**Integration of Repellency Effect of Neem-based Insecticide and Pheromone Bio-trap with *Beauveria bassiana* (Hypocreales: Cordycipitaceae) to Control the Red Palm Weevil, *Rhynchophorus ferrugineus*, (Coleoptera: Curculionidae)**

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Abstract

To control adults of the red palm weevil (RPW), *Rhynchophorus ferrugineus*, a pheromone bio-trap containing the commercial formulation of *Beauveria bassiana* (BroadbandTM) was integrated with neem-based insecticide, (azadirachtin). *Beauveria bassiana* at 40 ml suspension of 1 × 109 spores/ml caused 100% mortality of RPW. The LC50 of neem-based insecticide, (azadirachtin) to RPW by direct spraying was 1810.3 ppm, whereas that by feeding was 2117.80 ppm. The lowest concentration of neem that was 100% repellent to RPW was 800 ppm. The repellency effect of neem was integrated with the pheromone bio-trap in three net houses (5 × 5 m), each planted with five offshoots of palm trees (height, 1–1.5 m). In two of the net houses, wounds were made on offshoots and sprayed weekly with 800 ppm neem, and a pheromone bio-trap was introduced. The third net house was the control, in which wounds were made on the offshoots without the spraying of neem and a pheromone non-bio trap (without fungus) was introduced. Thirty adults were introduced, and after 30 d in the net houses with the pheromone bio-trap, all RPW died because of contamination by fungi. The repellency of neem prevented the insects from laying eggs in the wounded offshoots, and no larvae were found. However, in the control net house, only 60% of the adults were trapped and killed in the pheromone non-bio trap, and two of the five offshoots were infested with 26 - 34 larvae.

Keywords: botanical insecticide, neem, *Beauveria bassiana*, red palm weevil, *Rhynchophorus ferrugineus*, pheromone bio-trap.

Résumé

Pour contrôler les adultes du charançon rouge des palmiers *Rhynchophorus ferrugineus* (RF), un bio-piège à phéromones contenant la formulation commerciale de *Beauveria bassiana* (BroadbandTM) a été intégrée à l’insecticide à base de neem (azadirachtine). *Beauveria bassiana* à 40 ml de suspension de 1 × 109 spores/ml a causé une mortalité totale de RF. La CL50 de l’insecticide à base de neem, (azadirachtine) pour RF était de 1810.3 ppm par pulvérisation directe, et de 2117.80 ppm par alimentation. La plus faible concentration de neem qui était 100% répulsive à RF était de 800 ppm. L’effet répulsif du neem a été intégré au piège biologique à phéromones dans trois maisons en filet (5 × 5 m), chacune plantée de cinq ramifications de palmiers (hauteur, 1–1,5 m). Dans deux de ces maisons, des blessures ont été faites sur des ramifications et pulvérisées chaque semaine avec 800 ppm neem, et un piège biologique à phéromones a été introduit. La troisième maison en filer était le témoin, dans lequel les blessures ont été faites sur les ramifications sans la pulvérisation de neem et un piège non biologique à phéromone (sans champignon) a été introduit. Trente adultes ont été introduits, et après 30 j, dans les maisons munies d’un piège biologique à phéromones, tous les RF étaient morts à cause de la contamination par des champignons. La répulsion du neem a empêché les insectes de pondre des œufs dans les ramifications blessées, et aucune larve n’a été trouvée. Cependant, dans la maison témoin, seulement 60% des adultes ont été piégés et tués dans le piège à phéromone non biologique, et deux des cinq ramifications ont été infestées by 26 - 34 larvées.

**Introduction**

Date palm is the main crop in countries of the Arabian Gulf and the production of dates is an important part of the food security, particularly for rural communities. Culturally, dates are one of the foods consumed daily in these arid regions. The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), (Coleoptera: Curculionidae), is an invasive species that attacks date palms, which was discovered in the Arabian Gulf countries in mid-1985 and in Saudi Arabia in early 1987 (Al-Abdulmohsin 1987). Because the pest management tools were insufficient and phytosanitary measures were weakly implemented, RPW spread rapidly in Saudi Arabia, primarily through infested plant materials transported from one area to another (Faleiro et al. 2019). The RPW has become the most destructive pest affecting palm trees, causing serious damage that ultimately leads to the complete removal of infested palm trees. As a result, the numbers of palm trees are decreasing in the countries of the Arabian Gulf (Faleiro 2006). This pest is expanding its geographical range, becoming a worldwide invasive pest species in the last four decades. The RPW has been recorded in the Middle East, Europe, North Africa, and the Caribbean Islands (Faleiro 2006, Giblin–Davis et al. 2013, Faleiro et al. 2019).

Chemical pesticides are the most common tool used to control pests and weeds in both undeveloped and developed countries (Whitten and Oakeshott 1991). Pesticides are widely used because they are toxic to a wide range of pests, and are cheap and easily applied using spraying equipment. Globally, $25­–30 billion is spent annually on pesticides (Pretty and Hine 2005). In Saudi Arabia, approximately 3,700 tons of pesticides are used annually, (personal communication, Ministry of Agriculture, 2018). Although chemical pesticides are effective in protecting crops, there is a concern about negative effects on the environment and human health. Because of the intensive use of chemical pesticides, RPW has recently developed resistance to the commonly used insecticides such as organophosphorus and synthetic pyrethroid insecticides, impairing the efficacy of RPW control in the field (Al-Ayedh et al. 2016, Wakil et al. 2018). Therefore, alternative techniques to protect plants, including botanical insecticides and biological methods (Pretty and Hine 2005), are necessary.

The weevils of *Rhynchophorus* genus are attacked by more than 50 different natural enemies, including viruses, bacteria, yeast, fungi, nematodes, mites, insects, and vertebrates (Mazza et al. 2014). Both fungi and nematodes are promising biological control agents against RPW (Dembilio et al. 2010, Manachini et al. 2013, Hussain et al. 2017). The insect pathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* can infect and kill a variety of arthropod species. Both fungi are used as insect pathogens in the biocontrol of agricultural pests. In particular, *M. anisopliae* is used to control grasshoppers and locusts in different parts of the world. Contact with or intake of these two entomopathogenic fungi reduces the populations of target insects (Bateman at al. 1996, Clarkson and Charnley 1996, Wraight et al. 2010, Hussain et al. 2017). In particular, the entomopathogenic fungus, *B. bassiana* has shown great promise in the control of RPW (Güerri–Agulló et al. 2011). Laboratory and semi-field cage studies show the possibility of infecting and killing RPW adults with *B. bassiana* by using pheromone traps (Hajjar et al. 2015). However, hot and dry conditions are very unfavorable towards the implementation of entomopathogenic fungi, which require warm and moist conditions. Faleiro et al. (2019), in an overview of the gaps, challenges, and prospects of RPW management, found that the known entomopathogenic agents (fungi and nematodes) are not adequately exploited in the control of the RPW. In Saudi Arabia and the Arabian Gulf countries, the use and implementation of these agents in date palm fields have not been promising, which is undoubtedly due to the extremely hot and dry conditions that prevail in the arid regions of the world in which date palms are cultivated. In addition, larval stages in the life cycle of the RPW are hidden in the trunks of date palms and the use of biological agents in the field is challenging (Al-Dosary et al. 2016). Therefore, insecticides remain the main tool used to control this pest, in addition to some cultural protective measures (Faleiro 2006). In developing integrated pest management (IPM) program to control the RPW, an important alternative to the commonly used insecticides is the use of a botanical insecticide that is less harmful to the environment and humans. The botanical insecticide neem-based, azadirachtin is an alternative, environmentally friendly insecticide that is used to control many harmful pests. The active substance azadirachtin is extracted from neem seeds, producing an organic insecticide that has strong effects on pest behavior, with antifeedant and repellent effects affecting pest growth (Schmutterer 1990, Ruckin 1992, Lowery and Isman 1994, Tunca et al. 2014). Neem extracts affect more than 200 species of insects, including white flies, thrips, beetles, and moths (Miller and Uetz 1998). Various malformations were recorded on adult weevils treated with azadirachtin. A negative correlation was observed between the concentration of azadirachtin and the rate of adult emergence of RPW (Bream et al. 2001, Abahussain 2008). Neem is primarily injected into infested palms, and this method of application is effective in killing various stages of the RPW in the trunks of palms, thereby reducing the infestation (Ghoneim et al. 1998, Azam et al. 2001, Abahussain 2008, Sujatha et al. 2009). Furthermore, Al-Shawaf et al. (2013) reported that azadirachtin kills 96.7% of RPW eggs in treated sugarcane pieces. In addition (Merghem, and Mohammad, 2017) reported the potential repellent effect of Neem on other insects and especially on the RPW. In an effort to reduce the reliance on chemical insecticides and protect date palms for RPW, the study objectives were to evaluate the integration of a pheromone bio-trap with *B. bassiana* with the repellent effect of neem on trunks of date palms to kill RPW adults and prevent oviposition in trunk wounds.

**Materials and Methods**

**Test Insects**

The adults and larvae of RPW were obtained from the laboratory of the Date Palm Research Center of Excellence (DPRCE), King Faisal University, Hofuf, Saudi Arabia. Pineapple was used as the food medium because it is attractive to females for oviposition. All larvae were reared in the laboratory (25–30°C) on pineapple in cages (45 × 38 × 38 cm) made of transparent polyethylene with wire mesh on the top, whereas the adults were maintained in cages on fresh palm fronds and dates.

**Test Materials**

Becker Underwood, Inc., (West Sussex, UK) supplied the liquid formulation of spores suspension *B. bassiana* (BroadbandTM). Astrachem, Saudi Arabia, supplied Azdar 10EC, the neem-based botanical insecticide, azadirachtin. ChemTica International, S.A., Costa Rica, supplied the ferrugineol pheromone lure.

**Pheromone Bio-trap of the RPW**

The RPW trap was designed as described by Hajjar et al. (2015) to attract and contaminate RPWs with the conidia of *B. bassiana* from the commercial product (BroadbandTM). The transfer of the conidia from the infected (directly exposed) adults to uninfected adults was expected during mating (Hajjar et al., 2015). The trap was a small bucket (height, 25 cm; diameter of circular bottom, 15 cm) with four symmetric holes (4 × 4 cm) to allow entry for beetles. Food bait, pieces of palm fronds, and date fruits were placed in the bottom of the bucket. The ferrugineol pheromone lure (ChemTica International, S.A., Costa Rica) was hung internally from the bucket lid. A layer of sackcloth that covered the internal surface of the bucket was soaked with 40 ml of BroadbandTM suspension at 1 × 109 spores/ml. The moist sackcloth in the bucket helped the fungus to overcome the extremely hot and dry conditions that exist in Saudi Aribia.

**Preliminary test to prove the pathogenicity of fungi**

The dead red palm weevils collected from the net house with bio-trap were put into sterile petri dishes with high humidity and incubated at 25 ± 1°C, to confirm fungal infection.

**Neem Bioassays**

The neem botanical insecticide was tested in in a range of dose–response bioassays against adult RPWs. Six serial dilutions of neem were tested, ranging from 500 to 5000 ppm; controls were treated with distilled water only. Three replicates of 10 adults (five males + five females) were treated topically with each dilution by using a spray chamber, which was fitted with an air-atomizing nozzle connected to a regulator valve providing a constant airflow of 10 l/min. The target surface in the spray chamber was a rotating turntable (30 rpm). In addition, a bioassay test was conducted on the oral administration of neem by using treated pieces of palm frond as food. Three replicates were used for each dilution, and each replicate was 10 adults (five males + five females) placed with treated food. Bioassays were conducted at ambient laboratory temperature (25°C), and mortality was recorded after 24 h for the topical application (direct spay) and daily for 14 d for the oral administration. Adults with no signs of movement were recorded as dead. Probit statistical analysis was used to determine the LC50 and LC90 values of treated insects.

**Repellency Effect of Neem on the RPW**

This test was carried out to determine the repellent concentration of neem that could overcome the effect of other attractive factors to RPW, whereas the attractive factor to the weevil (pheromone) was the same in all treatments, but the concentration of the neem-based insecticide, was variable. Filter papers were placed in glass petri dishes (20 cm); a piece of treated frond and date fruit was added. In the control dishes, food was treated with distilled water. The attractive pheromone was placed in the dishes of both the treatments and controls. In a glass box (30 × 30 × 20 cm), the petri dishes with treated frond and date were placed at one end and the control dishes at the opposite end. To determine the lowest concentration repellent to the RPW, several concentrations of neem below the value of the LC50 were prepared, starting from 100 ppm and increasing in increments of 100 ppm to 800 ppm. Four boxes were used as replicates for each concentration, and twenty of numbered labeled adults were placed inside each box in the middle between treated and control petri dishes. The tests were conducted at ambient laboratory temperature (25°C). The behavior of the weevils was monitored daily for at least 6 h by using a digital video camera (Sony, Japan) and the number of adults avoid movement across to the treated food under different concentrations of the neem was recorded for 6 hours.

**Persistence of the repellent effect of Neem to the RPW**

This trial was conducted to determine the period for which a concentration of neem could repel the RPW. The lowest repellent concentration that led to 100% repellency of the RPW was used. The 20 labeled weevils were renewed daily in each replicate (glass box), and the same treatment and control petri dishes were used from the first day of the test, which was also conducted at ambient laboratory temperature (25°C). The duration of the repellent action of the tested concentration, and the rate of decrease in repellent effect over time were determined by monitoring weevil behavior. The number of adults avoid movement across to the treated food under different concentrations of the neem was recorded for at least 6 h by using a digital video camera (Sony, Japan) and for duration of 8-day post treatment.

**Integration of the repellency effect of Neem with the Pheromone Bio-trap of *B. bassiana* to Control the RPW**

This trial was conducted in field and integrated the efficacy of the bio-trap with *B. bassiana* (BroadbandTM) in attracting, infecting, and killing the RPW as described by Hajjar et al. (2015) with the repellent effect of neem in protecting palms from infestation by the RPW. Four net houses (5 × 5 m) were used, and five palms (height, 1­–1.5 m) were planted in each house. Cuts were made in the trunks to artificially wound the palms. In two of the houses, the palms were sprayed weekly with neem as a repellent at the concentration of 800 ppm, and the pheromone bio-trap was placed in the middle of each house. The other two net houses was considered as the control. Where, the common bucket pheromone trap without the fungus, that recommended by Agriculture authority in KSA, was placed in the middle of control net house, which contained in the bottom of the bucket an attractive food bait of palm frond pieces and date fruits that was mixed with the insecticide (Cypermethrin). The palms in the control net houses were not sprayed. Thus, in both treatment and control, the ferrugineol pheromone lure was hung internally from the bucket lid. In each net house, 30 adult RPWs (15 males + 15 females) were introduced. The effectiveness of the treatment integrating the repellent concentration of neem and the pheromone bio-trap of *B. bassiana* was evaluated under the prevailing environmental conditions of 40 -48°C in Alhasa, Saudi Arabia,. The death of weevils was recorded daily for 30 d after introduction, in addition to tracking the presence of RPW larvae in the wounds on trunks.

**Statistical Analyses**

The lethal concentration that caused 50% mortality (LC50) was determined by probit analysis using the PoloPlus software program (Le Ora software, Berkley, California) (Robertson et al. 2003).

Daily and cumulative mortality caused by the integration of the pheromone bio-trap with neem at the end of the trial were subjected to analysis of variance (ANOVA), and the means were compared by the least significant differences (LSD) at P ≥ 0.05 using the SAS 9.3 statistical software package (2007).

**Results**

In preliminary test, the commercial fungus (BroadbandTM) infected RPW by contact. The morphological characteristics of mycelia and spores retrieved from dead weevils confirmed infection by the fungal species *B. bassiana*.

**Neem Bioassays**

According to the probit analysis, the LC50 of topical application (direct spay) was 1810.3 ppm and that of oral administration was 2117.8 ppm and the two LC50 values were not significantly different (Table 1). The LC95 values were 6855.6 ppm with topical application and 6947.8 ppm with oral administration, which were also not significantly different at the confidence limit (CL) of 95%. However, the value of the slope of the LDP line was greater with oral administration than with topical application, and the value of the R factor was higher with topical application (0.982) than with oral administration (0.926), but there was no significant difference between the two slop values.

**Repellent Effect of Neem**

The repellent effect of neem was determined based on concentration below the LC50 values. The number of weevils repelled increased as the concentration of the insecticide increased; the repellency of neem began at 400 ppm and continued up to 800 ppm (Table 2). The repellency effect at 800 ppm was 100%, which lasted for 2 d of treatment. The efficiency of repellency then decreased with time, and 80% of the weevils were repelled from the treated surface at 3 d, 60% at 4 d, 40% at 5 d, 25% at 6 d, and 5% at 7 d.

**Integration of the Repellency Effect of Neem with the Pheromone Bio-trap of *B. bassiana* to Control the RPW**

This trial studied the effectiveness of the pheromone bio-trap in the field to kill weevils by contaminating them with spores of the fungus *B. bassiana*, in addition to the possibility of transferring the spores to other weevils during mating as indicated by Hajjar, et al. (2015). Additionally, the effectiveness of neem as a repellent in preventing weevils from laying eggs on palms was examined by creating artificial wounds on the palm trunks. However, at the lowest concentration of neem that was 100% repellent to adult RPWs (800 ppm), the 100% repellency effect persisted for 2 d. Therefore, in the field trial, neem at 800 ppm was sprayed weekly to obtain a longer effective period in repelling weevils from treated palms.

The results from the two treated net houses with the introduction of the pheromone bio-trap of *B. bassiana* are shown in Table 3. The accumulative death rate was 100% after 13 d, and the cause of death was infection by the spores of *B. bassiana* from the pheromone bio-trap. To confirm death by the fungus and retrieve the mycelia, the dead adults were transferred to sterile petri dishes and incubated at 25 ± 1°C and 75% ± 5% humidity. By contrast, in the control net house, the accumulative death rate reached only 60% after 30 d, and all the dead weevils were attracted to the pheromone in the common trap used by Agriculture authority in KSA, and killed by insecticide mixed with food, in the trap.

In addition to determining the effect of using the pheromone bio-trap, the repellent effect of neem to protect palms was studied by measuring the ability of weevils to lay eggs in wounded and treated trunks after 30 d. The high repellency of neem prevented females from laying eggs on the treated trunks, and compared with the palms in the control net house, the trunks of treated palms were 100% free of infestation by RPW larvae. However, in the control net house, four of the five palms had RPW larvae in the trunk wounds (Table 4). The mean number of larvae found in the control was significantly greater than the treatment with neem at the repellent concentration with the pheromone bio-trap (F = 20.018; df = 9; p = 0.0003).

**Discussion**

Neem insecticide is used to control the RPW either as a direct spray or as an injection in the trunk of palms (Bream et al. 2001, Abahussain 2008, Sujatha et al. 2009, Al-Shawaf et al. 2013, Porcelli et al. 2013, Merghem, & Mohamed, 2017 ). The repellent effect of neem to the RPW is rarely studied, this study may be the first to investigate the repellency effect of sub lethal doses of neem to the RPW, where the concentration of 800 ppm caused 100% repellency to adults for 2 day after treatment and then the effect was decline to 80% after 3 days and to 40% after 5 days. However, the repellency effect of neem extract to RPW was also reported by Merghem, & Mohamed (2017), with both remedial and protective effects, against RPW in field trials at Ismailia governorate in Egypt. Where a considerable reduction of infestation was 60.8% and 53.1% repellency on average for all applied treatments. In addition, the antifeedant and repellency effects of neem have been studied in many insects. According to Isman (2006), neem remains the most potent desert locust antifeedant discovered. A sublethal dose of azadirachtin affects the development of insects such as the spotted bollworm, *Earias vittella* (Gulzar et al. 2019), and the emergence ratio during development and the longevity of beneficial insects such as *Chelonus oculator* (Tunca et al. 2014) and *Habrobracon hebetor* Say (Abedi et al. 2014). In this study, neem repellency and *B. bassiana* with pheromone traps together achieved good control in a field control program of RPW. Whereas in the 30-d field test, neem at the repellent concentration of 800 ppm treated to trunk of palm , in combination with the pheromone bio-trap of *B. bassiana*, was highly effective in preventing females from laying eggs in the wounded trunks of treated palms, when compared with untreated palms in the control. The high efficacy in the field trial of the pheromone bio-trap of *B. bassiana* is consistent with the finding of Güerri–Agulló et al. (2011), who reported great promise with *B. bassiana* for the control of RPW. Similarly, laboratory and semi-field cage studies indicate that the pheromone bio-trap of *B. bassiana* is effective in infecting and killing RPW (Hajjar et al. 2015). In addition the field repellency effect of neem was also compatible with the repellency effect of neem to RPW reported by Merghem, & Mohamed (2017). The results of this work revealed that the application of the fungus *B. bassiana* could be adequately exploited to control RPW by keeping the fungus moist by lining the bucket of the pheromone bio-trap with sackcloth, which helped to overcome the extremely hot and dry conditions that exist in the arid regions of the world, where date palms are cultivated.

**Conclusions**

In this study, the integration of the pheromone bio-trap of *B. bassiana* with the repellent effect of a sub lethal concentration of the insecticide neem sprayed on palms under field conditions was effective in the control of the RPW and killing all weevils. Neem was successful in repelling the RPW and prevented the females from laying eggs in wounded and treated palms.

**Acknowledgments**

This study supported by the Date Palm Research Center of Excellence (DPRCE), King Faisal University.

References

Abahussain, M. O. 2008. The effects of neem seed extract (Azadirachtin) on the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Rynchophoridae: Coleoptera). Int. J. Sci. Res. 17: 89–94.

Abedi, Z., M. Saber, G. Gharekhani, A. Mehrvar, and S. G. Kamita. 2014. Lethal and sublethal effects of azadirachtin and cypermethrin on *Habrobracon hebetor* (Hymenoptera: Braconidae). J. Econ. Entomol. 107: 638–645.

Al-Abdulmohsin, A. M. 1987. First record of red date palm weevil in Saudi Arabia. Arab World Agriculture. 3: 15–16.

Al-Ayedh, H., A. Hussain, M. Rizwan-ul-Haq, and A. Al-Jabr. 2016. Status of insecticide resistance in field-collected populations of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). Int. J. Agric. Biol. 18: 103–110.

Al-Dosary, N. M., S. Al-Dobai, and J. R. Faleiro. 2016. Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. Emir. J. Food Agric. 28: 34–44.

Al-Shawaf, A. M., Y. Al-Suleiman, E. Al-Abdullah, A. Al-Shagag, A. Al-Dandan, M. Al-Bagshi, S. Al-Saroj, S. Al-Bather, A. Ben Abdallah, and J. R. Faleiro. 2013. Can azadirachtin deter red palm weevil, *Rhynchophorus ferrugineus*, (Coleoptera: Curculionidae) from laying eggs? Entomology 2013, ESA Annual Meeting, 10–13 November 2013, Austin, Texas. United States of America.

Azam, K. M., S. A. Razvi, and I. Al-Mahmuli. 2001. Survey of red palm weevil, *Rhynchophorus ferrugineus* Oliver. Infestation in date palm in Oman. pp. 25–27. In Proceedings, Sec. Int. Conf. Date Palms, 25–27 March 2001. College of Food and Agriculture. UAEU, Al-Ain, UAE.

Bateman, R. P., M. Carey, D. Batt, C. Prior, Y. Abraham, D. Moore, N. Jenkins, and Y. Fenlon. 1996. Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskål). Biocontrol Sci. Technol. 6: 549–560.

Bream, A. S., K. S. Ghoneim, M. A. Tanani, and M. I. Nassar. 2001. The disruptive effects of Azadirachin and Jojoba on development and morphogenesis of the red palm weevil, *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera). pp. 280–303. In Proceedings, Sec. Int. Conf. Date Palms, 25–27 March 2001. College of Food and Agriculture. UAEU, Al-Ain, UAE.

Clarkson, J. M., and A. K. Charnley. 1996. New insights into the mechanisms of fungal pathogenesis in insects. Trends Microbiol. 4: 197–203.

DeBach, P., and D. Rosen. 1991. Biological control by natural enemies. Cambridge University Press, Cambridge, UK.

Dembilio, Ó., E. Quesada–Moraga, C. Santiago–Alvarez, and J. A. Jacas. 2010. Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana*, as a biological control agent against the red palm weevil, *Rhynchophorus ferrugineus*. J. Invertebr. Pathol. 104: 214–221.

Faleiro, J. R. 2006. A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. 26: 135–154.

Faleiro, J. R., M. Ferry, Th. Yaseen, and S. Al-Dobai. 2019. Overview of the gaps, challenges and prospects of red palm weevil management. Arab Journal Plant Protection 37: 170–177.

Ghoneim, K. S., A. S. Bream, and H. A. Mohamed. 1998. Bioactivity the ecdysteroid agonist *Tebufenozide* (RH-5992) on the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd). (Lepidoptera: Noctuidae). Al-Azhar Bull. Sci. 9: 947–963.

Giblin–Davis, R. M., J. R. Faleiro, J. A. Jacas, J. E. Peña, and P. S. P. V. Vidyasagar. 2013. Biology and management of the red palm weevil, *Rhynchophorus ferrugineus*, pp. 1–34, In J. E. Peña (ed.), Potential invasive pests of agricultural crops. CABI, Wallingford, UK.

Güerri–Agulló, B., R. López–Follana, P. Asensio, P. Barranco, and L. V. Lopez–Llorca. 2011. Use of a solid formulation of *Beauveria bassiana* for biocontrol of the red palm weevil (*Rhynchophorus ferrugineus*) (Coleoptera: Dryophthoridae) under field conditions in SE Spain. Fla. Entomol. 94: 737–747.

Gulzar, A., M. M. Ali, M. Tariq, I. Bodlah, K. Tariq, and A. Ali. 2019. Lethal and sublethal effects of *Azadirachtin indica* seed extract on the development of spotted bollworm *Earias vittella* (Fab.) Gesunde Pflanzen 71: 19–24.

Hajjar, M. J., A. M. Ajlan, and M. H. Al-Ahmad. 2015. New approach of *Beauveria bassiana* to control the red palm weevil (Coleoptera: Curculionidae) by trapping technique. J. Econ. Entomol. 108: 425–432.

Hussain, A., M. Rizwan-ul-Haq, and A. Al-Jabr. 2017. Susceptibility, antioxidant defense, and growth inhibitory response of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) against the virulence of *Metarhizium anisopliae* isolates. Univers. J. Plant Sci. 5: 17–23.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture, and an increasingly regulated world. Annu. Rev. Entomol. 51: 45–66.

Lisansky, S. 1997. Microbial biopesticides, pp. 3–10. In H. F. Evans (ed.), Microbial insecticides: novelty or necessity? British crop protection council symposium proceedings No. 68, Farnham, UK.

Lowery, D. T., and M. B. Isman. 1994. Effects of neem and azadirachtin on aphids and their natural enemies, pp. 78–91. In P. A. Hedin (ed.), Bioregulators for crop protection and pest control. ACS Symposium Ser. 557, American Chemical Society, Washington, D.C., USA.

Manachini, B., D. Schillaci, and V. Arizza. 2013. Biological responses of *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera) to *Steinernema carpocapsae* (Nematoda: Steinernematidae). J. Econ. Entomol. 106: 1582–1589.

Mazza, G., V. Francardi, S. Simoni, C. Benvenuti, R. Cervo, J. R. Faleiro, E. Llácer, S. Longo, R. Nannelli, E. Tarasco, and P. F. Roversi. 2014. An overview on the natural enemies of *Rhynchophorus* palm weevils, with focus on *R. ferrugineus*. Biol. Control 77: 83–92.

Merghem, A., & Mohamed, A. A. R. 2017. Impact of Neem Extracts, Azadirachta indica A. Juss Induced against Red Palm Weevil, Rhynchophorus ferrugineus (Olivier) Attacking Date Palm Orchards in Egypt. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 9(3), 109-117.

Miller, F., and S. Uetz. 1998. Evaluating biorational pesticides for controlling arthropod pest and their phytotoxic effects on greenhouse crops. Horttechnology 8: 185–192.

Porcelli, F., F. Valentini, R. Griffo, E. Caprio, and A. M. D’Onghia. 2013. Comparing insecticides and distribution techniques against red palm weevil. AFPP – Colloque Méditerranéen Sur Les Ravageurs des Palmiers, Nice: 16, 17 ET 18 Janvier, 2013.

Pretty, J., and R. Hine. 2005. Pesticide use and the environment, pp. 1–22. In: J. Pretty (ed.), the Pesticide Detox. Earthscan, London, UK.

Robertson, J. L., H. K. Preisler, and R. M. Russell. 2003. PoloPlus probit and logit analysis user’s guide. LeOra Software, Berkeley, CA.

Ruckin, F. R. 1992. Neem, a tree for solving global problems. National Academy Press, Washington, D.C., USA.

SAS 9.3. 2007. Interface to PC files for microsoft windows, SAS user’s guide. SAS Institute, Cary, NC.

Schmutterer, H. 1990. Properties and potential of natural pesticides form the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35: 271–297.

Sujatha, A., M. S. V. Chalam, and S. Arulraj. 2009. Status of red palm weevil damage in East Godavari district and strategies for control with eco-friendly methods. J. Plant Crops 37: 206–211.

Tunca, H., N. Kilinçer, and C. Özkan. 2014. Toxicity and repellent effects of some botanical insecticides on the egg-larval parasitoid *Chelonus oculator* Panzer (Hymenoptera: Braconidae). Sci. Res. Essays 9: 106–113.

Wakil, W., M. Yasin, M. A. Qayyum, M. U. Ghazanfar, A. M. Al-Sadi, G. O. Bedford, and Y. J. Kwon. 2018. Resistance to commonly used insecticides and phosphine fumigant in red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Pakistan. PLoS ONE 13: e0192628.

Whitten, M. J., and J. G. Oakeshott. 1991. Opportunities for modern biotechnology in control of insect pests and weeds, with special reference to developing countries. FAO Plant Prot. Bull. 39: 55–181.

Wraight, S. P., M. E. Ramos, P. B. Avery, S. T. Jaronski, and J. D. Vandenberg. 2010. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J. Invertebr. Pathol. 103: 186–199.

Table 1. LC50 and LC95 values (ppm) of the neem insecticide for the RPW

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Slope ± SE | LC50(ppm) | 95% CL \*\*\* | LC95 (ppm) | 95% CL \*\*\* | Chi Square | R |
| Topical application\* | 2.84 ± 0.52 | 1810.3 | 1346.5–2323.3 | 6855.6 | 4698.5–14149.5 | 0.142 | 0.98 |
| Oral administration\*\*  | 3.19 ± 0.59 | 2117.8 | 1629.6–2664.8 | 6947.8 | 4907.8–13596.8 | 7.840 | 0.93 |

\*Topical application was in a spray chamber, and mortality was recorded after 24 h.

\*\*Oral administration was through contact with and oral ingestion of treated pieces of palm fronds, and accumulative mortality was recorded for 14 d.

\*\*\*Upper and lower limit at CL 95%

Table 2. Average number of RPW repelled under different concentrations (ppm) of the neem insecticide.

|  |
| --- |
| Mean ± SE\* |
| 800 ppm | 700 ppm | 600 ppm | 500 ppm | 400 ppm | 300 ppm | 200 ppm  | 100 ppm | Day |
| 20.00±0.0 | 20.00±0.0 | 18.00±0.18 | 9.50±0.13 | 5.75±0.21 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 1 |
| 20.00±0.0 | 18.00±0.18 | 13.25±0.21 | 8.25±0.21 | 2.00±0.18 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 2 |
| 16.00±0.41 | 12.00±0.32 | 9.00±0.18 | 3.50±0.29 | 1.00±0.18 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 3 |
| 12.00±0.32 | 8.80±0.21 | 5.25±0.21 | 1.00±0.18 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 4 |
| 8.00±0.26 | 5.80±0.34 | 1.25±0.11 | 0.50±0.13 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 5 |
| 5.00±0.18 | 3.00±0.18 | 0.00±0.00 | 0.58±0.33 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 6 |
| 1.00±0.18 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 7 |
| 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 8 |

The numbers of repelled adults were recorded daily from monitoring the insects in the boxes for 6 h daily by using a digital video camera

\*All values represented as Mean ± SE, n = 20 adults, No. of replicates = 4.

Table 3. Mortality rate (%) of RPW infected with *Beauveria bassiana* in the treated net houses with a bio-trap and in the control net house with a pheromone trap during 30 d

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Control net house | Treated net houses | Day after treatment | Control net –house\*\*  | Treated net houses\* | Day after treatment |
| 50% | 100% | 16 | 0% | 0% | 1 |
| 50% | 100% | 17 | 0% | 0% | 2 |
| 50% | 100% | 18 | 0% | 20% | 3 |
| 50% | 100% | 19 | 10% | 30% | 4 |
| 50% | 100% | 20 | 10% | 40% | 5 |
| 60% | 100% | 21 | 15% | 60% | 6 |
| 60% | 100% | 22 | 20% | 70% | 7 |
| 60% | 100% | 23 | 20% | 70% | 8 |
| 60% | 100% | 24 | 20% | 80% | 9 |
| 60% | 100% | 25 | 30% | 90% | 10 |
| 60% | 100% | 26 | 40% | 90% | 11 |
| 60% | 100% | 27 | 40% | 95% | 12 |
| 60% | 100% | 28 | 40% | 100% | 13 |
| 60% | 100% | 29 | 40% | 100% | 14 |
| 60% | 100% | 30 | 50% | 100% | 15 |

\*Treatment; % of mean death, n= 30, No. of replicates in treated net house = 2

\*\*control % of death of n=30

"T" value for the difference between the death rates of treated net houses with a bio-trap and the control net house with a pheromone trap (df = 29; T value = 16.62; significant at p =0.01)

Table 4. Number of larvae of RPW after 30 d in wounded palms in net houses. Palms were either treated with neem at a repellent concentration of 800 ppm or not treated (control)

|  |  |
| --- | --- |
| Palm tree | No. of larvae |
| Control net house | Treatment net house |
|  | No. 1 | No. 2 | No. 1 | No. 2 |
| 1 | 15 | 0 | 0 | 0 |
| 2 | 12 | 15 | 0 | 0 |
| 3 | 9 | 26 | 0 | 0 |
| 4 | 24 | 17 | 0 | 0 |
| 5 | 0 | 7 | 0 | 0 |
| Mean | 12.5 a ± 2.8  | 0.00 b ± 0.00 |
| L.S.D (0.05) | \* 5.07 |

* Mean represented for 10 trees ± SE;
* \*: significant values at (F = 20.018; df = 9; P = 0.0003)
* LSD: Lowest significant differences at p ≥ 0.05
* Means in the same row without the same alphabetical letter are significantly different at p ≥ 0.05.