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Ecological and Biocontrol Studies on the Red Palm Weevil

Rhynchophorus ferrugineus the Main Insect Pest of Date Palm Trees in the Arabian Gulf Region

Arab Organization for Agricultural Development Project of transfer of biological control technology to integrated pest management of the red palm weevil in the Middle East.

Key words: Rhynchophorus ferrugineus. Red palm weevil. Entovmopathogenic control. Beaveria bassiana. Metarhizium anisopliae . Date palm trees.

Abstract

The present study was conducted at Al-Qatif region in the Kingdom of Saudi Arabia through a program of the Arab Organization of Agricultural Development.

The population density of the red palm weevil, Rhynchophorus ferrungineus was surveyed in two years 2000 and 2006 to determine the weevil abundance during the 12 months of those years. In both considered years, two major peaks were detected, a higher peak was observed in the middle of June, which was higher in the year 2000 than that in 2006, i.e. a mean of 15.52 and 11.44 weevils/trap, respectively. A lower second peak occurred in October and November in 2000 and 2006, respectively. The lowest population density of weevils was recorded in the winter months starting from December up to February.

Susceptibility of date palm tree varieties exhibited different degrees of infestation by the red palm weevil; a high infestation percentage ranging between 23.8 and 27.3 % was observed in Azaba Banat, Reziz and Hagab. The varieties Fahel, Bekera, Halow Ahmer, Awinate, Shehl and Kesba were moderately infested (ranging between 10 and 17.5 %). The varieties Goara,

Kenezi and Magi exhibited approximately

Photo 2. Emergence of Beauveria bassiana on red palm weevil larvae after spraying

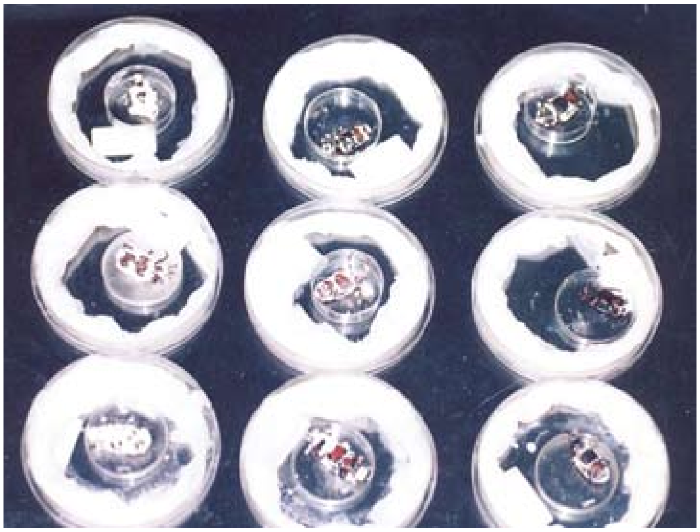


Photo 1. Emergence of Beauveria bassiana on red palm weevil

a 10% infestation percentage; meanwhile, Nashwo was quite resistant to infestation by the red palm weevil.

Furthermore, rate of infestation by the red palm weevil differed on the date palm tree trunk, at a height of 50 cm above ground level, the highest percentage of infestation was detected (i.e. 40%). This was followed by a 20% infestation at ground level, while minimal percentage of 7% was found at the height above 1 meter on the tree trunk from ground level.

Two new Saudi Arabia isolates of the entomophathogenic fungi Beauvaria bassiana (BSA 3 Saudi isolate) and Metarhizium anisopliae

were successfully isolated and recorded, both of which were mass cultured in the laboratory on a Sabouraud plus yeast extract medium and a solid rice grain

medium. A bioassay was conducted to determine the efficiency these two Saudi isolates B.bassiana and M.anisopliae prepared as fungi-codacide oil suspension. In the laboratory experiment, according to the calculated $LT_{50,}$ M.anisopliae proved more effective than B.bassiana as it depicted a shorter LT_{50}. Under semi field B.bassiana proved to be more effective than M.anisopliae as LT_{50} was 121.89 and 132.7 hours, respectively.

In a field experiment, B.bassiana fungi-codacide oil suspension was sprayed at a concentration of $5x10^8$ conidia/ ml on infested date palm trees. One month following the first spraying in December the number of weevils was reduced from 16.5 to 6 weevils/ trap giving a reduction of 63.64%. Meanwhile, following the second application of this fungi suspension in April, low of only 9.09% reduction in the insect population.

Introduction

The red palm weevil Rhynchophorus ferrugineus (Coleoptera: Curculionidae), is a most destructive pest to palm trees in many countries. This weevil attacks coconut, oil and date palms throughout South East Asia (Kalsohven, 1950). The red palm weevil was accidentally introduced for the first time in the Arabian Gulf region in 1985 in the United Arab Emirates, then in 1986 it appeared in Saudi Arabia. In 1992, it spread to Iran, followed by Egypt in 1993, Jordan and occupied Palestine in 1998 and 1999 respectively (Abraham et al., 1998; Murphy and Briscoe, 1999 and Soroker, et al. 2005). In 1997, the Arab Organization for Agricultural Development (AOAD) based in Sudan set up a project with the aim of controlling this destructive insect pest . Efforts for control of R. ferrugineus 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 entomopathogenes of which the fungi Beauveria bassiana and Metarhizium anisopliae proved to be most effective. These two fungi have been reported to be successful in the control of several coleopteron insects, a survey conducted by Li (1988) showed 200 species of coleopteron and lepidopteron insects were infected by these fungi. Subsequently, successful control of many insect pest have been reported by the use of these entomopathogenic fungi; e.g. potato beetle, Leptinotarsa decemlineata (Miranpuri et al., 1992a); flea beetles, Phyllotreta crucifera (Miranpuri et al. ,1992b); blister beetle, Lytta nuttali

Photo 3. Discovering the isolation of Emergence of Beauveria bassiana in Kingdom of Saudi Arabia in the soil under the date palm farms

Photo 4. Impact of the discovered Beauveria bassiana on red palm weevil

(Miranpuri and Khachatourians, 1994), and banana weevil (Nagra et al., 2004).

The present investigation was undertaken was carried on date palm trees in Al-Qatif Region, Saudi Arabia by a project sponsored by AOAD. The experiments were concerned with the following:-

1- Survey of the seasonal fluctuation in the population densities of the red palm weevil. This part of the work is significant for choosing the appropriate time for control of this insect pest.

2- Susceptibility of the different varieties of date palm tree to infestation by the red palm weevil.

3- Infestation by the red palm weevil on the vertical height of the date palm tree trunk as well as the age of the trees.

Photo 5. Employee training on proliferation of the pathogenic fungi (Beauveria bassiana) to insects on environment of Sapurad Dexter's Agar

4- Evaluating the efficiency of two new entomopathogenic fungi isolates Beauveria bassiana (BSA3) and Metarhizium anisopliae (Saudi isolate) under semi field condition for the control of the red palm weevil.

5- Efficiency of B. bassiana (BSA3) for the control of red palm weevil in the field. It was necessary to establish means for the mass production of these fungi in the laboratory so as to supply sufficient amounts needed for experiment.

Materials and Methods

Experiment 1: For the collection of the red palm weevils from infested date palm trees an improved insecticide free food baited aggregation pheromone / kairomone traps were used (Hanounik et al., 2000) and approved by the project AOAD. These traps were placed partially buried around the trunk of the date palm trees at the plantations in Al-Qatif region. Approximately 12 traps were placed (one trap/ hectare), the traps were investigated at a weekly interval throughout the year, the number of collected weevils were recorded and mean number of weevil/ month determined. It was noteworthy, that the traps were replaced with fresh ones at each weekly inspection.

Photo 6. Installment of Pheromone-kairomone trap that was used to collect red palm weevil from date palm farms

Photo 7. Search for red palm weevil under date palm trees after 48 hours from spraying, then put them into incubation under humidity conditions to know cause of death (See Photo 1)

Experiment 2: It is well established that all date palm tree varieties are liable to infestation by the red palm weevil. However, liability of infestation was noticed to be higher in some varieties and lower in others, also, the age of the palm tree showed some variation. A total of 3017 date palm trees presenting 16 varieties were considered in this part of the work in Al-Qatif region. The palm trees were divided into two groups, the first presented wide spread popular varieties ,e.g. Reziz, Shishni, Klas, Fahl, Bekera, Hellaw Ahmer, Goarra , Kinizi and Nashow .The second group was expressed by less common varieties e.g. Asaba Banat, Hogob, Awinate, Shehl , Kesba and Magi . Other varieties presented by 15 or less palm trees were not considered in this survey.

Photo 8. Spraying Date Palm farms with the fungi's solution product (Beauveria bassiana)

Experiment 3: The vertical distribution of infestation by the red palm weevil on the trunk of date palm trees was also studied. The distance (cm) between symptom of infestation on the trunk at ground level and different heights of the palm tree was measured as well as under the soil. In this investigation only signs of fresh infestations were considered.

Furthermore, approximately 200 trees regardless of their variety were categorized according to their ages; 1-5, 6-10, 11-15, 16-20, 21-25, 26-30 and 31-40 year old tree, and rate of their infestation by the red palm weevil determined.

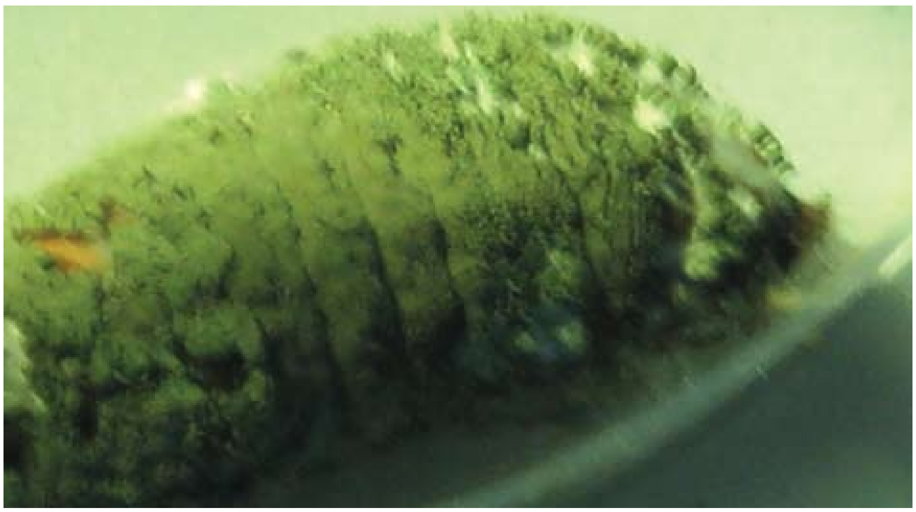
Photo 9. Half-circled Experiments boxes before spraying the farms

improved insecticide free food baited aggregation pheromone - kairomone traps were used

Experiment 4: Two new Saudi Arabia isolates of entomopathogenic fungi were evaluated for their efficiency in the control of the red palm weevil, (i) Beauveria bassiana (BSA3 Saudi isolate), Hegazy et al. (2007) working at

AOAD project successfully isolated this fungus from infested red palm weevils collected at Al-Qatif region, it was recorded after being verified by CABI Bioscience, UK.

(ii) Metarhizium anisopliae was isolated by Hegazy and Al –Muhanna (2006), from the soil under date palm trees at Al-Qatif region .This fungus was confirmed by Biological Control Institute, at Dermchtate, Germany. These two entomopathogenic fungi were mass produced in the laboratory of the AOAD project at Al-Qatif region.



Larvae of Rhynchophorus ferrugineus sprayed with entomompathogenic fungus Metarhizium anisopliae

For the production of the two fungi a liquid Sabouraud Broth plus yeast extract medium (per litre: Mycological Peptone 10gm, Dextrose 40 gm, yeast extract 2.5 gm) was prepared and 60 ml of the medium is then distributed into 250 ml Erlenmeyer flasks.. The flasks were plugged with cotton bungs, covered with aluminium foil and autoclaved at 121ºC for 20 min. Once cool, each flask was inoculated with 1 ml of a spore suspension (6×10^6 conidia/ml) in 0.05% Tween 80 using fresh conidia obtained from SDYA slopes. The flasks containing inoculated media were placed on a rotary shaker (110 rpm) for 3 days at 27± 2º C. This procedure provides an inoculum of suspended mycelial fragments in active growth phase for transfer onto the solid substrate. The mycelial inoculum was diluted by 50% with cold sterile water before transfer to the solid substrate (Jenkins et al., 1998).

A solid medium made with rice grains was also tested for the mass production of the considered fungi according to the method of Jenkins et al. (1998). From a preliminary experiment the Egyptian variety of rice proved to be more efficient than the American rice Uncle Ben for the maintenance of the fungi (Hegazy and Al-Muhanna, 2006). Rice grains were washed in running water for 10 minutes then well drained, fresh water was then added to the rice at the rate of 300 and 200 ml/ kg for that to be inoculated with B. bassiana and M. anisopliae, respectively. The rice was par boiled for 10 min to accelerate the absorption of the water before distributed into autoclaveable sacs (1 kg dry rice/ sac) and autoclaved for 60 min at 121^{0}C. Once cool, the bags were transferred to a laminar air flow cabinet and 150 ml of ready diluted liquid inoculum was added to each sack and thoroughly mixed. The sacs were blown by sterile oxygen and sealed then placed on

shelves in a disinfected air conditioned room at 27 ± 2^o C for 15 days. On completion of the incubation period the sacs were opened and the inoculated rice was spread at a 3 cm layer thickness on disinfected plates to allow the substrate to dry. The conidia were observed to form a white layer on the surface of the conidiated rice and by means of a sieve it was separated and collected as a dry powder. This powder was placed in disinfected plastic sacs with non indicating silica gel at a rate of 20% w/w .The sacs were sealed and stored at -4^{o}C until needed for the experimental work.

The fungi B. bassiana and M. anisopliae are hydrophobic and do not form a suspension in water, therefore Codacide oil was added to the dry conidia to overcome this issue. Codacide is a natural vegetable oil adjuvant produced by Dow Agro Sciences which when mixed with plant protection pesticide (e.g. herbicide, fungicide insecticide, IGR…etc) increase their efficiency by improving their deposition, spread and reduces spray drift. Also, it improves deposition adhesion on plant surface or insect cuticle. Therefore, the addition of codacide to the prepared B. bassiana and M. anisopliae spray formulation was evaluated to establish if its addition would

Mass production of entomopathogenic fungus Metarhizium anisopliae on rice

improve the characteristic of the tested entomopathogenic fungi. Suspensions of 5x10^8 and 5x10^9 conidia /ml of either M .anisopliae or B.bassiana, were added to 10 cm of codacide oil in one liter distilled water and blended well on an electric rotator. It is noteworthy, that these two concentrations were chosen from the bioassay conducted by Hegazy et al. (2007) to determine the fungi LC_{50}.

Mass production of inoculated rice by entomompathogenic fungus B. bassiana (BSA isolate)

To establish the homogeneity in distributions of the fungi-conidia suspension, it was sprayed on glass slides and the droplets were investigated by a microscope. The viability of the fungi was then determined by cutting off the antennae of treated weevil and fixing it on the inner side cover of SDA Petri dish and inoculation of the fungus was followed.

Experiment 5:- A laboratory experiment was conducted to determine LT_{50} (mean lethal time) from the accumulative mortality of the red palm weevil treated with the concentration of 5x10^8 and 5x10^{9}conidia/ml of both B.bassiana and M.anisopliae fungi-conidia suspension. Results were presented graphically as log/probit lines and LT_{50} values calculated by the program Sigma plots for windows (version 2).

Experiment 6:- A semi field experiment was carried out in a date palm nursery, uninfested young shoots were chosen, each of which was covered

by a mesh netting. To each shoot ten adult red palm weevils were introduced, they were left for 24 hours to assure their boring in the tree trunks. Subsequently,

Mycosis of treated R. ferrugineus sprayed with

Semi field application of entomompathogenic fungi on red palm weevils

six plots, each presented by ten date palm shoot (still enclosed in the mesh net) were considered. The first and second plots were sprayed with $5x10^8$ and $5x10^9$ conidia/ml, respectively, of B.bassiana fungi-codacide suspension. The same procedure in the third and fourth plots was sprayed with M.anisopliae at the respective above mentioned concentrations. As a control, date palm shoots were sprayed with water and another control was sprayed with water mixed with codacide oil. Mortality of the weevils was calculated from which the lethal time LT_{50} determined.

Experiment 7 :- Field application of the fungus B. bassiana (BSA3):

Two adjacent date palm trees farms, each of approximately 5 hectares infested by the red palm weevil were selected, one presenting a control and the other specified for the spray application of the entomopathogenic fungus B. bassiana Saudi Arabia isolate (BSA3). One month prior to the application of the fungus B. bassiana at a concentration of $5x10^8$ conidia/ml the mean monthly population density of the red palm weevils was determined from the weekly catch by the pheromone/ kairomone traps; the traps were distributed at a distance of 100 meters apart. In the experimental farm, date palm trees were sprayed by means of 600L mechanical sprayer with $5x10^8$ conidia/ml of B.bassiana obtained from the maintained mass culture. The nozzle of the sprayer was modified to give a slow coarse spray and spraying of the fungus suspension was directed to the crown of the tree where weevils are known to aggregate. Two applications, with the same concentration, were administered to each palm tree, the first application was conducted in December 2005 and the second in April 2006. Following each application of the fungus the mean monthly population density of the red palm weevil was again determined by the use of the pheromone/ kairomone traps, so as to evaluate the efficiency of the fungus spraying in reducing the population of this weevil.

The same procedure of the above mentioned experiment was repeated in the year 2007; however, the first spraying of the fungi suspension was carried out in February and the second in April. The numbers of weekly caught weevils were counted and the monthly mean number of the caught insects determined.

Percentage reduction in the numbers of the red palm weevil was calculated by Henderson and Tilton equation (Henderson and Tilton, 1955).

$$R = \left[1 - \frac{Ta \ x \ Cb}{Tb \ x \ Ca} \right] x \ 100$$

Where:

R = Percentage of population reduction

Tb = Number of insects in treated plot before treatment.

Ta = Number of insects in treated plot following treatment.

Cb = Number of insects in control plot before treatment.

Ca = Number of insects in control plot following treatment.

This experiment was important so as to determine the appropriate time of the year for the control of this insect pest. It was necessary to determine if the death of the weevil was due to infection by the fungus or other reason. For this purpose, the caught weevils as well as any dead ones found on the trees or soil were also picked and taken to the laboratory for investigation. These weevils were individually placed in humid Petri dishes, after seven days the insects were investigated to determine mycosis on their cadavers.

Results

Population densities of the red palm weevil R. ferrugineus

In the survey conducted during the two years 2000 and 2006, it was noticed that the mean monthly abundance of the red palm weevil fluctuated during the different months. Furthermore, both years understudy exhibited a similar trend and also at a much lower abundance in the year 2006. In both years under investigation the highest number of insects on the palm trees was detected during the month of June.

As depicted in Fig.1, in the year 2000 a gradual slow rate of increase in the abundance of weevil started from the beginning of January up to the middle of April. This was followed by a sharp increase to reach a peak in the weevil population by the middle of June reaching a mean of 15.52 weevil/ trap/ month. In the subsequent months, the insect's population sharply decreases to reach a mean of 6.32 weevil/ trap/month in September. Subsequently, a second peak in the insect's population was observed in the middle of October giving 8.8 weevil/ trap/month. The number of collected insects then slowly gradually declines up to the end of December, (i.e. 5.6 weevils/ trap/ month).

In the second year, 2006, under investigation, a relatively similar trend to that observed in 2000 in regard the number of red palm weevils collected by the traps, although at a lower rate. In this year a sharp drop in the number of weevils occurred from 7.72 to 2.88 weevil/ trap/ month from January to February. This was followed by a slow gradual increase to reach a first peak of 11.44 weevil/ trap/month in middle of June which then dropped between to 3.6 to 3.12 weevil/ trap/month in September and October 2006, respectively. A second peak was detected in the middle of November reaching 9.64 weevil/ trap/ month followed by a sharp decrease of 5.6 weevils / trap/month in December.

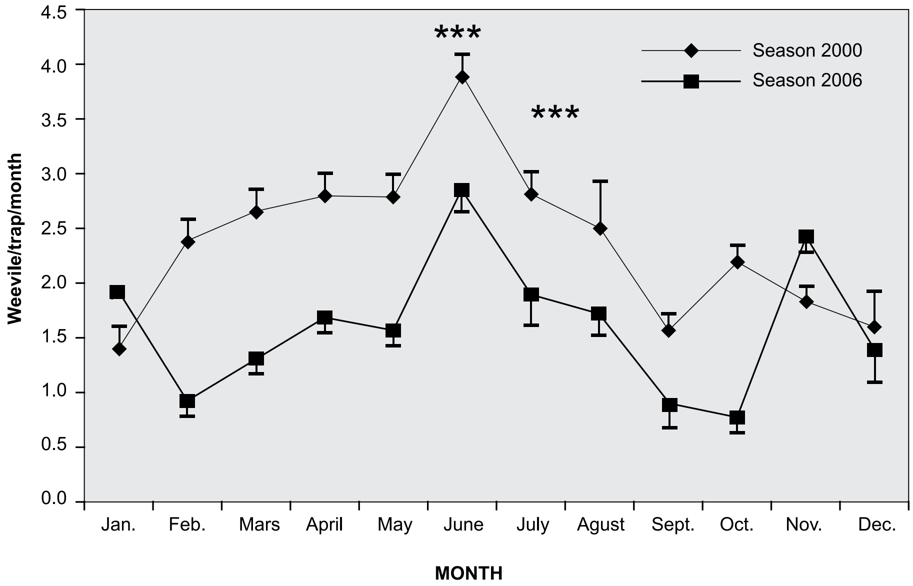


Fig. 1: Monthly fluctuation in the population density of the red palm weevil at Al-Qatif province, Saudi Arabia in two different years 2000 and 2006.

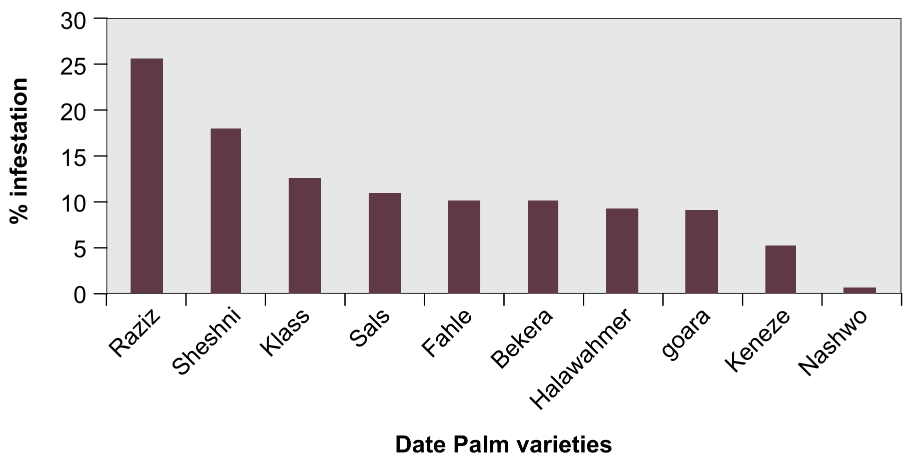


Fig :2.Percentage infestation by R. ferrugineus on popular wide spread date palm tree varieties.

Susceptibility of date palm varieties to infestation by the red palm weevil Rhynchophorus ferrugineus

In the popular wide spread varieties, Reziz was the most susceptible date palm variety to infestation by red palm weevil reaching 25.9 %, this was followed by 17.9 % in Shishni variety In the varieties Klas ,Sals ,Fahl, Bekera, Hallow ahmer and Goarra the rate infestation was much lower and ranged between 9 to 12.5%.

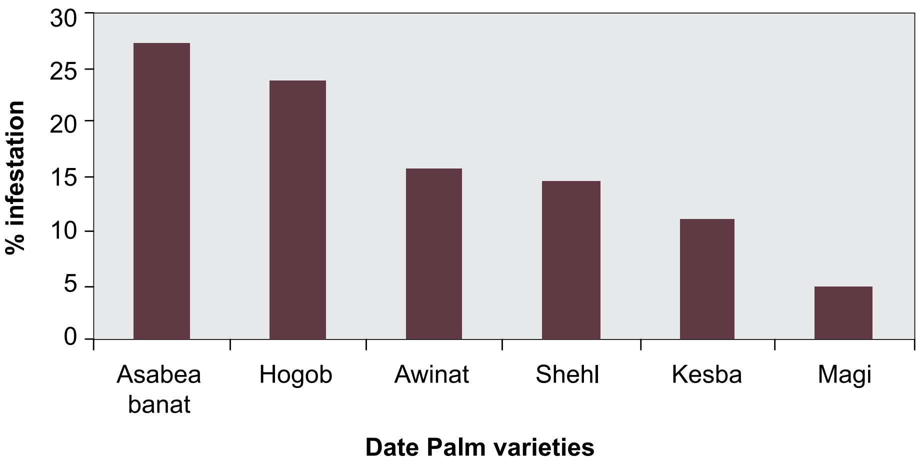


Fig 3.Percentage infestation by R.ferrugineus on less common wide spread date palm varieties.

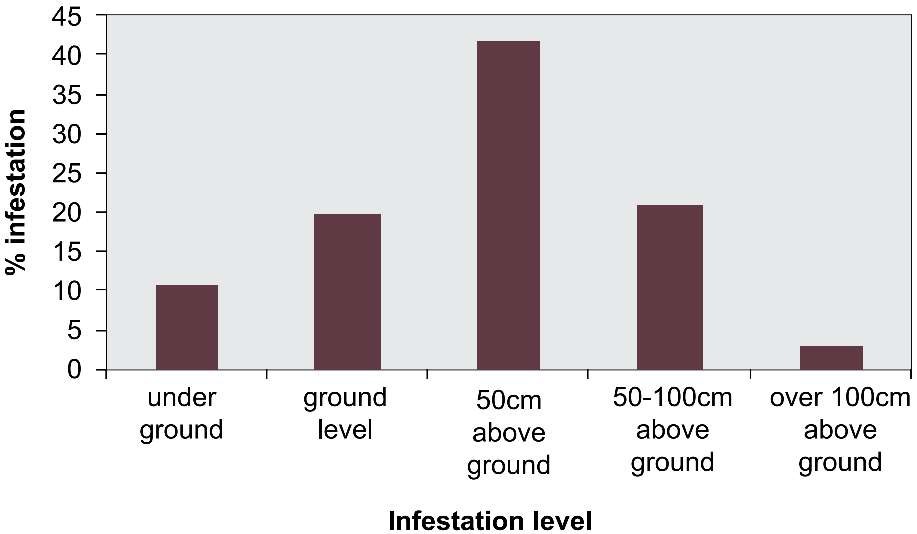


Fig.4: Vertical distribution of infestation by the red palm weevil on date palm tree trunk

The least infestation was detected in Kinizi and Nashow varieties, i.e. 5.1 and 0.5 % respectively (Fig. 2).

In the less popular varieties, the variety Asaba Banat was the most susceptible to infestation by the red palm weevil giving 27.3% closely followed by Hogob (23.8 %).The ratio of the infestation in Awinate, Shehl and Kesba varieties were 15.0, 14, 6 and 11.1% respectively. In this group the least ratio of infestation of 4.8% was recorded on Magi variety (Fig. 3).

Vertical distribution of infestation:

From a survey conducted on nearly 200 date palm trees infested by the red palm weevil and ranging in age between 1 to 10 years, it was observed that 11 % of weevil infestation was below soil level. Meanwhile, a higher level of infestation reaching 20 to 21% occurred on the tree trunk at ground level

and between 50 to 100 cm height. The highest infestation percentage of 40% was detected at the height of 50cm on the tree trunk from ground surface. Meanwhile, minimal infestation of 7% was found above 100 cm level (Fig.4).

The highest percentage of 2.75% infestation by R.ferrugineus was detected on date palm trees ranging in age between 6 and 10 years, this was followed by 1.1% in those aged 11and 15 years. Younger date palm trees i.e. 1 to 5 years exhibited 0.4 % infestation by the weevil. It is noteworthy, that weevil infestation in older trees (above 16 years old) was minimal.

Efficacy of the entomopathogenic fungi B.bassiana and M. anisopliae for the control of the red palm weevil:

(i)Fungi–codacide suspension: It was necessary to determine the distribution of the sprayed fungi-codacide suspension. Using an atomizer, the fungi suspension mixed with 10% codacide oil was sprayed on a glass sheet. Observation with a microscope exhibited an even distribution of the conidia of both B.bassiana and M. anisopliae as they appeared as a homogenous layer with no cells clumping or clusters.

To examine the viability of the fungi-codacide suspension, red palm weevils were sprayed with 5x 10^8 conidia /ml, subsequently, the antenna of the weevils were severed and fixed on the inner surface of a SDA Petri dish. After approximately 7days, the antennae were investigated, in all cases, the fungi was evident on these antenna and the grown conidia had dispersed on the SDA medium and subsequently grew normally as colony forming unit proving the viability of the fungi-codacide suspension.

(ii)Laboratory experiment: Two concentrations, $5x10^8$ and $5x10^9$conidia /ml of each of the two fungi-codacide suspensions B. bassiana and M. anisopliae were tested to determine their LT_{50} under laboratory conditions (Table 1 and Fig.5). When the higher concentration, i.e. $5x10^9$ conidia /ml of B. bassiana or M. anisopliae

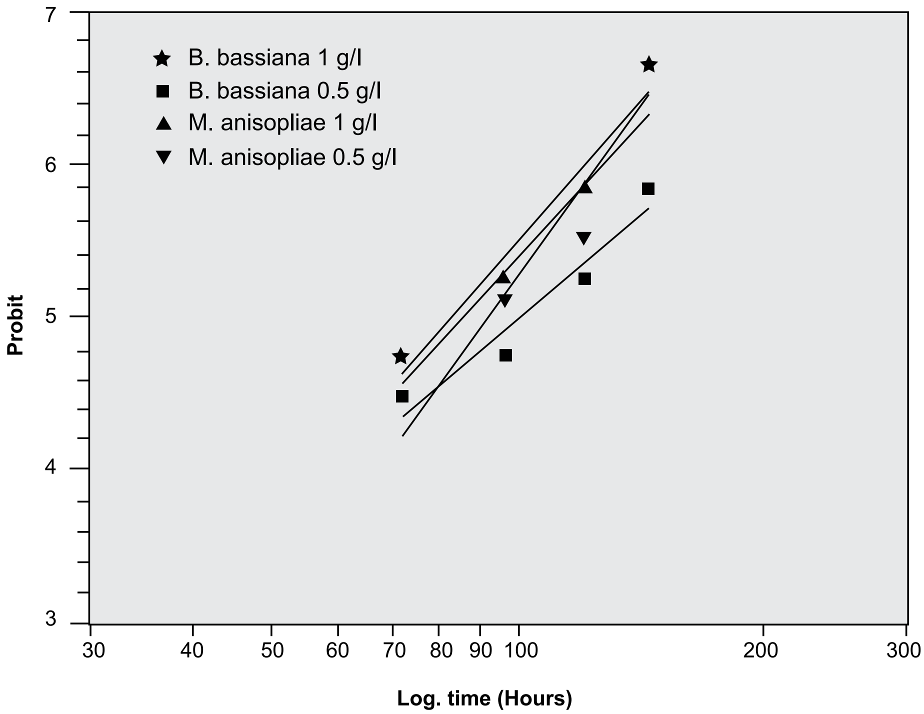


Fig. 5: LT_{50} calculated from accumulative mortality of the red palm weevil treated by two concentrations of B. bassiana and M. anisolpiae spores under laboratory conditions.

Fungi	Concentration	LT_{50} (hours)	Slope	r
B. bassiana	$5x10^9$	91.20	7.39	0.95
	$5x10^8$	100.44	4.48	0.96
M. anisopliae	$5x10^9$	82.79	6.15	0.97
	$5x10^8$	85.11	5.86	0.93

Table 1: LT_{50} values of the red palm weevil treated by two concentrations of B. bassiana and M. anisopliae under laboratory conditions.

were tested, LT_{50} was found to be 91.20 and 82.79 hours, respectively. When the lower concentration of the respective mentioned fungi was applied, i.e. $5x10^8$ conidia /ml, the calculated LT_{50} were slightly longer i.e. 100.44 and 85.11 hours, respectively. These values show that M. anisopliae had a more rapid effect than B.bassiana in the laboratory experiment. The regression values estimated from the regression lines ranged between 0.93 and 0.97.

Semi field experiment: Table 2 and Fig. 6 show the values of the calculated LT_{50} of the red palm weevil following treatment with the two fungi under

study conducted under semi field conditions. LT_{50} was 121.89 and 132.70 hours for B. bassiana and M. anisopliae, respectively, in addition, the slope values of the probit regression lines were 3.23 and 2.72 while the regression values were 0.98 and 0.97 to the two respective mentioned fungi. These values show that under semi field conditions B. bassiana exhibited a higher potency and a more rapid action than M.anisopliae against this weevil. In addition, according to the fungi conidia it was observed that the dimension of B. bassiana spores was 3.5 micron. Meanwhile, M. anisopliae spores were elongated with a dimension of 9 micron, signifying that B. bassiana spores exhibited a more surface area contact than that offered by M. anisopliae spores which may in turn may affect the potency of the fungi.

(vi)Field application in the season 2005-2006:

Prior to the application the fungus B. bassiana on date palm trees infested by the red palm weevil, a mean of 16.5 weevil/ trap/month were caught by the pheromone/kairomone traps (Fig.7). After the first spraying in December 2005 by this fungus at a concentration of $5x10^8$ conidia/ml, the monthly mean number of collected weevils after one month following application was reduced to 6 weevil/ trap giving a total 63.64% percentage reduction in their population. Prior to the second application in April 2006 by B.bassiana, 11 weevils/ trap were collected, after one month from this application, the population of the weevils was reduced by only 9.09%, (Fig7).

Dead R. ferrugineus weevils collected from the field following the first application of B. bassiana, showed that 88% of these collected weevils died as a result of their infection by this fungus .This was exhibited by the mycosis on weevil cadavers.

The previous experiment was repeated in 2007 but by applying the first spray treatment by $5x10^8$ conidia /ml of B.bassiana in the second week of February instead of December and the second application during the second week of April. The number of collected weevils

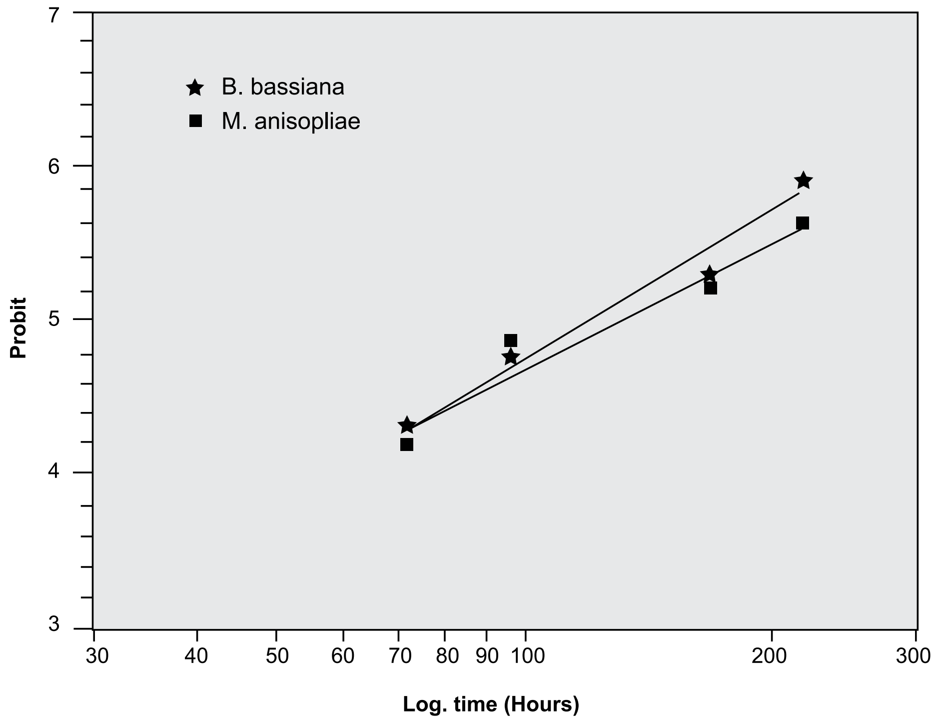


Fig. 6: LT_{50} calculated from the accumulative mortality of the red palm weevil treated by B.bassiana and M.anisopliae at $5x10^8$ conidia /ml under semi field conditions.

Fungi	LT_{50} (hours)	Slope	r
B. bassiana	121.89	3.23	0.98
M. anisopliae	132.70	2.72	0.97

Table 2: LT_{50} values of the red palm weevil treated with $5x10^8$ conidia/ ml of B. bassiana and M. anisopliae under semi field conditions.

prior to the first application was 8 weevils/ trap. One week following the fungus spraying the number of collected insects was markedly reduced to 3 weevils/ trap, with no change in the weevil population in the control, giving a 70% reduction in the population of the red palm weevil. However, in the second week following the fungi spraying the number of trapped weevils in the treated plot had risen to 10 weevils/ trap, comparable to their number detected in the control plot (i.e. 11 weevils/ trap). At this period percentage reduction in the insect population was only a low of 9.09%. A mean reduction of 38.1% in the weevil population was calculated in these two week period in February.

In the second week of April, the population of R.ferrugineus in the control plot was 8 weevils /trap, however this number increased to 13 weevils /trap in the third week of this month. Meanwhile, in the plot treated with B.bassiana there was a reduction in the population of the red palm weevil from 12 to 8 weevils/ trap.

Discussion

The Saudi Arabia isolates of the two entomopathogenic fungi B.bassiana and M. anisopliae were successfully cultured in the laboratory on the standard semi liquid medium prepared from dextrose and peptone. Also, a solid medium prepared from rice grains was tested and proved its high efficiency in producing a high yield. The component of the latter medium was much more economical than the former one, therefore, it should be more suitable for the mass production of these two entomopathological fungi. Godonou (1999) working with a rice medium achieved $5x10^5$ conidia per gram of B.bassiana.

In the laboratory test, M.anisopliae had a much more rapid effect on the red palm weevil than B.bassianna as exhibited by LT_{50} values. Meanwhile, when the same test was conducted under semi field conditions, B.bassiana was much more potent as a lower LT_{50} value was observed. This signifies that the potency of M.anisopliae in the field was more affected by some environmental factor to which B.bassiana was much more tolerant.

Codacide oil was added to the two prepared suspension of both fungi to increase their efficiency. Bateman et al. (1998) and Luze and Batagin (2005), indicated that the use of oil enhanced the efficiency of mycoinsecticide, furthermore, the addition of oil enables fungi pathogens to remain active under conditions of low humidity.

Before implicating any control program, it is necessary to determine the population of the insect so as to choose the appropriate time for the application of control measures. El-Garhy (1996) conducted experiments in Egypt and found that population of the red palm

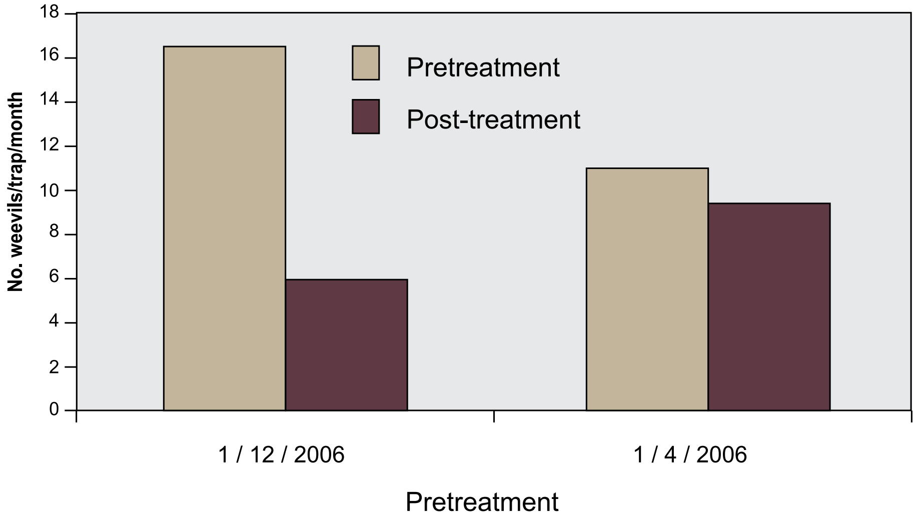


Fig.7: Numbers of R.ferrugineus weevils prior and post treatment with $5x10^8$ conidia/ ml B.bassiana (BSA3) on date palm trees at Al-Qatif region.

weevil increased with the onset of warmer weather, starting from the month of April. Meanwhile, also in Egypt, Hussein (1998) stated that environmental changes do not appear to have any remarkable influence on the population growth of the red palm weevil. In the present survey conducted in Al-Qatif (KSA), it was observed that the highest population density of the red palm weevil was detected in June and in October-November; meanwhile relatively low infestation was found in December up to February. This shows that this weevil can tolerate warm and moderate high temperatures more than their toleration to lower temperatures. Meanwhile, the weevil's population declined with the high temperatures in the months of July, August and September. It seems reasonable to assume that as only adult weevils were considered in the trapping, it could be suggested that development of this insect in the larval stage could have been extended in the cooler months of the year, and therefore delayed the appearance of the adult weevil. Or that due to rise in temperatures, the insect life span was shortened and adult weevils emerged in search of their mates. However, further studies are needed to establish the life cycle of this insect in the field.

Choosing the appropriate time to conduct a biological control program by the use of fungi is most important for it to succeed. The potency of fungus B.bassiana sprayed in the field, apparently, was affected by several environmental factors. It was found most efficient in reducing the population of the weevils in the first and second week following application, subsequently; the fungus gradually loses its viability. Bernal et al. (1999) found that infection levels of B.bassiana and M.anisopliae were highest during the first five days following application. Costa et al. (2001), found that the persistence of viable B.bassiana spores was significantly longer when a greater portion of the UV spectrum was blocked. Hegazy et al. (2007) showed that germination of B.bassiana conidia was more affected by sunlight than temperature.

Most date palm tree varieties were infested by the red palm weevil but by different rates. This observation is most significant; therefore, relatively resistant varieties are to be chosen when planting new fields, without neglecting the yield and economical value of the date palm varieties. Infestation by the red palm weevil was relatively low in young trees (1 to 5 year old), older ones (6 to 10 year old trees) were liable to a higher rate of infestation. Meanwhile, in 16 and older trees negligible infestation was detected. These observations may signify the preference of the red palm weevil to certain nutritional factors found in some palm tree varieties and not in others; also the penetration of this weevil in the trunk of old trees might be hindered to certain

Treat ment	N° of insects		% Reduc tion	Number of insects after 2 weeks	% Reduc tion	Mean number of insects	% Reduc tion
	Before treat ment	After 1 week					
Control	8	10	------	11	------	10.5	-----
Treated	8	3	70	10	9.091	6.5	38.1

Table 3: Number of Rhynchophorus ferrugineus weevils caught by in date palm tree plantation treated with Beauveria bassiana (BSA 3)

factors in the tree structure.

The two considered entomopathogenic fungi B.bassiana and M. anisopliae proved their efficiency in the control of the red palm weevil, therefore should be included in an integrated pest management program.

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