapid among nymphs kept at 25°C, where insect death started at 4 days after treatment, while started at5 and 6 days after treatment at 28°C and 22°C respectively. The mortality among those nymphs kept at 25°C reached to 100 % after 7 days of treatment, the medial lethal time LT_{50} was 5.3 days table (1). While, in case of those nymphs kept at 22, 28°C the mortality reached to 100 % after 15, 16 days after treatment, and the LT_{50} were 10.1 and 10.6 days respectively, with no significantly difference between 22 and 28°C. Although the mortality among nymphs kept at 31°C reached to 40% after 20 days of treatment and the LT_{50} was 28.6 days. There was no mortality among the nymphs kept at 34 °C.

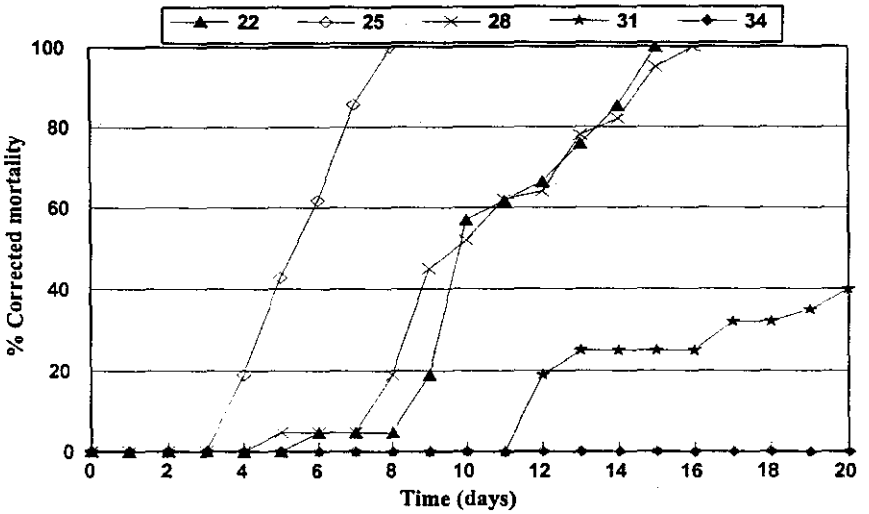

Fig (1): Effect of temperatures on efficacy of *B.bassiana* against of the 5th nymphal instars of the desert locust, *S. gregaria*

1-2- *Metarhizium anisopliae:*

Data illustrated in figure (2) showed the effect of different temperatures on the efficacy of *M. anisopliae* against the fifth nymphal instar of the desert locust, *S.gergaria.* These data clearly showed that there were significant differences between mortalities of the nymphs kept on different temperature. Disease development was more rapid at temperature 28 and 31°C. The death started at 4th day when nymphs kept at 25, 28, 31°C and 34°C while started at 9th day for nymphs kept at 37°C. It's obvious that the mortality among treated nymphs reached to 100 % after 11, 8,7,11, and 17 days of treatment when kept at 25, 28, 31, 34, and 37°C respectively. The LT_{50} was 5.1 days for nymphs kept at 28 and 31°C table (1). On the other hand nymphs that kept at 25 and 24°C, the LT_{50} were 10.1 and 10.6 days respectively. In contrast the LT_{50} was 28.6 day for the nymphs kept at 34 °C. From figures 1 and 2 concluded that the optimum temperature for the efficacy of *B. bassiana* is 25°C, while for *M. anisopliae* was between 28-31°C.

These findings go in line with the results of Shashi-Sharma *et al.*, (1998), who found that *B. bassiana* could grow within the temperature range 20-28°C, but 25°C was the most suitable temperature for conidial production. Ekesi *et al.*, (1999), studied in the laboratory the effect of temperature on germination, radial growth and pathogenic activity of two strains of *B. bassiana* and four strains of *M. anisopliae* on the legume flower thrips, *Megalurothrips sjostedti.* Germination, radial growth and pathogenic activity were low for all strains at 15°C. Optimum temperature for germination, radial growth and pathogenic activity ranged between 25-30 °C. The fastest growing strain at 25-30°C was *M. anisopliae* strain ICIPE 69, compared to other strains. Berlanga-Padilla *et al.*, (2002) determined the optimal temperature for germination and growth *B. bassiana* isolates was between 24

and 30 °C. At 26°C, *B. bassiana* caused 88% mortality in *S. p. piceifrons*, and LT_{50} were 5.9 days. Inglis *et al.*, (1997) also observed that at constant 35 °C, over 80% mortality was obtained in *Melanoplus sanguinipes* (Fab.), whereas less than 10%mortality was obtained when the grasshoppers were kept at 40°C for 12 h /day. Interestingly, Fargues *et al.*, (1997) determined the effects of temperature on conidial germination and susceptibility of adults of *S. gregaria*, to four isolates of *Metarhizium flavoviride*. There were differences among the isolates in the effects of temperature on germination of conidia after a 24-h incubation period. Over 90% of conidia of all isolates germinated after 24 h at 30°C. In contrast, at 40°C, none of the isolates germinated for up to 72 h. However, there were differences in germination between the isolates at 35°C. Locust mortality and disease progression were significantly affected by temperature. At both 25°C and 30°C, all isolates induced 98-100% mortality within 8 days; however, there were differences between isolates at 35°C. None of the isolates caused significant mortality at 40°C.

Table (1): LT_{50}, LT_{90}, values of 5th nymphal instars of the desert locust, *S.gregaria*, treated with *B.bassiana* and *M. anisopliae*, at different temperature.

Temp.	*Beauveria bassiana*		*Metarhizium anisopliae*	
	LT_{50}	LT_{90}	LT_{50}	LT_{90}
22	10.5^{b}	14.7	-	-
25	5.3^{a}	7.7	7.6^{b}	10.7
28	10^{b}	15	5^{a}	7.9
31	28.6^{c}	106.2	5.1^{a}	6.3
34	0^{d}	0	7.1^{b}	11
37	-	-	12.4^{c}	16.5

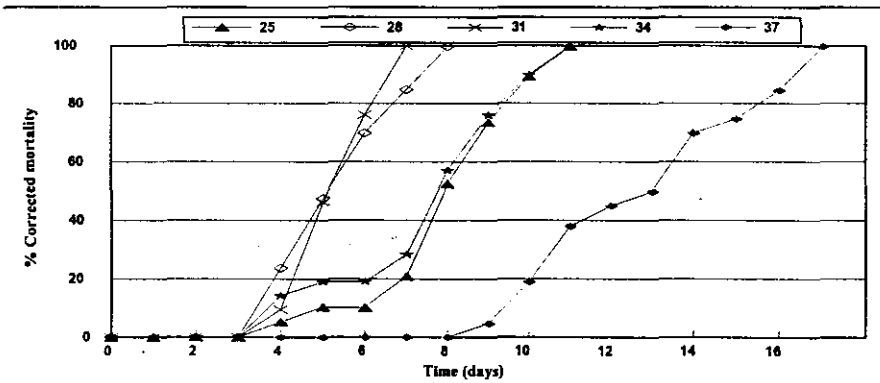

Fig (2): Effect of temperatures on efficacy of *M.anisopliae* against the 5th nymphal instars of the desert locust, *S. gregaria*.

2-Bioassay of *B. bassiana, M. anisopliae* against the 4th, 5th nymphal instars of the desert locust.

2-1-fourth nymphal instar:

Table (2) showed the time mortality response of *B. bassiana* and *M. anisopliae* on the 4th nymphal instar of *S. gregaria*, expressed of the time required to kill 50 and 90% of the treated nymph (LT_{50} and LT_{90}) . It's clear that LT_{50} of treated nymph with *B. bassiana* were: 5.51, 6.33, 7.5 and 8.97days, while these values for nymphs treated with *M. anisopliae* were 4.47, 5.28, 6.32 and 12.03 days after treatment with doses 1.5×10^5, 1.5×10^4, 1.5×10^3, 1.5×10^2 spore/nymph, respectively. The slopes of the linear regression of the mortality versus time for *B.bassiana* were 3.5, 7.84, 6.29 and 4.14 and for *M. anisopliae* were 6.9, 9.02, 10.8 and 3.3. It could be concluded that *M. anisopliae* caused rapid mortality among infected 4th instar nymph comparing with *B. basiana* at all the tested doses except incase of dose1.5×10^2 spore /nymph. Treated nymph with *B. bassiana* died faster than those treated with *M. anisopliae*. It's obvious that dose1.5×10^5 of *B.bassiana* has significantly lower LT_{50} than dose1.5×10^3 and significantly lower than of dose1.5×10^2. While in case of *M. anisopliae* there were significant differences between each dose and other and could by arranged in ascending order as follows1.5×10^5> 1.5×10^4>.5×10^3>.5×10^2. The slope of *B.bassiana* regression line with dose1.5×10^5 was the lowest value indicating the lowest degree of homogeneity of these insects for their susceptibility to this fungus. On contrary, the slope regression line with dose1.5×10^4 was the highest value indicating the highest degree of homogeneity for susceptibility of the 4th nymphal instars of the locust, *S.gregaria* to this fungus

Table (2): LT_{50}, LT_{90}, values of 4th nymphal instars of the desert locust, *S.gregaria*, treated with *B.bassiana* and *M. anisopliae*, at different doses.

Doses*	*B.bassiana*			*M.anisopliae*		
	LT_{50}	LT_{90}	Slope	LT_{50}	LT_{90}	Slope
1.5×10^5	5.51 ^{cA}	12.67	3.55±0.6	4.47 ^{dB}	6.85	6.9±0.48
1.5×10^4	6.33 ^{cA}	9.22	7.84±0.57	5.28 ^{cB}	7.33	9.02±0.71
1.5×10^3	7.5 ^{bA}	12	6.29±0.57	6.32 ^{bB}	8.43	10.8±0.58
1.5×10^2	8.97 ^{aA}	18.28	4.14±0.22	12.03 ^{aB}	29.41	3.3±0.14

*** Conidia/nymph**

LT_{50} with same small letters did not differ significant in the same fungi.

LT_{50} with same cab letters did not differ significant in the same dose.

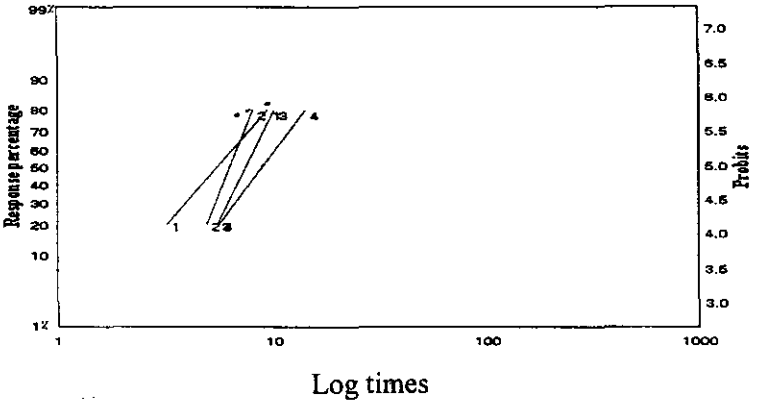

Fig. 3: Toxicity regression lines of *B.bassiana* against the 4th nymphal instars of the desert locust, *S.gregaria*.

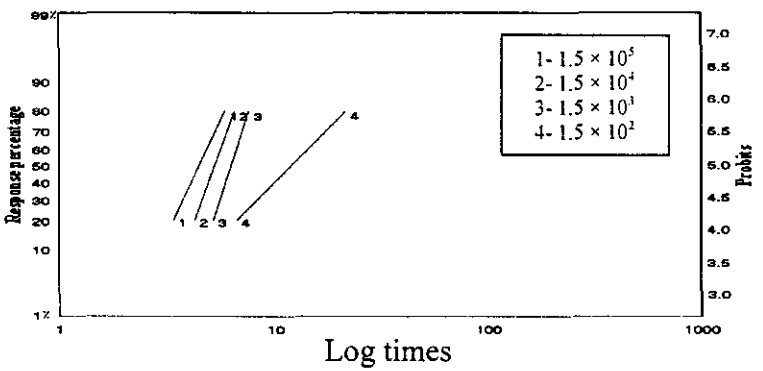

Fig. 4 : Toxicity regression lines of *M.anisopliae* against the 4th nymphal instars of the desert locust, *S.gregaria*..

2-2-fifth nymphal instar:

Table (3) shows the time mortality response of *B. bassiana* and *M. anisopliae* against the 5th nymphal instar of *S. gregaria*, expressed of the time required LT_{50} and LT_{90}. Its clear that LT_{50} of treated nymph with *B. bassiana* were: 4.51, 4.16, 6.66 and 14.2 days while these values for treated nymphs with *M. anisopliae* were 4.84, 5.36, 6.53 and 9.12 days after treatment with doses 1.5×10^5, 1.5×10^4, 1.5×10^3, 1.5×10^2 respectively. The slopes of the linear regression of the mortality *versus* time for *B.bassiana* were 7.3, 7.5, 7.5 and 4.3 and for *M. anisopliae* were 6.72, 7.45, 7.79 and 10.08. It's clear that *M. anisopliae* caused significant rapid mortality comparing with *B. bassiana* in case of dose1.5×10^2spore/nymph. While *B. bassiana* caused significant rapid mortality than *M. anisopliae* in case dose1.5×10^4. In both fungus, doses 1.5×10^5, 1.5×10^4 have significant lower LT_{50} than dose 1.5×10^3. While 1.5×10^3 was significant lower than dose1.5×10^2spore/nymph. The slope of *M.anisopliae* regression line with dose1.5×10^2 was the lowest value indicating

the lowest degree of homogeneity of these insects for their susceptibility to this fungus. On contrary the slope regression line with dose 1.5×10^{3} was the highest value indicating the highest degree of homogeneity for susceptibility of the 5th nymphal instars of the desert locust, *S.gregaria* to this fungus.

Table (3): LT_{50}, LT_{90}, values of 5th nymphal instars of the desert locust, *S.gregaria*, treated with *B.bassiana* and *M. anisopliae*, at different doses.

Doses*	*B.bassiana*			*M.anisopliae*		
	LT_{50}	LT_{90}	Slope	LT_{50}	LT_{90}	Slope
1.5×10^{5}	4.51^{cA}	6.75	7.3±0.63	4.84^{cA}	6.72	8.99±0.74
1.5×10^{4}	4.16^{cB}	6.15	7.5±0.58	5.36^{cA}	7.45	8.98±0.88
1.5×10^{3}	6.66^{bA}	9.85	7.5±0.5	6.53^{bA}	7.79	16.72±1.22
1.5×10^{2}	14.2^{aA}	28.18	4.3±0.19	9.12^{aB}	15.08	5.86±0.29

*Conidia/nymph

-LT_{50} with same small letters did not differ significant in the same fungi.

-LT_{50} with same cab letters did not differ significant in the same dose

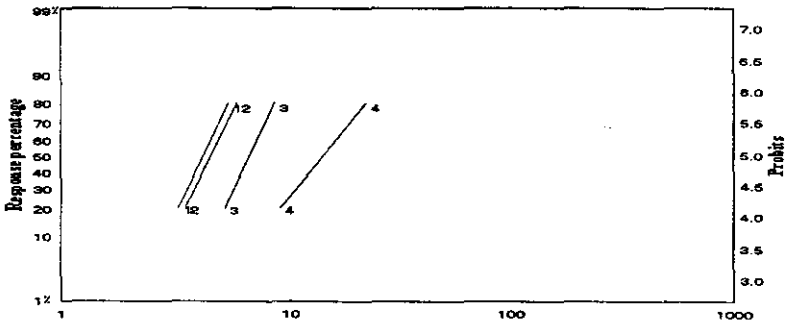

Fig. 5: Toxicity regression lines of *B.bassiana* against the 5th nymphal instars of the desert locust, *S.gregaria*.

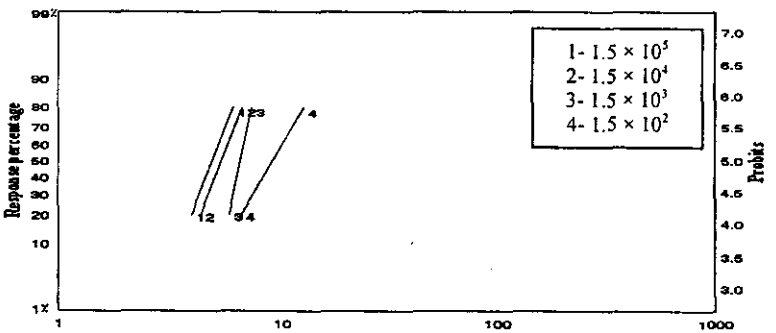

Fig. (6): Toxicity regression lines of *M.anisopliae* against the 5th nymphal instars of the desert locust, *S.gregaria*.

These findings go in line with the results of Moore and Erlandson, (1988), who assessed *B.bassiana* against the nymphs of the grasshopper, *Melanopuls sanguiniipes* (Fabricius) by topical oral and injected applications 1.5×10^3, 1.5×10^4 and 1.5×10^5 spore/nymph. *B.bassiana* caused high mortality (82-100%) at all doses tested, between 3-5 days after applied the fungus. Tefera and Pringle, (2003) tested isolates of *B.basiana* and *M.anisopliae* against the spotted stalk borer, *Chilo partellus* (Swinhoe). All isolates induced 100% mortality to *C. partellus* larvae in six days. Moore *et al.*, (1992) showed that *M.flavoviride* killed adults' of *S.gregaria*, with dose 80.000 conidia /insect at 30°C in 5-9 days. Prior *et al.*, (1992) used 75000 spore / insect of *M.anisopliae* against of *S.gregaria*. At this dose the fungus killed 50% of the test insect in 4-5 days. Also this results corresponds exactly with (Lomer *et al.*, 1999) when they used spraying the Niger isolate of *M.anisopliae* against fourth instars hopper bands of the desert locust , *S.gregaria* .The nymphs of the desert locust gave up to 90% mortality in 9^{th} day after spraying. On the other hand, work carried out on *B.basiana* in the United States (Foster *et al.*, 1992) and in Canada (Inglis *et al.*, 1993) and on *M.flavoviride* in Africa (Bateman *et al.*, 1994) has indicated that secondary pick- up takes place .In a trial on *Zonocerus variegates* (L.) where application was very satisfactory .The speed kill in the field has only been 1-2 days slower than expected from laboratory. These findings go in line with the results of (Lobolima *et al.*, 1992; Johnsone *et al.*, 1992) who tested the fungus *B.basiana* against *Oedaleus senegalensis* .The fungus caused a statistically significant mortality when sprayed directly against the insects in the field. Also these results are consistent with those of (Berlanga-Padilla and Hernandez, 2002) who showed that at 26 degree *B.bassiana* caused 76%mortality in adults of *Schistocerca piceifrons piceifrons* (Orthoptera: Acrididae) and their half lethal time (LT_{50}) was 5.2 days. Milner and Prior, (1994) stated that most mortality with two isolates of *M.flavoviride* against 4th instars of *Chortoiecetes terminifera* with dose 3.75×10^7 occurred between 4-6 days, while LT_{50} was the 4.3 days for isolate FI985.While isolates FI610 killed almost all the insects at 5 days. LT_{50} was 6.7. Also (Bateman *et al.*, 1996) screened the pathogenicity of 159 isolates of *Metarhizium* spp and *Beauveria*. spp against *Schistocerca gregaria* adults. All screens included a standard strain of *Metarhizium spp.* (from a single spore of IMI 330189ss), which gave an average LT_{50} value of 4.4 days in 46 assays. Approximately 50 isolates, all belonging to the genus *Metarhizium*, showed virulence that was comparable with this strain.

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تأثير درجات الحرارة على فاعليـــة فطـــرى البيوفريـــا باســـيانا و الميتـــاريزيوم انيسوبلاى على الجراد الصحراوى

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تعتبر حشرة الجراد الصحراوي من أهم الحشرات الاقتصادية واكثرها ضـــراراوتغزو مناطق عديدة من العالم معضمها في افريقيا والشرق والاوسط,واتجهت الابحاث الحديثة لاســـتخادم المكافحة الحيوية وبشكل خاص الممرضات بسبب الاثار الضارة للمبيدات, هذا وتعتبر درجـــات الحراة من أكثر العوامل المحددة لفعالية الفطريات الممرضة للحشرات لذلك تم دراسة فعالية كل من فطري البيوفاريا باسيانا والميتاريزم انيزوبليا بجرعة 1.5×10^{3} جرثومة/حورية على العمر الحوري للخامس للجراد الصحراوي في المعمل.وكانت درجة الحرارة المثلـــى لفطـــر البيوفاريـــا باسيانا 25م° ولم تسبب درجة الحرارة 34 اي فعالية للفطـــر. بينمابالنـــسبة لفطـــر الميتـــاريزم انيزوبليا كانت درجة الحراة المثلى بين 28-31م°.عند هذه الدرجات الحرارة المثلى للفطرين تم اختبار فعالية كل من الفطرين وبجرعات مختلفة عاىالعمرين الحوريين الرابع والخامس وقد اثبت فطر الميتارزيم فعالية اعلى من فطر البيوفاريا عند الجرعات الاعلى بينما في الجرعة الاقل حدث العكس.