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ASSESSMENT OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* SAUDI ARABIAN ISOLATE (B - SA3) AGAINST THE DEVELOPMENTAL STAGES OF THE RED PALM WEEVIL, *RHYNOCHOPHORUS FERRUNGINEUS* (OLIV.)

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ABSTRACT

The efficacy of the entomopathogenic fungus *Beaveria bassiana* Saudi Arabian isolate (B-SA3) was evaluated against the red palm weevil, *Rynochophorus ferrungineus* (Oliv.). The mentioned isolate was assessed on the different developmental stages of the red palm weevil through toxicity tests, i.e. determination of lethal concentration and lethal times to kill 50% of treated insects. The results showed that 8th instar larvae were the most tolerant followed by pupa to *B.bassiana* as their LC_{50} values were $3.75x\ 10^8$ and $3.78x\ 10^7$ conidia/ ml, respectively. Meanwhile, 4th instar larvae were the most susceptible to infection by *B.bassiana* (B-SA 3), as LC_{50} value was $3.25x\ 10^6$ conidia/ ml, which proved insignificantly different to that determined in adult weevils, i.e. $4.18x\ 10^6$ conidia /ml. Furthermore, LT_{50} values were very similar in these latter mentioned developmental stages in any considered concentration; meanwhile, LT_{50} was much longer in 8th instar larvae than the other considered instar and/or developmental stages. The fungus *B. bassiana* was most virulent to eggs of the red palm weevil as none of the eggs hatched following their treatment with any of the considered concentrations (ranging between $5x\ 10^9$ to $5x10^5$ conidia /ml). Moreover, germination viability of harvested conidia of *B.bassiana* stored at -4°C was insignificantly affected up to the 10th month storage period and was well above 90%. However, germination percentage of conidia then decreased to 70.27% after 16 months of storage. Also, the virulence of the stored conidia was tested after 1, 6, 12 and 16 months on adult red palm weevils, the LC_{50} values were $3.75x10^6$, $4.66x10^6$, $4.17x10^7$ and $3.37x10^5$ conidia/ ml, respectively. These results show that there was a significant decrease in the virulence of the tested fungus when the duration of storage period was more than 10 months.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) is a major insect pest and widely accepted as being the most devastating insect pest of date, coconut and oil palm trees throughout Asia (**Kalshoven, 1966** and **Wattanaapongsiri, 1966**). In the mid 1980's *R.ferrugineus* was introduced to date palm trees in the Arabian Gulf Region. It quickly spread to Saudi Arabia, Iran and Egypt and many other countries in North Africa (**Abraham *et al* 1998**; **Murphy and Briscoe, 1999**). Due to the insect feeding habits inside the palm tree trunks, its control has been quite difficult; furthermore, infestation can not be discovered until damage has already been inflicted.

Efforts for the control of the red palm weevil were focused on the use of traditional chemical insecticides or by eliminating infested trees. Control of this pest is now more concerned with the use of biological control agents, such as the use of entomopathogenic bacteria, viruses, fungi or

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nematodes. *Beauveria bassiana* is well known as an entomopathogenic fungus with worldwide distribution. It is the anamorph stage of *Cordyceps bassiana*, a teleomorph in the ascomycetous family, Clavicipitaceae (**Sung *et al* 2007**). This fungus has proven to be effective for the control of many Coleopteran species, (**Miranpuri, *et al* 1992a** and **1992b, Miranpuri** and **Khachatourians, 1994; Athanassiou and Steenberg, 2007).**

The present investigation was therefore undertaken to assess the efficiency of the fungus *B. bassiana* Saudi Arabian Isolate 3, (B-SA 3) for the control of the four developmental stages of the red palm weevil. Also, the infectivity of *B. bassiana* conidia stored for 16 months was assessed at monthly intervals by a bioassay trials conducted on adult weevils. Furthermore, the viability of stored conidia was determined by the germination test.

MATERIALS AND METHODS

Insect culture

The culture of the red palm weevil *Rhynchophorus ferrugineus* was conducted under laboratory conditions of 25± 2^0 C and 70± 5% RH. Adult red palm weevils were collected from infested date palm trees at a plantation located at El-Kassasine, Ismailia Governorate, Egypt, by means of insecticide free food baited aggregation pheromone/ kaironome traps (**Hanounik *et al* 2000**). The traps were partially buried around the trunk of the date palm trees at a distance of 100 m apart. The traps were inspected every week and the live trapped weevils were collected and transferred to the laboratory. In the laboratory the weevils were sexed; every 2 pairs were placed in plastic cups, 9 cm in diameter. In each cup succulent tissues of date palm wood was placed as a source of food and which also served as an oviposition site. Adults were monitored and any laid eggs were collected daily by means of a soft brush and placed on moist filter paper in small Petri dishes. The eggs were monitored and upon hatching the newly hatched larvae were transferred to holes drilled in short pieces of soft date palm wood. After 10 to 14 days larvae were removed and placed in larger containers and allowed to feed on similar succulent date palm wood. Larvae were constantly observed and prior to their pupation, the full grown larvae were placed in clean moist plastic trays and provided with mat of dry palm fibres and left undisturbed to pupate. Subsequently, constructed cocoons were observed and upon adult emergence the weevils were placed in pairs as previously mentioned.

Entomopathogenic Fungus *Beauveria bassiana*

The entomopathogenic fungus Saudi Arabian isolate No 3 of *Beauvaria bassiana (*B-SA3) was evaluated for its efficiency in the control of the red palm weevil *R. ferrugineus*. The local strain of this fungus was isolated from dead red palm weevils *R. ferrugineus* collected from date palm plantations at Al-Qatif province, at the Kingdom of Saudi Arabia by **Hegazy *et al* (2007)** this isolate was identified and confirmed by CABI Bioscience UK.

Maintenance of *B. bassiana*

Fresh slant cultures (B-SA3) were obtained from the Arab Organization for Agricultural Development Collection. The fungus was sub-cultured on Sabouraud dextrose agar plus yeast extract (SDYA) medium (mycological peptone 10gm, dextrose 40 gm, yeast extract 2.5 gm and agar 15 gm per litre of distilled water) and incubated at 25 °C for 14 days. Subsequently, the conidia were harvested in sterile vials with screw caps by scraping the surface of agar plates, silica gel was then added at a rate of 20% w:w before the vials were sealed. Vials were held at -4 ° C until needed for the experimental work.

Bioassay of *B. bassiana* (B-SA 3)

The infectivity of *B.bassiana* (B-SA 3) was evaluated on the different developmental stages of *R. ferrugineus* according to the method described by **Finney (1952)** and **Marannino *et al* (2006)**. A stock solution of the fungal formulation was prepared from 1gm *B. bassiana* spores suspended in 100 ml sunflower oil and sterilized distilled water with 0.05% Tween 80 was added to make one litre. The suspension was well mixed using magnetic stirrer for 1 min to break spore chains into individual spores and assure uniform mixing. The spore concentration in the resulting suspension was determined by the use of a haemocytometer slide and adjusted to the desired concentration. A series of considered concentrations i.e. $5x10^9$, $5x10^8$, $5x10^7$, $5x10^6$ and $5x10^5$ conidia / ml were prepared in distilled water. The suspensions were used for inoculation within 1 hour (**Goettel and Inglis, 1997**).

Treatment was carried out on the different developmental stages of the red palm weevil which were selected from the maintained insect stock culture:-.

(i) Newly laid eggs (0-24 hours old).
(ii) 4th and 8th instar larvae; the larval instars were determined by the width of their head capsule (**El-Muhanna *et al* 2000**)
(iii) Pupae, 7days post pupation.
(iv) Full grown adults, approximately one week post emergence.

The dipping technique was used, where 20 eggs and insects in each of the mentioned developmental stages were dipped for 20 seconds in one of the prepared concentrations. Treated specimens were then maintained separately in Petri dishes measuring 15cm in diameter and provided with small soft pieces of date palm wood as a source of food. Twenty specimens were used for the treatment of each mentioned developmental stage and each replicated three times; a control presented by untreated insects was included for each experiment where specimens were treated in a mixture of sunflower oil, water and 0.05% Tween 80.

Insect mortality was recorded daily and corrected by **Abbott's** formula **(1925).** Toxicity regression lines were plotted in form of Log concentration / probit relationship as described by **Finney (1952)** to determine LC_{50}, as well as the slope and regression values. In addition, the time required to kill 50% of the target insect (i.e. LT_{50}) was also calculated for each treated developmental stage using log time/ probit relationship.

Following the death of the treated insects their cadavers were incubated individually at 25±2°C in sealed moist Petri dishes. The specimens were examined microscopically, daily and for one week, to determine if the death of the insect was a result of it's infection by *B. bassiana* as confirmed by the appearance of the fungus aerial mycelium and/or 'mummification'.

Effect of storage periods on the viability and virulence of *B.bassiana* (B-SA 3) dry conidia

From the stock culture of *B. bassiana,* (B-SA 3) dry conidia were harvested and packed in several sterile glass vials with screw caps as previously mentioned, they were then stored in a freezer set at -4° C. At a monthly interval and for 16 consecutive months the viability of the stored conidia was determined by their germination after defrosting them at room temperature.

For this experiment, at every monthly investigation, 0.1 gm of the stored conidia was weighed and added to 250 ml of sterilized SDYA medium in 1000 ml Erlenmeyer flask, the flask was then incubated on a rotary shaker (120 rpm) for 18 hours at 25±2°C. After incubation 4 droplets of *B. bassiana* spore suspensions were placed on 4 slides and percentage of spore germination was calculated by counting 25 spores in 4 different fields of view for each slide (e.g. 400 spores) using a phase contrast microscope at 400X magnification. A spore with a germ tube longer than its width was considered germinated.

Moreover, the virulence of the stored conidia was further evaluated on their efficiency to infect adult date palm weevils. For this experiment five concentrations were prepared, $5x10^9$, $5x10^8$, $5x10^7$, $5x10^6$ and $5x10^5$ conidia/ ml of *B. bassiana*. Ten adult weevils in five replicates were treated in one of the prepared concentrations using the dipping technique (20 sec); a control was included in each case. The mortality percentage of weevils was recorded and corrected according to **Abbott's** formula, **(1925)** and LC_{50} and LT_{50} values determined according to **Finney (1952).** Subsequently, any dead weevil was incubated as previously mentioned, to determine if its death was as a result of fungus infection by the appearance of fungus aerial mycelium on its cadaver.

RESULTS

I- Susceptibility of the red palm weevil to *Beauvaria.bassiana* (B-SA 3)

Under conditions of the present work, the incubation period of untreated *R.ferrungineus* eggs was approximately 4 days and percentage of egg hatchability was 100%. When newly laid eggs were treated with any of the prepared concentrations of *B.bassiana* none of them hatched, giving 100% unhatchability to all of the treated eggs. All unhatched eggs treated by any of *B.bassiana* concentrations exhibited growth of aerial mycelium on their chorion which was first apparent on the 5th day post treatment.

The conducted bioassay of *B.bassiana* on *R.ferrugineus* post embryonic developmental stages revealed that 4th instar larvae were more susceptible than 8th instar larvae as determined from the plotted toxicity regression lines of the tested fungus against the targeted larval instars. The LC_{50} values were $3.25x\ 10^6$ and $3.75x10^6$ conidia/ ml to the respective mentioned instars, **Table(1) and Fig (1).** As seen in Table 2, the calculated LT_{50} value was always lower when 4th instar larvae were treated, i.e. 93.97, 118.58, 140.28 and

168 hours when the concentrations $5x10^9$, $5x10^8$, $5x10^7$ and $5x10^6$ conidia/ ml were used. Meanwhile, the LT_{50} values were 128.52, 153.46, 200 and 303 hours in 8th instar larvae treated with the respective mentioned concentrations.

The LC_{50} value for pupae and adults of the red palm weevil was $3.78x10^7$ and $4.18x\ 10^6$ conidia/ ml, respectively. These results shows that pupae were more tolerant to *B.bassiana* than either adult weevils or 4th instar larvae, however, pupae were more susceptible than 8th instar larvae **Table (1)**.

It is of interest to note, that as depicted in Table 1, the susceptibility of adult red palm weevils to *B.bassiana* was relatively comparable to that of 4th instar larvae. This fact is seen by the insignificant difference in the determined LC_{50} which was $4.18x10^6$ conidia/ ml and $3.25x\ 10^5$ conidia/ ml in adult and 4th instar larvae, respectively. Furthermore, the LT_{50} values in these two mentioned developmental stages were nearly identical, **Table (2)**.

Observing the growth of the fungus aerial mycelium on the cadavers of treated insects 7 day following their death, it was found that percentage of its appearance was higher when adult weevils were treated , followed by 4th instar larvae then pupa. As seen in **Table (3)**, the percentage of aerial mycelium ranged between 100- 84.62, 95-70 and 82.35-54.55% on the cadavers of adult weevils, 4th instar larvae and pupa, respectively, treated with *B.bassiana* at the concentration between $5x10^9$ to $5x10^7$ conidia/ ml. Meanwhile, this percentage was much lower, i.e. 57.14- 28.57% when 8th instar larvae were treated with the respective mentioned concentrations.

Time-Mortality Relationship

From the fore mentioned results regarding the calculated mortality parameters, an additional parameter was considered. A mathematical estimation was used to determine the time required to kill the target insect if 4th and 8th instar larvae as well as adults of the red palm weevil were each treated by their corresponding determined LC_{50} value. Accordingly, the logarithmic values of the considered concentration (conidia/ ml) of *B.bassiana* were plotted versus the absolute values of exposure time. The obtained regression lines, exhibited in **Table (2)**, show the response of the treated developmental stage to the applied concentration. Hence, in spite of the differences in the calculated LC_{50} values of the red palm weevil treated as an adult weevil and 4th or 8th instar larvae, it could be mathematically estimated that no significant differences will be detected in the calculated lethal time values. This assumption is depicted in **Fig.(2)** as the absolute lethal time values were found to be 171, 172 and 166 hours in 4th, 8th instar larvae and adult weevils, respectively, i.e. 7.13, 7.16 and 6.9 days, respectively, with an average of 7.06 days.

II- Effect of storage periods on the viability and virulence of *B.bassiana* conidia.

It was important to establish the viability of *B.bassiana* conidia cultured on the SDAY media and stored for a period up to 16 months at -4^0 C. As exhibited in **Table (3) and Fig. (3)**, the viability of the conidia depicted by its percentage germination was over 95% when stored for the first 6 months from their initial storage, giving the highest germination percentage of 98.9% after one month of storage. This was followed by a slow gradual decrease in conidia germination in the following month to reach 95.63% at the end of 6th month storage period. Furthermore, in the subsequent 7th to the 10th month from storage, conidia germination was still above 90% (i.e. ranging between 94.8 to 90.16%), However, by the 11th month from initial storage, the viability of the conidia was observed to decline from 86.48% to a relative low of 70.27% at the termination of the experiment i.e.16 month. Statistically, no significant difference was found in the germination of conidia stored up to 10 months, but significant differences were detected for those stored for an extended period.

Bioassay of stored *B.bassiana* conidia

For the further evaluation of the viability of stored *B.bassiana* conidia a bioassay was conducted to determine their virulence in infecting *R. ferrugenius* adult weevils. As seen in **Table (4)** and **Fig. (4)**, the LC_{50} values were $3.75x10^6$, $4.66x10^6$, $4.17x10^7$ and $3.37x10^8$ conidia/ ml when the *B.bassiana* concentrations were prepared from conidia stored for 1, 6, 12 or 16 months, respectively. This shows that the virulence of fungus spores slowly decreased with increase in duration of storage period, this was further confirmed by the determined LT_{50} values **Table (5)**.

As seen in **Table (6)**, following treatment with *B.bassiana* conidia that had been stored for 1 or 6 months at the concentrations of either $5x10^8$ or $5x10^9$ conidia/ml 100% of dead weevils exhibited visible fungus aerial mycelium of their cadavers. A slightly lower percentage of 88.89 and 85.72%

Table 1. LC_{50} values of *Beauveria bassiana* (B-SA 3) against larvae, pupae and adults of the red palm weevil

Developmental stage treated	LC_{50} (conidia/ ml)	Slope	*r*
4th instar larva	3.25×10^6	0.517	0.967
8th instar larva	3.75×10^8	0.519	0.994
pupa	3.78×10^7	0.523	0.992
adult	4.18×10^6	0.616	0.982

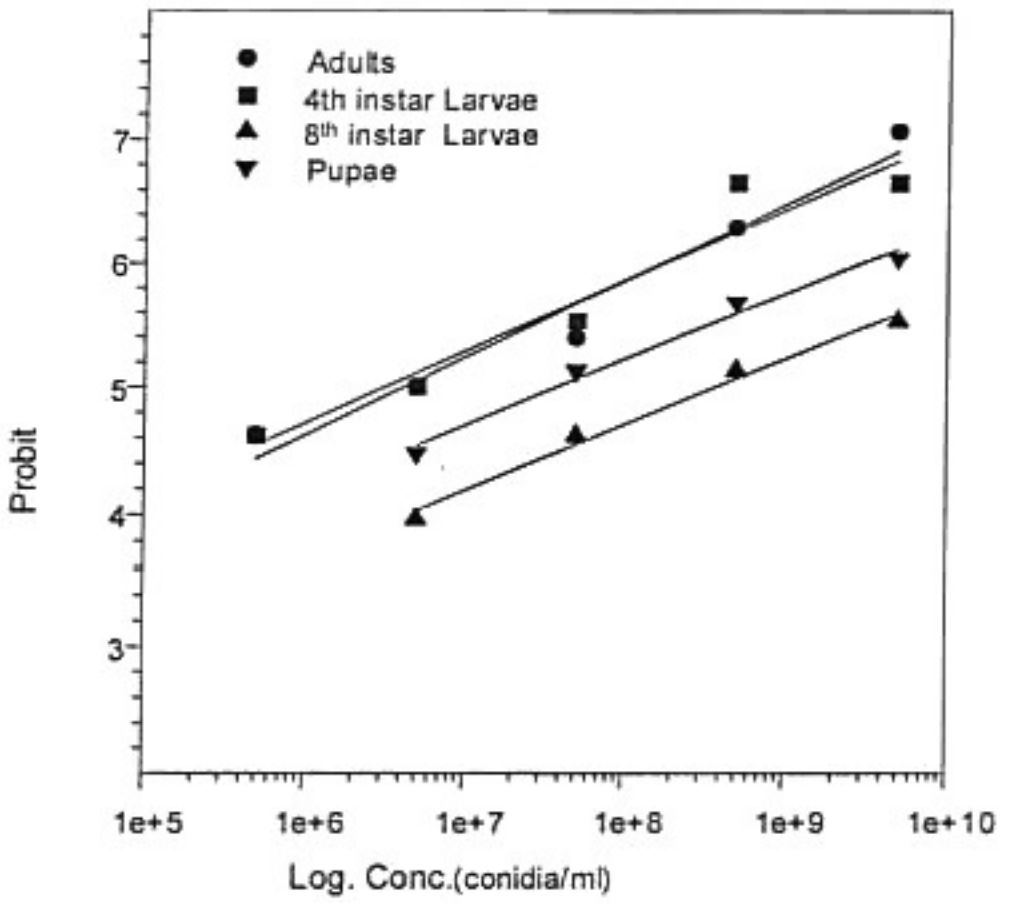

Fig. 1 . LC_{50} values of the red palm weevil to *Beauveria bassiana* (B-SA3)

Table 2. LT_{50} values in adults, 4th and 8th instar larvae of the red palm weevil treated with different concentrations of *Beauveria bassiana* (B-SA 3)

Concentrations (conidia/ ml)	LT_{50} (hours)		
	4th instar larva	8th instar larva	adult
5×10^9	93.97	128.52	87.3
5×10^8	118.58	153.46	106.66
5×10^7	140.28	200	129.72
5×10^6	168.27	303	168.27

Table 3. Percentage of dead red palm weevil's treated with *B. bassiana* (B-SA 3) with visible aerial mycelium on their cadavers by the 7 days following their death

Concentration (conidia/ ml)	% red palm weevil cadavers bearing aerial mycelium				
	egg	4th instar larva	8th instar larva	pupa	adult
$5x\ 10^9$	100	94.74	57.14	82.35	100
$5x10^8$	100	88.89	45.45	66.67	88.89
$5x10^7$	100	71.43	28.57	54.55	84.62
$5x10^6$	100	60	0	50	60
$5x\ 10^5$	100	42.86	0	0	28.57

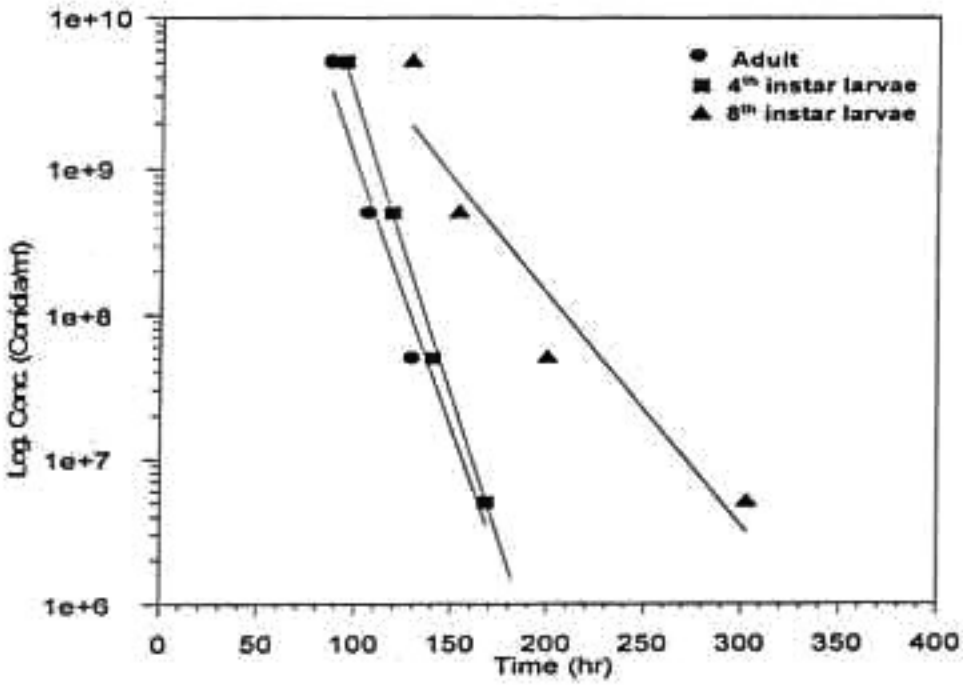

Fig 2. Regression lines of concentrations of *B. bassiana* (B-SA-3) conidia versus exposure time in considered larval instars and adult weevils of *R. ferrugineus*

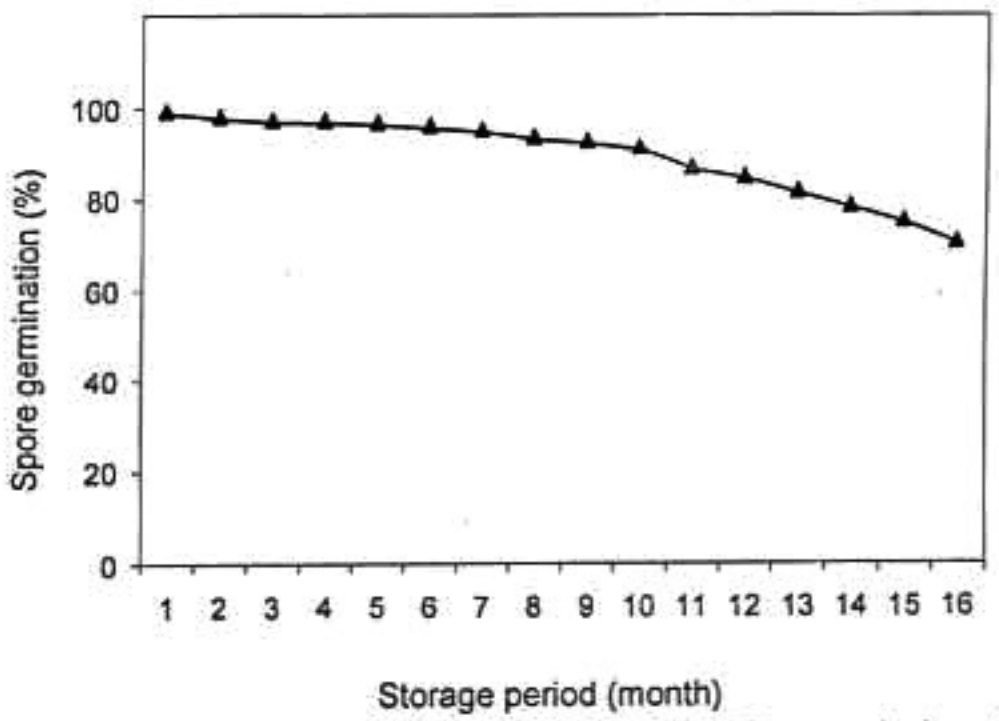

Fig. 3. Percentage of germinated conidia of *Beauveria bassiana* (B-SA 3) stored at -4^{0}C for up to 16 months

Table 4. LC_{60} values of *Beauveria bassiana* (B-SA 3) conidia stored at -4°C for up to 16 months to adults of the red palm weevil

Storage period (months)	LC_{50} (conidia/ ml)	Slope	*r*
1	$3.75x\ 10^6$	0.594	0.98
6	$4.66x\ 10^6$	0.644	0.99
12	$4.17x\ 10^7$	0.617	0.99
16	$3.37x\ 10^8$	0.592	0.96

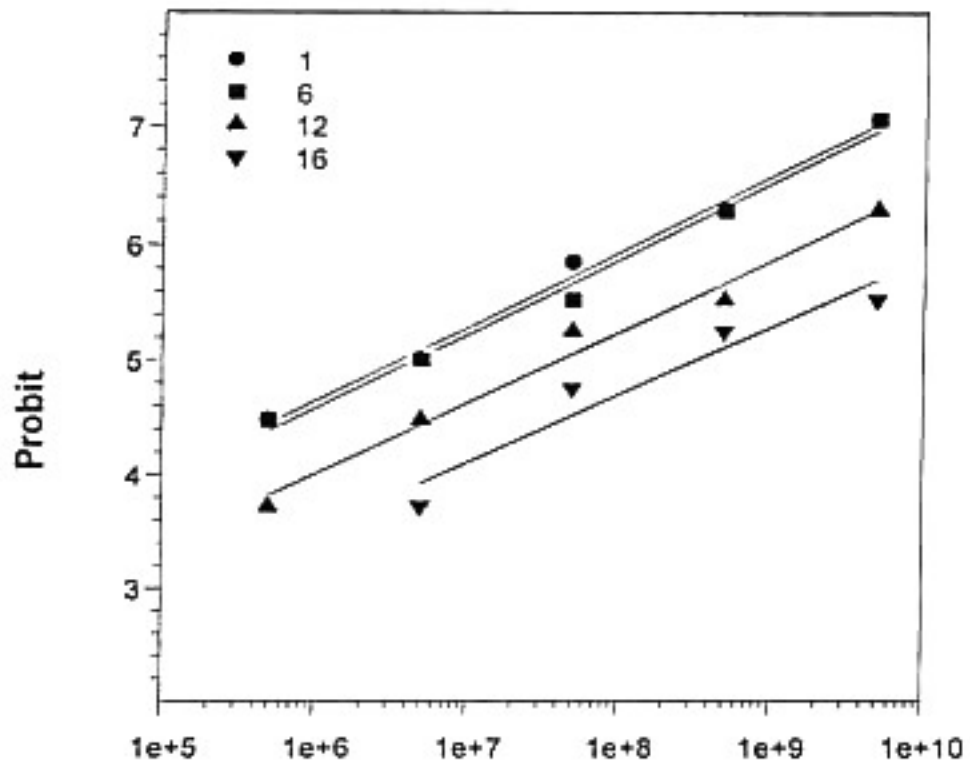

Fig. 4. Regression lines of the red palm weevil treated with *Beauveria bassiana* (B-SA 3) conidia stored from 1- 16 months at -4°C

Table 5. LT_{60} values of the red palm weevil treated with *Beauveria bassiana* (B-SA 3) conidia stored for 1 to 16 months at -4°C

Concentration (conidia/ ml)	Storage period (months)	LT_{50} (hours)	Slope	*r*
$5x\ 10^9$	1	73.74	9.14	0.984
	6	78.89	10.2	0.966
	12	82.6	5.15	0.996
	16	102.8	5.02	0.970
$5x\ 10^8$	1	75.51	9.02	0.937
	6	89.95	6.21	0.989
	12	123.59	3.82	0.993
	16	131.2	3.48	0.973
$5x\ 10^7$	1	85.6	5.33	0.965
	6	104.6	3.61	0.985
	12	151	3.68	0.964
	16	220	4.22	0.999

Table 6. Percentage of dead red palm weevil treated with *B.bassiana* (B-SA 3) conidia stored for up to 16 months at -4°C with visible aerial mycelium on their cadavers by the 7th day following treatment

Concentration conidia/ ml	Percent of mortality at different storage period (months) of *B. bassiana* conidia			
	1	6	12	16
$5x\ 10^9$	100	100	77.78	42.86
$5x\ 10^8$	100	100	71.43	33.33
$5x10^7$	88.89	85.71	50	25
$5x10^6$	83.33	60	33.33	0
$5x10^5$	50	33.33	0	0

of dead weevils exhibited a similar feature following their treatment with $5x10^7$ conidia/ ml that was stored for 1 and 6 month, respectively. These percentages were much lower when the lesser concentrations were used and were most evident when the concentrations were prepared from conidia stored for 16 months as it ranged between 42.86- 25 % when $5x10^9$, $5x10^8$ and $5x10^7$ conidia /ml were tested. Meanwhile, no aerial mycelium was evident on the weevils cadavers treated with the other lower concentrations.

DISCUSSION

Entomopathogenic fungi as biological control agents have been successfully used in reducing insect pest populations in different ecosystems (**Inglis *et al* 2001**). *Beauveria bassiana* has been and still is evaluated and targeted against a wide range of pests, e.g. the Andean potato weevil, white flies, the cabbage looper pine caterpillar and corn borer, (**Evans, 2003**).

In the present investigation the toxicity of a Saudi Arabia isolate of *Beauveria bassiana* (B-SA 3) was evaluated on the eggs, 4th and 8th larval instars, pupa and adult of red palm weevils, *R. ferrungineus* (Oliv.). The ability of entomopathogenic fungi to infect the egg stage is a promising potential and a high susceptibility to *B.bassiana* (B-SA 3) was detected in the eggs of the red palm weevil which might suggest the virulence of fungus conidia in penetrating the egg chorion. The addition of sunflower oil in the formulation of *B. bassiana* might have influenced the virulence of fungus and enhanced adhesive properties. **Samuels *et al* (2002)** showed that a high percentage of egg infection was only obtained following application of oil formulated conidia. It could also be suggested that the growth of the fungus mycelium on the egg chorion might have hindered respiration of the embryo and subsequently caused its death; further studies are needed to verify these assumptions. In a similar study, **Gindin *et al* (2006)** recorded 80% mortality to eggs of the red palm weevil treated with the fungus *Metarhizium anisopliae*.

Fourth instar larvae and adult weevils were more susceptible to *B.bassiana* than older 8th instar larvae as well as pupae. Furthermore, the LT_{50} values at each tested concentration of *B.bassiana* were insignificantly different in 4th instar larvae and adult weevils, signifying the high virulence of this fungus in penetrating the cuticle of these two mentioned developmental stages.

In the present work, 8th instar larvae of the red palm weevil were observed to be markedly less active than younger 4th instars, this observation might have been a reason for their lesser exposure to the entomopathogenic fungus and therefore leading to a decrease in their infection. The tolerance of 8th instar larvae could also be due to the accumulation of fat body in their body cavity which could have protected or obstructed the penetration of the fungus. The fat body was often the site for the accumulation of toxins (**Casarett and Doulls, 1996**). A group of cyclotetra-depsipeptides (toxic metabolites) were isolated from *Beauveria* species. Beauverolides (**Elsworth and Grove, 1980**), beauverilide (**Isogai *et al* 1978**), and beauveriolides (**Mochizuki *et al* 1993**). However, *B. bassiana* is a source of various cyclodepsipeptide antibiotics. Beauvericin, the most commonly produced cyclodepsipeptide by strains of *B. bassiana*

(**Hamill *et al* 1969**). The low percentage of visible aerial mycelium on the cadavers of insects treated as 8th instar larvae with any of the considered concentrations following their death exhibits their tolerance to infection by the fungus. It is most significant for the appearance of aerial mycelium on insect's cadavers following their treatment with any entomopathogenic fungi so as to create an opportunity of spreading the conidia for infecting other insect pests. Therefore, this calculation is most important when conducting a control program for the red palm weevil especially that adults of this insect secrete an aggregation pheromone (**Al-Jabr and Al-Rajeh, 2000**) therefore allowing close contact and spreading of fungi infection between weevils.

The viability the Saudia Arabian strain of *B.bassiana* (B- SA 3) conidia when stored for up to10 months at -4°C was insignificantly affected and was well above 90% and, furthermore, it was still in the range of 80's% when stored for 13 months. This suggests the feasibility of having a constant stored stock of this entomophathogenic fungus so as to be available for use in a biological control program towards *R.ferrugineus* or any other insect pest. **El-Sufty *et al* (2007)** stored a local strain UAE-B2 of *B.bassiana* in the United Arab Emirates at -10° C for 13 months without decrease in its virulence.

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تقييم كفاءة الفطر بوفيرا باسيانا الممرض للحشرات (العزلة السعودية B-SA3) ضد سوسة النخيل الحمراء *Rhynochophorus ferrungineus* (Oliv.)

[٢٠]

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الملخـص العربـــي

تم تقييم كفاءة العزلة السعودية (B- SA3) من الفطر بوفيرا باسيانا الممرض للحشرات ضد أطوار سوسة النخيل الحمراء، ومن خلال اختبارات السمية تم تحديد التركيز القاتل ل 50% من أعداد الأطوار المختبرة والوقت اللازم لموت 50% من التعداد. وقد أظهرت النتائج أن العمر اليرقى الثامن هو الأكثر تحملا لتأثير الفطر يليه طور العذراء.

وكانت قيم التركيز القاتل لـــ 50% من تعداد اليرقات والعذارى المعاملة $3.75x\ 10^8$ ، $3.78x\ 10^7$ كونيديا/مل على التوالي. واتضح أن العمر اليرقى الرابع هو الأكثر قابلية للعدوي بالفطر بوفيرا باسيانا (العزلة السعودية B-SA3) ، حيث كان التركيز القاتل لـــ 50% من اليرقـــات المعاملة هو $3.25x\ 10^6$ كونيديا/مل والذى لم يختلف معنويا عن التركيز اللازم لقتـــل 50% من تعـــداد الطور البالغ وهو $4.18x\ 10^6$ كونيديا/مل.

وكانت قيم الوقت اللازم لموت 50% من تعداد الأطوار المعاملة (LT_{50}) متقاربة بالنسبة لنفس التركيز تحت الاختبار، وفى نفس الوقت فإن الزمن اللازم لموت العمر اليرقى الثامن كان أطول نسبيا مقارنة بذلك الوقت اللازم لموت بقية الأعمار اليرقية أو الأطوار الأخرى للحشرة.

تؤكد النتائج أن فطر بوفيرا باسيانا كان أكثر شراسة ضد بيض سوسة النخيل الحمراء، حيث لم يفقس أي من البيض المعامل بأي من التركيزات المختبرة والتي تراوحت بين $5x10^9$ -$5x10^5$ كونيديا/مل.

باختبار حيوية جراثيم فطر البوفيرا باسيانا المخزنة على درجة حرارة 4°- م أتضح عدم وجود اختلافات معنوية فى نسبة الإنبات للجراثيم المخزنة حتى عشرة شهور (نسبة الإنبات فوق 90 %) بينما انخفضت نسبة الإنبات تدريجيا بزيادة فترة التخزين حتى وصلت إلى 70.27% بعد ستة عشر شهراً من التخزين.

وتم اختبار شراسة الجراثيم المخزنة بعد 1 ، 6 ، 12، 16 شهراً ضد الحشرات الكاملة لسوسة النخيـــل الحمراء وكانت قيم التركيز القاتـــل لـــ 50% مـــن الحشرات المختبرة $3.75x10^6$ ، $4.66x10^6$ ، $4.17x10^7$ ، $3.37x10^8$ كونيديا/مل على التـــوالي. وتـــشير هـــذه النتائج إلى انخفاض شراسة الفطر بدرجـــة معنويـــة بزيادة فترة التخزين عن عشرة شهور.

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