EFFICIENCY OF NEW ISOLATES OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* AGAINST RPW, *RHYNCHOPHORUS FERRUGINEUS* IN SAUDI ARABIA

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Abstract

The red palm weevil *Rhynchophorus ferrugineus* is a destructive pest on date palm plantations in the Middle East. Traditionally chemical insecticide have been used to control this insect in the Arab region .Arab Organization for Agriculture development (AOAD) set up a project to use bioagents to control this pest. seven local strains of entomopathogenic fungus *Beauveria bassiana* were solated from naturally infected red palm weevils adults, pupae and from an adult of the mole cricket *Gryllotalpa* sp. for the first time in Eastern province, Saudi Arabia. Bioassay of three isolates, B-SA1, B-SA2 and B-SA3 on *R. ferrugineus* adults resulted values LC50 of 1.25x107 ,1x105 and 2x106 conidia/ml. Adults allowed to contact food sprayed with a formulation of conidia of B-SA3 and oil\water of the concentrations of 30, 20, 10, 9, 5 x 108 (conidia /ml) for three days showed 100% death in insects. however , 2.5 and 1.25 x 108 (conidia /ml) killed 50 and 57 % of the rpw. 100 % aerial fungus developed on insects at the concentrations of 30 and 20 x 108 conidia /ml . Only 75,60 , 50 , 50 and 12.5 % of the insects showed aerial growth of the fungus resulted from concentrations of 10, 9 , 5, 2.5 and 1.25 x 108 conidia /ml respectively . Stored dray conidia at - 4 C for 16 months, indicated 98.5 % germination during the first 6 months, then declined to 88 % up to the month 13. Germination of stored dray conidia for 14 to 16 months exhibited 74 and 61 % germination respectively. Effect of sunlight on the germination of the dray conidia during a period of 15 days exhibited reduction in germination from 95 .7 after one day to 91.2, 86.4 75, 70.7 and 67.57% at days 3,6,9,12 and 15 days respectively .Regration line showed that the half life of the fungus is about 22 days.

INTRODUCTION

The red palm weevil (rpw) *Rhynchophorus ferrugineus* is the most destructive pest of palm trees in many countries . It invades date palms coconut and oil palms through Southeast Asia (Kalshoven, 1950). Red palm weevil was accidentally introduced for the first time to the Gulf Region, Since its appearance in United Arab Emirates in 1985 ,then Saudi Arabia on 1986 Iran in 1992, Egypt in 1993, Jordon in 1998 and Israel in 1999 (Abraham *et. Al.* 1998,Murphy and Briscoe 1999, Soroker *et.*

al., 2005) . Since then efforts to control *R. ferrugineus* in the Arabian Gulf region focused mainly on traditional insecticide or elimination of infested trees, the attention since 1997 was paid on controlling this pest by use of biological Control .

The Arab Organization for Agriculture Development (AOAD) set up a project to study means for biological and ecological control of *R. ferrugineus* and to determine the elements of its biological control in the Arabian Gulf area. The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuil, has been successfully used as a bio-control agent for the management of a number of coleopteran insect pests, including Colorado potato beetles *R. Ferrugineus*(Hanounik *et. al.* 2000) ,*Leptinotarsa decemlineata* (Miranpuri *et. al.* 1992a) blister beetle, *Lytta nuttali* (Miranpuri and Khachatourians. 1994) and flea beetles, *Phyllotreta crucifera* (Miranpuri *et. al.* 1992b) .Li (1988) recording nearly 200 species of coleopteron and lepidopteron insect have been recorded as hosts to *B. bassiana*.

MATERIALS AND METHODS

Sample collection

Samples of rpw *R. ferrugineus* adults, pupae or larvae as well as other insect species were collected regularly from Al-Qatif date palm farms for detecting fungus infection Insect samples were incubated individually in moist plastic Petri dishes at 25 C , some of them were infected and exhibited external growth of white sporulation or hyphae. Aerial fungus from the insects was examined and then inoculated in SDAY Petri dishes and incubated at 25 C for 12 to 15 days ,series of process were made for fungal purification. Grown conidia were harvested and stored in sterilized sacs at - 4C . Furthermore soil samples from under palm trees were collected in plastic containers ,each sample was divided into four plastic cups of 15 cm. Collected soil samples were moisten and on each five last instar larvae of the great wax moths *Galleria mellonella* were placed to each container. Cups were maintained at 25+2 C for 15 days. Any wax moths larvae showing mycosis were removed from the containers and transferred into moisten plastic Petri dishes at 25C. the aerial fungi on the its external body of the wax moth larvae was examined and inoculated in SDAY Petri dishes and maintained at 25 C for 12 days. The formed dry conidia were harvested and microscopically examined and stored in the freezer at - 4 C until required for experimental work . Dry conidia were used for mass production using sub merged technique .Submerged media were accomplished in flasks contain the conidia and sterilized SDY with stirrer bars, shake flasks on rotary shaker at 25 C for 48 hours. primary identifications were done according to the characterization of the conidia and conidiophores before sending for final identification.

Insects

R. ferrugineus adults were collected from infested at a date palm plantations in Al- Qatif ,Eastern Province ,Saudi Arabia, by the use of insecticide free food baited aggregation pheromone\kairomone traps .Adults were maintained at room temperature 25+ 2 C in plastic containers and provided with small pieces of soft date palm wood as source of food. Adults were monitored for one week, dead or injured weevils discarded, and remaining healthy active ones used in this study.

Isolation and identification

Many as 1100 samples of adults,pupae or larvae of rpw were collected from date palm farms in Qatif were kept individually in humidified Petri dishes at 25 C degrees for detecting the entomopathogenic fungus. Samples of the detecting fungus (isolate B-SA3) were sent after has been purified to CABI Bioscience in UK for identification.

Bioassay

10 adults of *R. ferrugineus* were separately dipped for 30 second in different dilutions of *B. bassiana* isolates with various conidia concentrations, 5x109, 5x108, 5x107, 5x106 and 5x105 (conidia\ml). Treated adults then reared individually in plastic cups 15 cm diameter and provided with small pieces of soft date palm wood. Insect mortality was monitored daily and dead insects incubated individually at 25 + 2 C in sealed 10cm Petri dishes, conidia and conidiophores from cadavers were examined microscopically for confirmation of infection by *B. bassiana*, Probit analysis (Excel AAS 97) was conducted to compute values of LC50 .

Determination of economic spray

Oil\water formulations of.30x108, 20x108, 10x108, 9x108, 5x108, 2.5x108 and 1.25x108 conidia /ml *B. bassiana* with tween 80 were prepared .5 plastic cups of 15 cm diameter for each concentration supplied with pieces of soft date palm wood were sprayed by hand atomizer , then 10 red palm weevil adults were added to each cup. Insect allowed to fed on treated food for 3 days then treated pieces of food were removed and untreated small pieces of date palm wood were replaced .dead insects were monitored daily and incubated in moisten plastic Petri dishes at 25C. Insects used as control were sprayed with mixture of water and tween 80.

Effect of storage periods of the dry conidia on the germination of *B. bassiana*

Dray conidia of *B. bassiana* were stored at -4 C for different monthly periods of 2to 17 months. At a monthly interval percentage of conidia germination was determined by 0.1g of dry conidia from each stored products were added to 250 ml of submerged media accomplished in flasks contain the conidia and sterilized SDY with

stirrer bars ,shake flasks on rotary shaker at 25 C for 18 hours. germinated and non germinated conidia were counted by using the haemocytometer and light microscope , the percent of germinations were calculated.

Inhibition in the germination of the dray conidia exposed to sunlight for different periods

This experiment was conducted during second half of January 2007 where temperature was approximately less than 22 C., hence the effect of high temperature on germination was eliminated . Three replicates of 1g dray conidia of *B. bassiana* in sealed Petri dishes were inserted between date palm petioles for periods of 1,3,6,9,12,and 15 days. o.1g dray conidia of each Petri dish was Submerged in media, is usually accomplished in flasks contain the conidia and sterilized SDY with stirrer bars ,shake flasks on rotary shaker at 25 C for 18 hours. Germinations were then counted by light microscope.

RESULTS AND DISCUSSION

Bioassay of the fungus

Seven isolates of the entomopathogenic fungus *Beauveria bassiana* were collected, of wild, three isolates B-SA-1,B-SA-5and BSA-6 were found on the pupae of red palm weevil ,while other tow isolates B-SA-3 and B-SA-7 were found on larvae and adults of this weevil . A seventh isolate BSA-2 was detected on *Gryllotalpa sp.*. Bioassay of only the three isolates (B-SA-1,2,3) showed positive relation between concentrations and the percent of dead weevils .

The LC50 was found to be 1.25 x 107 ,1x 105 and 2 x106 conidia / ml for BSA-1 ,BSA-2 and BSA-3 respectively (figs. 1 ,2 ,3) .Only B-SA-3 was identified as *Beauveria bassiana* by CABI Bioscience, UK.

Beauveria bassiana was conducted and isolated by in an artificial rearing experiment, the fungus was associated with cocoon and the bodies of the adults of *Rhynchophorus ferrugineus* (Shaiju-Simon , *et. al.* 2003 , Ghazavi and Avand-Faghih 2002) . Effect of *Beauveria bassiana* on the RPW was studied in this project by (Hnounik, *et. al.*2000) using strain BOF-AG with 5x109 conidia ml. obtained from Live System Technology S.A., Colombia. The LC50 of the entomopathogenic fungus *Beauveria bassiana* can be different according to the strains and insect used ,no available data were found to show the LC50 of *B. bassiana* on red palm weevil. On the other hand , LC50 of 1.25 x105 (conidia /ml) was indicated when other strains of *B. bassiana* used on adults of cashew tree borer *plocaedeurs ferrugineus* (Ambethgar, 2003) , 3.5 x105 (conidia\ml) for the rice pest *Cnaphalocerocis medinalis* (Augada and Rombach ,1987) , 2.58 x105 conidia /ml to control *Lema pectoralis* Baly

(Coleoptera :Chrysomelidae) (Facundo *et. al*. 2001) , and range between 2x108 to 5x 108 to control *Sitophilus granaries* (Hluchy and Samsinakova 1989) and was 1.4 x108 and 6. 4x108 when adults and nymph of *helopetis antonii* sprayed (Sudarmdji and Gunwan ,1994) .

Determination of economic spray

Data indicated that the concentrations of (30 ,20,10,9 and 5 x108 (conidia \ml) caused 100% death to the red palm weevils *Rhynchophorus ferrugineus* (Fig.4).on the other hand the concentrations of 2.5 and 1.25 x108 conidia \ml caused death to 50 and 57 % of the insects respectively . Mycosis test on dead weevils indicated 100% when the concentrations of 30 and 20 x108(conidia \ml) were used. The percent of aerial fungus then declined to 75, 60 and 50 % when 10,9 , 5 and 2.25 x108 (conidia \ml) were applied respectively . The least mycosis of 12.5 % was found when the weevils treated with the concentration of 1.25 x108 (conidia/ml). It can be concluded that the less concentrations can caused death without mycosis appearance.

Effect of storage periods of the dry conidia on the germination of *B. bassiana*

The storage of dry conidia at -4 C for different monthly periods show that their germination was not significantly affected for up to six months showing 98.5% . this percentage slightly decreased to 88% after 13 months of storage which was further reduced to between 74 to 61 % after 16 months intervals (Fig.5) .This results is in agreement with (Gupta *,et. al.*2000) who indicated that the population of the formulation of Metarhizium anisopliae decreased significantly as the period of storage increased irrespective of the temperature, and conidia must be stored under low temperature (4 to 20 C) for the retention of high viability and virulence .

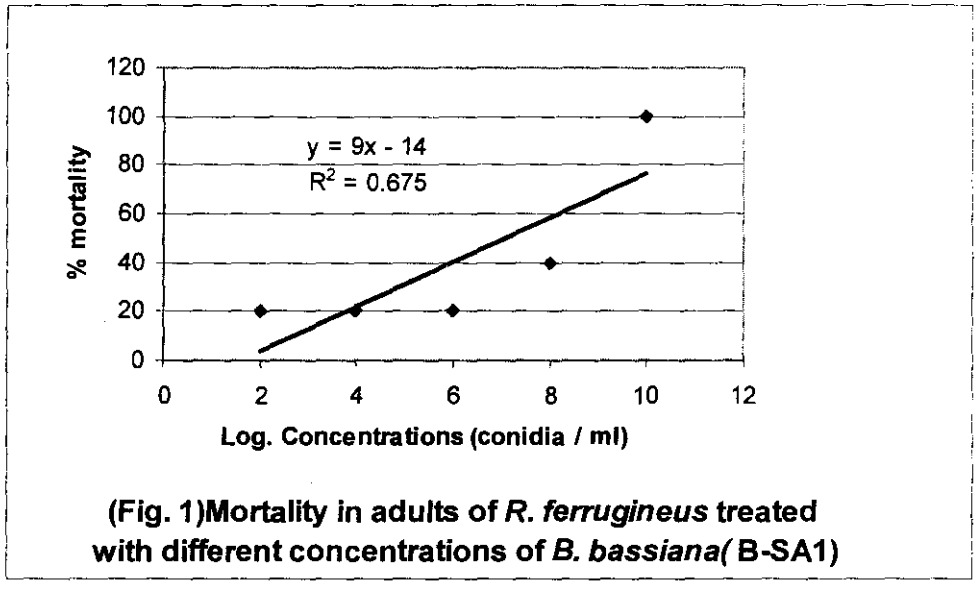
Effect of sunlight on germination of dry conidia

Exposure of conidia to the effect of sunlight and regardless of temperature show that germination was affected . Percentage of 95.7 % germination was recorded on the first day, this percentage was reduced to 91.2 and 86.4 % on the 3ed and 6^{th} day respectively. Exposure time to sunlight when increased to longer periods of 9 ,12 and 15 days reduced conidia germination to 75,70.7 and 67.57 % respectively.. Regration line of half life was calculated at day 22 (fig.6).

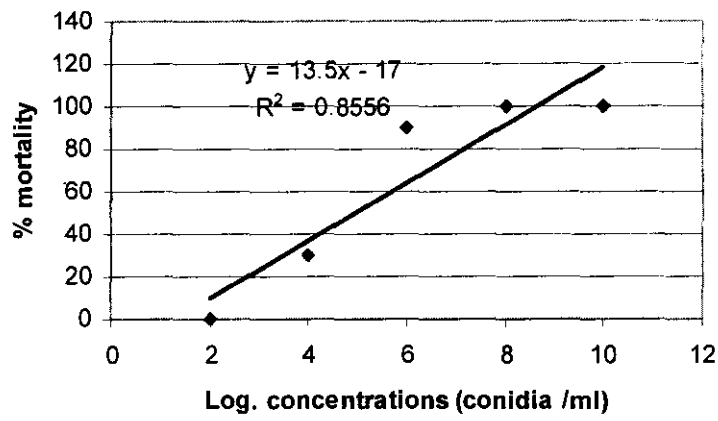
The viability of *B .bassiana* (Bb-9205) was studded by (Velez ,1996) in a coffee ecosystem, under sunlight and artificial shadow in Colombia ,he found no different between treatments in sunlight and shade at o,2,4,5,24 ,48 and 336 hours of exposure and the mean value of the relative ratio of reduction of viability was 45.97%. On the other side the effect of exposure to ultraviolet radiation in a sunlight simulator on the germination of conidia of *Beauveria bassiana* was also studded by (Velex and

Montoya, 1995) they found no direct germination of conidia sprayed on coffee leaf discs and exposed for 0-60 min .It also indicated that the persistence of viable *B. bassiana* spores was significantly longer under the plastic that blocked a greater portion of the UV spectrum (Costa *et. al.*, 2001) . In the fungus of *M. anisopliae* conidia viability was observed until the 10th day on the upper leaves and until the 14th day on the lower leaves of sugarcane plants (Riberio *et. al.*, 1992) . On the other hand exposure of ultraviolet light for 1 min. caused maximum sporulation to the colonies of *B. bassiana* (Diodato *at. al.* ,1993) .

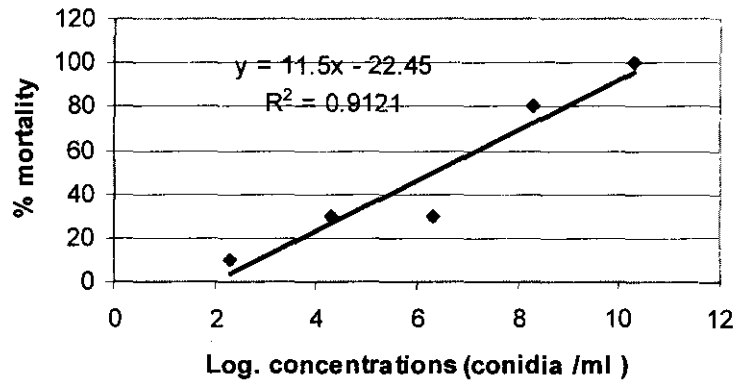
This work has been conducted through a project for biological control of red palm weevil (rpw) in Middle East sponsored by the Arab Organization for Agriculture Development (AOA D).



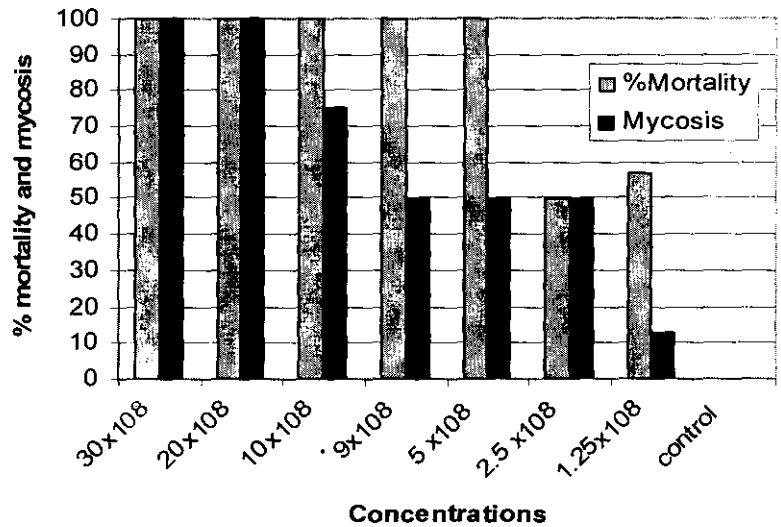
(Fig. 1)Mortality in adults of *R. ferrugineus* treated with different concentrations of *B. bassiana*(B-SA1)



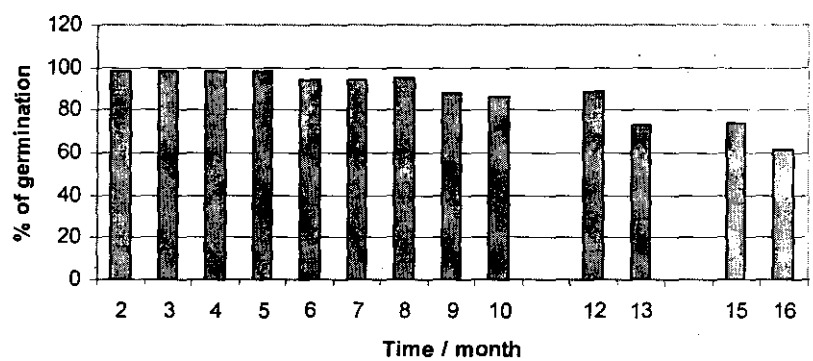
(Fig. 2)Mortality in adults of *R. ferrugineus* treated with different concentrations of *B. bassiana* (B-SA2)



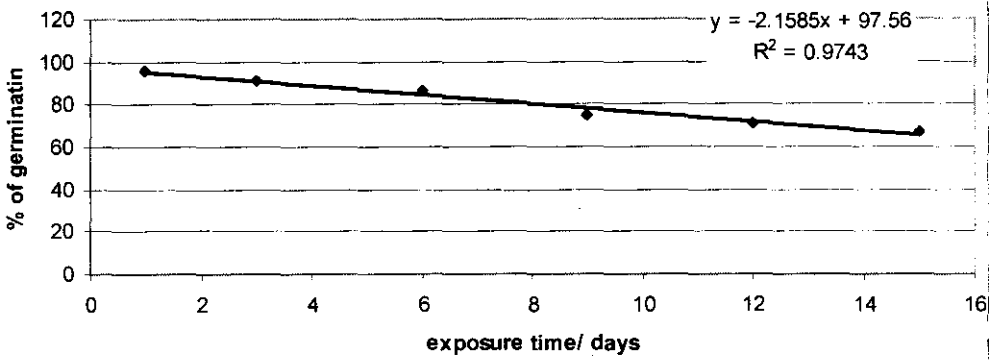
(Fig. 3) Mortality in adults of *R. ferrugineus* treated with different concentrations of *B. bassiana* (B-SA3)



(Fig. 4) Mortality and mycosis of *R. ferrugineus* treated with different concentrations of *B. bassiana* (B-SA3)



(Fig.5) Effect of storage periods on the germination of the dray conidia of *B. bassiana*



((Fig.6) effect of sunrays on germination of the dray conidia of *B.bassiana*

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فاعلية العزلات المكتشفة للفطر الممرض للحشرات بوفيرا باسيانا في مكافحة سوسة النخيل الحمراء في المملكة العربية السعودية

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مشروع مكافحة سوسة النخيل الحمراء – المنظمة العربية للزراعة والتنمية – وزارة الزراعة – القطيف – السعودية

تعتبر حشرة سوسة النخيل الحمراء من الحشرات المدمرة للنخيل في منطقة الشرق الأوسط .ولقد اعتاد المزارعون علي مقاومتها باستخدام المبيدات الحشرية . أسست المنظمة العربية للتنمية الزراعية مشروعا للمكافحة الحيوية لسوسة النخيل الحمراء الغرض منه اكتشاف ممرضات ومفترسات لمكافحة هذه الآفة . تم اكتشاف سبعة عزلات محلية من الفطر الممرض بوفيرا باسيانا من محافظة القطيف بالمنطقة الشرقية بالمملكة العربية السعودية ولقد أرسلت العزلة رقم ٣ ألي مركز كابي للعلوم الحيوية بالمملكة المتحدة للتعريف والتي أوضحت أن العزلة المحلية المكتشفة هي لفطر بوفيرا باسيانا *Beauveria bassiana* . ولقد تم دراسة التقييم الحيوي لثلاثة من عزلات الفطر المكتشف علي سوسة الخيل الحمراء , أوضحت نتيجة الدراسة أن التركيز القاتل إلي ٥٠% من الحشرات هو 1.25 x107 و 1x105 والأخير 2x106

(كونيديا\ مل) . كما تم دراسة الجرعة الاقتصادية لرش الفطر علي الغذاء ثم تطلق الحشرات علي الغذاء المعامل لمدة ثلاثة أيام , أوضحت النتائج أن رش الفطر بتركيز ٣٠ , ٢٠ , ١٠ , ٩ و ٥ x 108 (كونيديا \مل) أدت ألي موت جميع الحشرات في حين أدي التركيزي ٢,٥ و١,٢٥ (كونيديا\مل) إلي موت الحشرات بنسبة تراوحت بين ٥٠ إلي ٥٧ % . أدت المعاملات المختلفة إلي ظهور الفطر خارجيا علي أجسام الحشرات المعاملة بنسبة ١٠٠ % لكل من التركيزيين ٣٠ و ٢٠ x 108 (كونيديا\مل) في حين لم يظهر الفطر إلا بنسب تراوحت بين ٧٥ إلي ١٢,٥ % علي باقي التراكيز الأخرى تباعا . أظهرت الدراسة أيضا أن تخزين الجراثيم الجافة للفطر في درجة حرارة – ٤ ثم اختبار نسبة الإنبات علي فترات مختلفة وحتى ١٦ شهرا , أن نسبة الإنبات كانت ٩٨,٥ % خلال الستة أشهر الأول ثم انخفضت إلي ٨٨ % حتى الشهر الثالث عشر ثم سجلت انخفاضا أخر في الفترة مابين ١٤ إلي ١٦ لتصل نسبة الإنبات إلي ٧٤ و ٦١% علي التوالي . أدي تعريض جراثيم الفطر لأشعة الشمس في أبط سعف النخيل لمدة ١٥ يوما إلي انخفاض معدل الإنبات بنسب تراوحت بين ٩٥,٧ و٦٧,٥٧ % علي التوالي .