International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2381-3911 Vol. 2 (6), pp. 108-116, June, 2016.
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Full Length Research Paper

Efficacy of Abamectin (ABM) against various stages of R. ferrugineus under laboratory conditions

***Al-Waleed Albutairi, Nasser G. Laden and Yasser Hariri Al Saud**

Date Palm Research Center of Excellence, King Faisal University, Alahsa 31982, P.O. Box 400, Saudi Arabia.

Accepted 1 May, 2016

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, is one of the most devastating and invasive insect pests causing immense damage on date palms across the world. Natural insecticide Abamectin (ABM) has been evaluated against developmental stages of RPW under laboratory conditions. Insects were exposed to residual film of the insecticide on transparent cups using a Potter precision laboratory spray tower. Mortality of males, females, neonate larvae, larvae, eggs and pupae was recorded at different time intervals after treatment. Bioassay test showed that ABM at 600 ppm (µg/ml) caused 60% mortality against females and males after 24 h. By increasing the concentration to 1000 ppm, the mortality reached 100% for adults. The LC_{50}-values calculated for females and males were 582.9 and 502.1 ppm and that of LC_{95} was 1226.9 and 967.2 ppm, respectively. Neonate larvae exhibited more sensitivity toward ABM, where 200 and 500 ppm caused 80 and 100% mortality at 24 h after treatment with LC_{50} 98.7 ppm and LC_{95} 352 ppm. In the meantime, the toxicity effect of the treatment with ABM up to 500 ppm checked at the 24 h period was 0% mortality against 25-day old larvae, and then it reached 60% by the same concentration at 7 days after treatment. Other developmental stages, eggs and pupae, were dramatically affected by ABM, where 500 ppm caused 90.3% egg unhatchability and 100% mortality of pupae. Therefore, ABM can be a possible candidate to be applied on date palm by the Ministry of Agriculture after successful field experiments.

Key words: Abamectin, red palm weevil, bio-insecticide, bioassay.

INTRODUCTION

Abamectin (ABM) is a macrocyclic lactone product derived from the soil microorganism *Streptomyces avermitilis.* ABM is used as an insecticide and acaricide in many parts of the world, and acts as an agonist of γ-aminobutyric acid (Burg et al., 1979; Putter et al., 1981).

Saudi Arabia is one of the main date palm producers in the world, with her production amounting to 986,000 tonnes in 2008 (AOAD, 2009). The number of date palm trees is over 18 million, with about 400 different cultivars grown in different regions of the Kingdom of Saudi Arabia (Anon, 2004). Nearly 90 million date palm trees are grown in the Arab world which produced about 5.4×10^6 t in 2001 and increased to 6.7×10^6 t in 2004. This yield represents about 75 % of the world production (FAO statistics, 2009).

Based on the above information, it has become necessary to maintain this wealth of food in such arid environment and this can be achieved by protecting the date palm from serious pests. The red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the most invasive insect pests causing immense damage to date palms not only in Saudi Arabia and in the Arab countries, but all over the world (Abo-El-Saad et al., 2012).

The RPW is an economically important pest of date palm with its origin from South Asia and Melanesia, where it is a serious pest of coconut palms. The pest infestation has spread westwards very rapidly since the mid 1980s (Gomez and Ferry, 1999). It reached the Kingdom of Saudi Arabia, United Arab Emirates, and Oman in 1985 (Abozuhairah et al., 1996; El-Ezaby, 1997), Iran in 1996 (Faghih, 1996), Egypt in 1992 (Cox, 1993), Spain in 1994 (Barranco et al., 1996), and Israel, Jordan and Palestine in 1999 (Kehat, 1999). The RPW is

*Corresponding author. E-mail: waleed1962@gmail.com

a concealed tissue borer and all of its life stages are hidden inside the palm tree. Damage symptoms are indicated by the presence of tunnels in the trunk, and oozing of thick yellow to brown fluid from the tree.

Moreover, there is appearance of chewed up plant tissue in and around openings in the trunk, the presence of a fermented odor from the fluid inside infested tunnels in the trunk, and/or breaking of the trunk or toppling of the crown (Kaakeh et al., 2001).

The main element in date palm protection against RPW is the avoidance of progeny production, which can be achieved by many ways. Recent studies have focused on integrated pest management involving surveillance, pheromone lures (Faleiro et al., 2011), cultural control and chemical treatments (Abraham et al., 1998). Other techniques such as sanitation, or baited traps have been applied in infested areas in combination with chemical insecticides (Abraham et al., 1989; Moura et al., 1995). The choice of the chemical insecticides for field application is mainly based on the laboratory evaluation of certain insecticides (Abo-El-Saad et al., 2012), which indicated that beta-cyfluthrin possesses high efficacy against developmental stages of RPW under laboratory assays as well as dipping infested date offshoots in a 300 ppm beta-cyfluthrin. ABM is used as an insecticide and acaricide in many parts of the world. It inhibits the gamma-amino butyric acid (GABA), induces neurotransmission and causes paralysis in pests (Campbell et al., 1983; Shoop et al., 1995). ABM is also a chloride channel inhibitor, which makes it likely to affect the membrane stability (Korystov et al., 1999).

The translaminar insecticide, abamectin, has been a primary means of controlling *Liriomyza leafminers*. Furthermore, it was found that ABM exhibited dramatic effect against leaf mining Agromyzidae (Cox et al., 1995; Hara, 1986; Leibee, 1988; Parrella et al., 1988; Trumble, 1985; Weintraub, 1999, 2001). In addition to leafminer control, ABM is used for controlling mites, thrips, aphids, whiteflies, psyllids, diaspid scale insects, and lepidopteran pest species (Lasota and Dybas, 1991).

The aim of the current work is to assess the efficacy of ABM against various stages of *R. ferrugineus* under laboratory conditions as an attempt to find out a promising insecticide to be used in IPM program. According to our knowledge, this is the first investigation to show that ABM could be used against all stages of RPW.

MATERIALS AND METHODS

Insects

All stages of *R. ferrugineus,* adults, larvae, cocoons and eggs, were collected from infested date palm trees in Eastern Province fields of Saudi Arabia. Collected adults were kept under laboratory conditions (50-60% relative humidity), at 23±1°C with a photoperiod of 12 : 12 (light: dark) for 2 days as an adaptation period before the bioassay test.

Chemicals

The following chemicals were used in this study: Vermectin 1.8% EC (abamectin); $C_{48}H_{72}O_{14}$ (avermectin B_{1a}) + $C_{47}H_{70}O_{14}$ (avermectin B_{1b}). Abamectin used in bioassay was a generous gift from Agriculture Directorate at Alahsa, Ministry of Agriculture, Saudi Arabia.

Bioassay test against adults and larvae

Laboratory trials against various stages of *R. ferrugineus* were conducted in transparent plastic cups (250 ml). Three replicates of five 25-day larvae (n=15), males, females, cocoons and 10 eggs and neonate larvae of *R. ferrugineus* (Olivier) were exposed to various concentrations of ABM in the range of 200 - 1000 ppm for the adults and 100 - 500 ppm against larvae as a residual film on transparent plastic cups using a Potter precision laboratory spray tower. Similar transparent plastic cups sprayed with distilled water were run as a control. In order to assess the effect of the insecticide, the insects were examined at different exposing periods after treatment and the percentages of mortality and hatchability were recorded. An insect was considered dead if it neither moved nor responded by reflex movement, when gently touched with a brush. Every insecticide concentration was replicated three times. Normal Equivalent Deviates, Chi Square, 50% and 95% lethal concentrations and their Fiducial limits were calculated according to Finney (1971) using MINITAB Statistical software, version 13.30, Copyright 2000, Minitab Inc.

Oviposition

Equal number of males and females were kept in glass jars (500 ml) for mating. Only one slice of sugarcane was provided in each jar to concentrate the eggs and facilitate their collection. Two days later, the fertile eggs were selected and collected by hand using camel hairbrush after peeling off the fibers. Collected eggs were used in a bioassay test as subsequently described.

Egg treatments

Ten one-day old eggs of RPW were transferred to Petri dishes containing filter paper and were considered as the experimental unit. Each treatment was replicated three times in completely randomized design. Moisten pieces of cotton were placed along the inside of the dishes to maintain humidity. Petri dishes containing eggs were treated with 100, 200, 300 400 and 500 ppm of ABM using a Potter precision laboratory spray tower, subsequently; they were incubated at 37°C for 3 days to

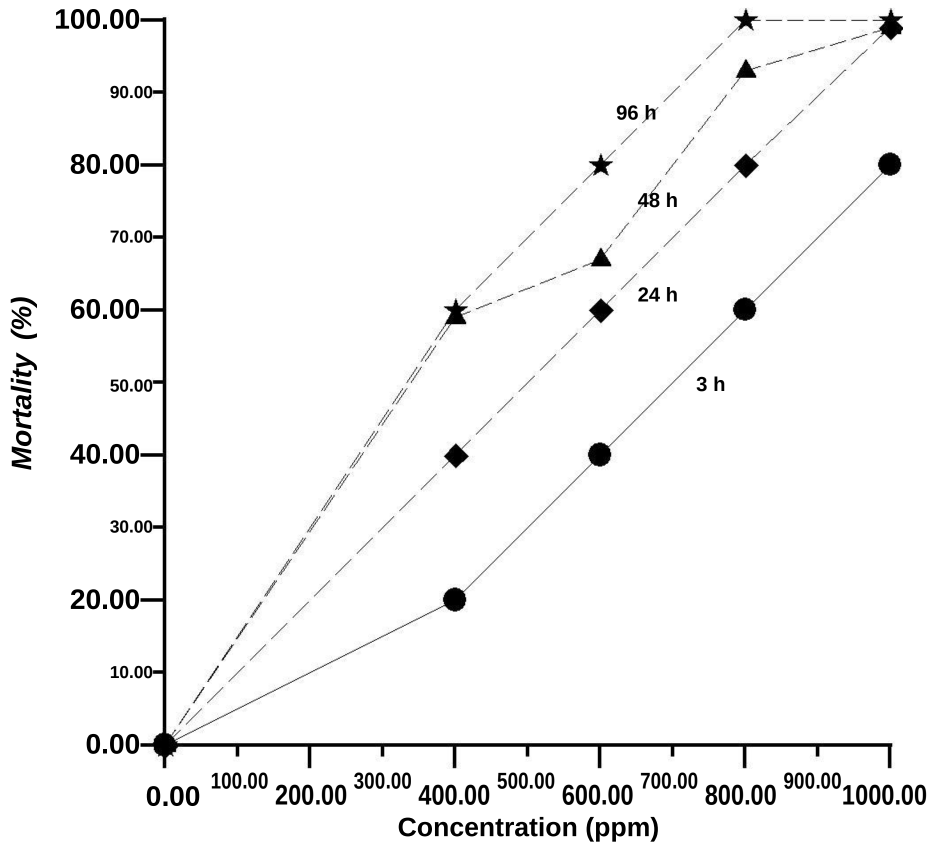

Figure 1. Percent mortality of adult male RPW after exposure to ABM for various time periods.

hatch. Similar Petri dishes sprayed with distilled water were run as a control. The number of hatched eggs was counted using probit analysis for LC_{50} and LC_{95} calculation as described by Finney (1971).

Cocoon treatments

Field cocoons of RPW were distributed in transparent plastic cups (250 ml). Three replicates of five cocoons (n=15) were used in each treatment. Each cocoon was immersed for 30 seconds in one of the following concentration of ABM (100, 200, 300 400 and 500 ppm), and then placed in plastic cup for incubation at 37°C for 2-3 weeks in dark and damp conditions (50-60% relative humidity, at 29±1°C). Similar number of cocoons was treated with distilled water and run as a control. The number of emerged adults was counted using probit analysis for LC_{50} and LC_{95} calculation as described by Finney (1971).

RESULTS AND DISCUSSION

Effect of ABM on adults of RPW

The data clearly showed that ABM had dramatic effects against both male and female of RPW. Figures 1 and 2 show that ABM at 600 ppm after exposure periods of 3, 24, 48 and 96 h caused respectively 40, 60, 67 and 80% mortality against the males of RPW and 40, 60, 60 and 80% against the females of RPW. LC_{50} and LC_{95} values of ABM against male at 24 h after treatment were 502.07 and 967.2 ppm with fiducial limits of 322.7-598.2 and 802.3-1605.3 respectively (Table 1). Furthermore, these values at the same exposure period against female were respectively 582.9 and 1226.9 ppm with fiducial limits of 386.9-699.4 and 966.4-2576.9 (Table 1). Moreover, by increasing the concentrations and exposure periods, mortality significantly increased to reach 100% at 800 ppm against male and female after 96 h. It is worth mentioning that one of the key elements in date palm protection against RPW is the avoidance of progeny production. The choice of the chemical insecticide for field application is based mainly on series of laboratory evaluation of certain insecticides (Abo-El-Saad et al., 2012). The results of this study indicated that ABM is a suitable bio-insecticide for killing RPW adult as a contact poison. Consequently, the life cycle of the insect will be broken down and likewise the rest of the developmental stages including egg, larva, and pupa. The second advantage of applying a bioagent such ABM is to avoid

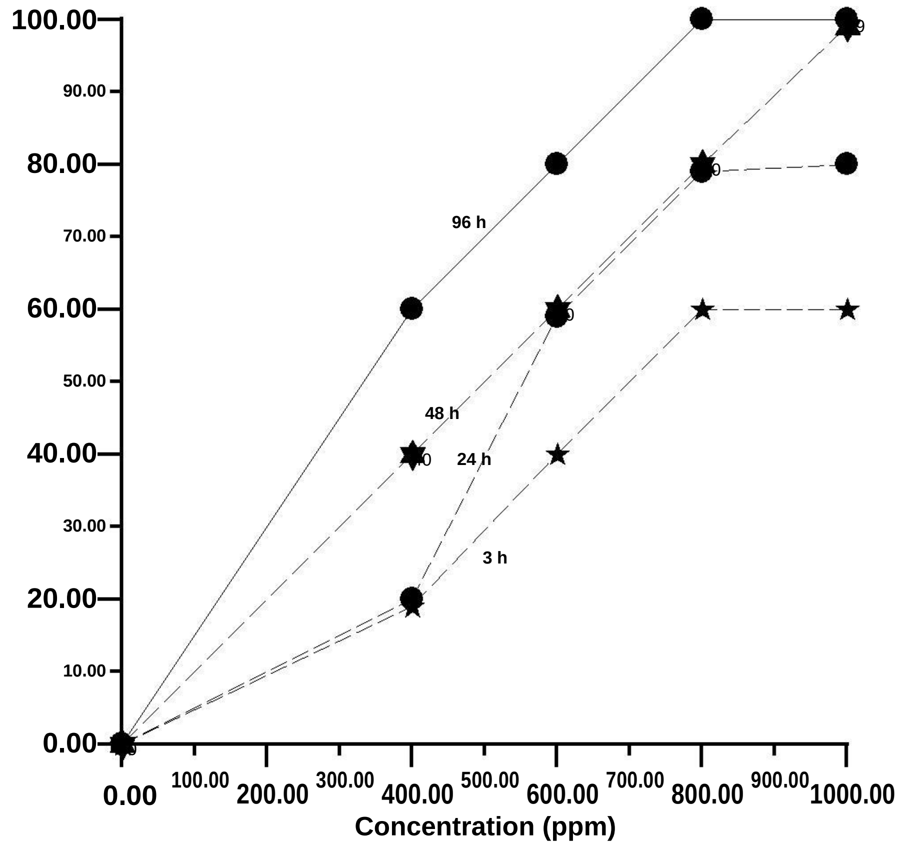

Figure 2. Percent mortality of adult female RPW after exposure to ABM for various time periods.

hundreds of highly toxic chemical pesticides extensively used to control pests to increase agricultural production (Fisher, 1993; Isman, 2006). Bio-pesticides such as abamectin has been reported as broad-spectrum pesticides with high biological activity with dosage as low as 1-3 g active ingredient per hectare. With such a low rate, such pesticides not only provide protection to the environment but also are safe to human beings (UNIDO, 2008).

Effect of ABM on larvae of RPW

ABM was examined against the newly hatched larvae (neonate) and 25-days old larvae of RPW. Figure 3 shows that ABM exhibited potential effects against neonate larvae at various concentrations of 100, 200, 300 and 500 ppm at 24 h after treatment. ABM at 100 ppm gave 30% mortality and by increasing the concentration to 200 and 500 ppm, mortality has increased to 80 and 100% respectively. LC_{50} and LC_{95} were respectively, 98.7 and 352 ppm with fiducial limits of 0.1-170.8 and 263.2-1407.2 (Table 1). On the contrary, ABM shows less influence toward older larvae at the same concentrations mentioned above. The results indicated that there was no larval mortality with various concentrations of ABM at 24 h after treatment, so the exposure period had continued for 7 days and the mortality reached only 20% at the concentrations after 72 h. At day 7 after the treatment, percent mortality reached a plateau of 60% with concentration in the range of 300 to 500 ppm. Accordingly, it is clear that older larvae had exhibited more tolerance for this insecticide compared with neonate larvae. The different effect of ABM on larvae was most likely due to the body weight of the larva in this age, which is about 0.0015 g for neonate larvae, and 2.9 g for 25-days old larvae respectively. The main component of the difference in the body weight is fat, which may be responsible for blocking of the insecticide from reaching the nervous system of the larva. The mechanism of ABM action is related to its effect on the γ-aminobutyric acid (GABA) system and Cl-channels (Turner and Schaeffer, 1989). Furthermore, it has been found that ABM inhibits the activity of F_oF_1ATPase, an enzyme present in the inner mitochondrial membrane that is responsible for ATP synthesis driven by the proton electrochemical gradient generated in the respiratory chain (Castanha-Zanoli et al., 2012, Hatefi, 1993; Pedersen, 1996). According to the current results, the negative impact of ABM on neonate larvae seems to be very high, and can be applied preferably on date palm as protective agent particularly

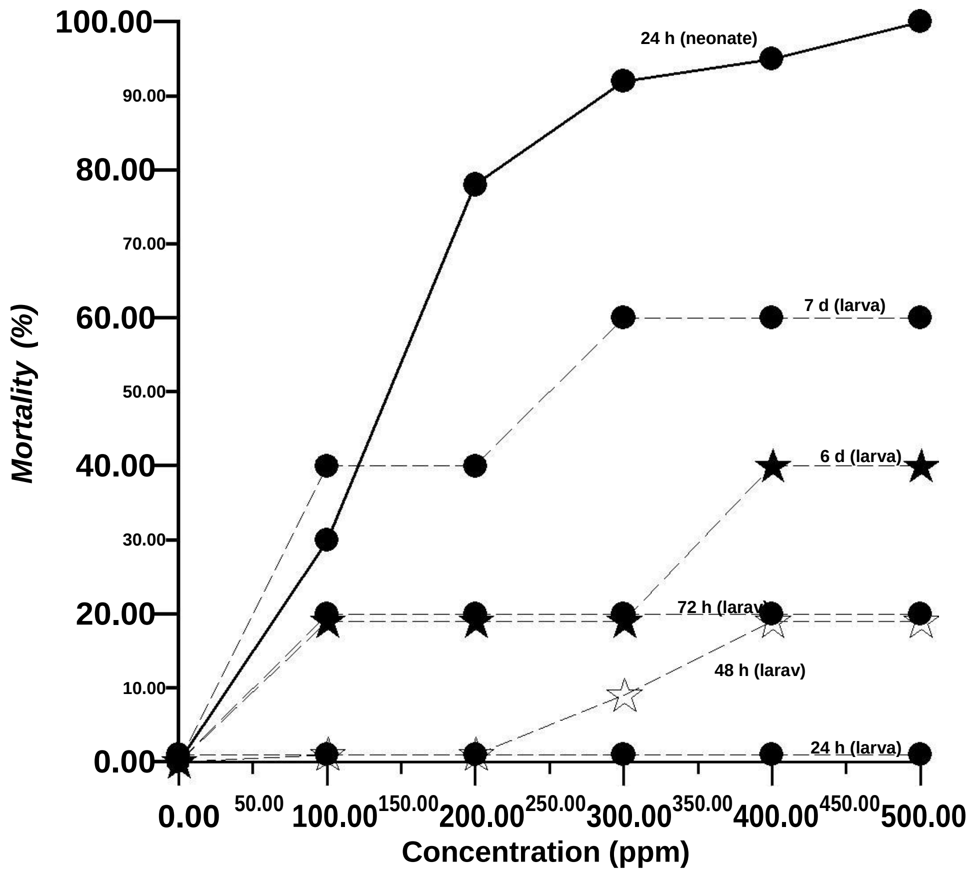

Figure 3. Percent mortality of larvae RPW after exposure to ABM for various time periods.

Table 1. Estimated LC_{50} and LC_{95} values of ABM against various stages of RPW at different time intervals after treatment.

Stage	Time	LC_{50} (ppm) (95% fiducial limits)	LC_{95} (ppm) (95% fiducial limits)	Chi square (X^2)	Degrees of freedom
Egg	3 days	123.6 (47.1-175.4)	756.4(563.9-1666.3)	1.3	2
Larva	48 h	739.9 (522.3-1220.5)	1497.1(1332.1-1670.8)	0.96	3
Neonate	24 h	98.7 (0.1-170.8)	352.0(263.2-1407.2)	0.55	2
Male	24 h	502.07 (322.7-598.2)	967.2(802.3-1605.3)	1.41	2
Female	24 h	582.9(386.9-699.4)	1226.9 (966.4-2576.9)	1.84	2
Cocoon	3 weeks	65.1(43.3-110.9)	709.7(533.4-1019.9)	0.92	1

after pruning. In this respect, the cut palm emits highly volatile organic materials (Kairomones) which attract the weevil for laying eggs inside wound tissues (Murphy and Briscoe, 1999).

Effect of ABM on egg-hatch

Egg-hatch of RPW after exposure to ABM was highly affected (Figure 4). At 200 ppm, egg hatch was reduced to 36.5%. By increasing the concentrations, the hatchability decreased to 9.7% by 500 ppm after exposing to residual film of the insecticide for 3 days as compared with control. LC_{50} and LC_{95} were 123.6 ppm and 756.4 ppm with fiducial limits of 47.1-175.4 and 563.9-1666.3 (Table 1). Most likely, the effect of such insecticide was due to the lack of energy resultant in the inhibition of F_oF_1ATPase activity in the inner mitochondrial membrane that is responsible by ATP synthesis driven by the proton electrochemical gradient generated in the respiratory chain (Castanha-Zanoli et al., 2012; Hatefi, 1993; Pedersen, 1996). Subsequently, blockage of insect embryonic development and

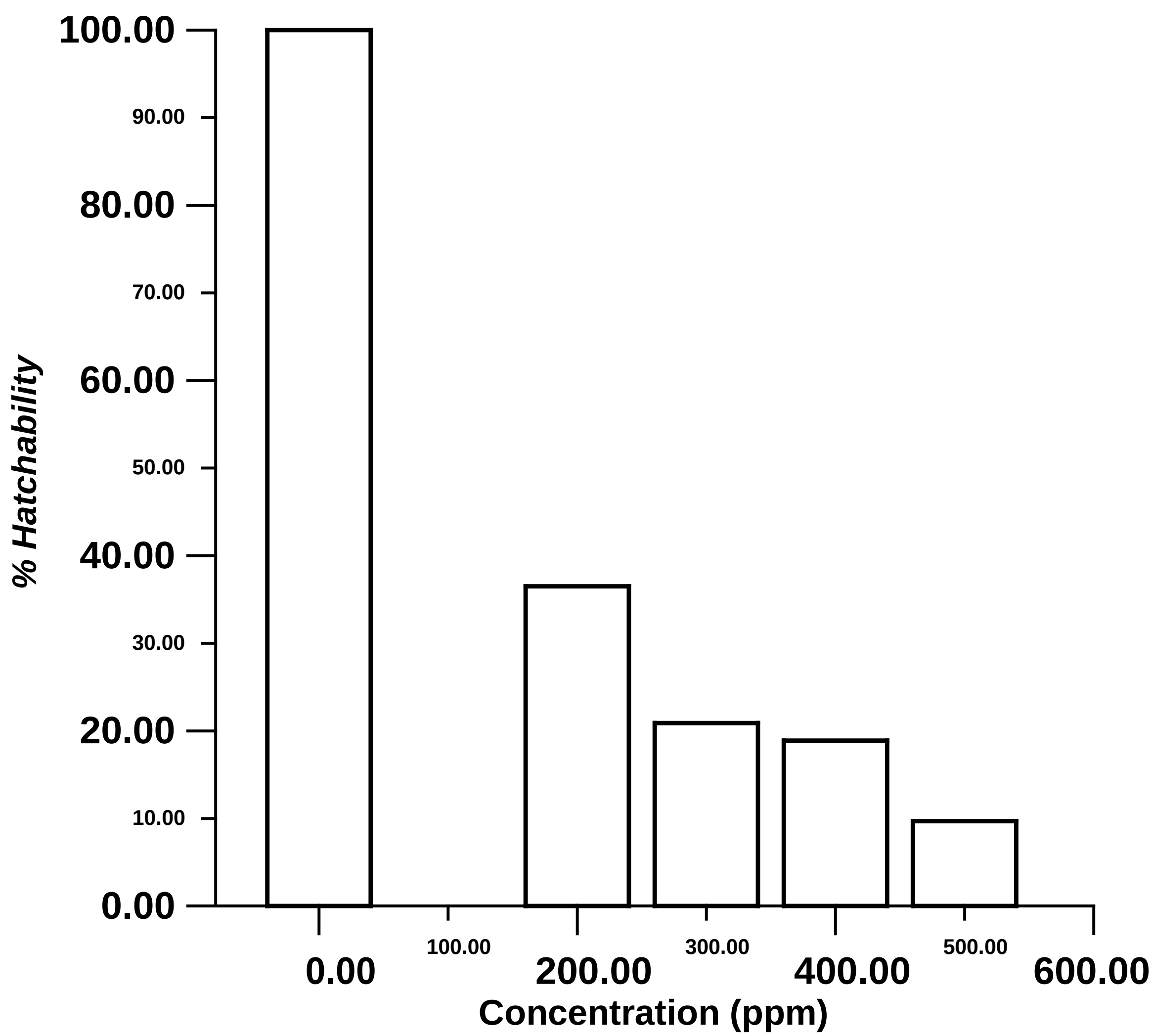

Figure 4. Percent hatchability of eggs RPW after exposure to ABM for 3 days.

eventually no eggs would hatch. Our data are consistent with the data of other investigators who indicated that blockage of insect embryonic development occurred both when the insecticide was applied directly to the egg stage soon after oviposition (Kathuria et al., 2000) and when it was applied to the female during egg formation (Reissig et al., 1998), or at maturation (Ahmed et al., 1990). Likewise, these results make it advantageous for curative and protective treatments applied to the trunk of palm trees. So, when female RPW would lay eggs on ABM treated tissues, the contact effects on eggs would inhibit embryonic development and therefore stop new infestation.

Effect of ABM on cocoon-emergence

Results clearly show that ABM is markedly effective against RPW pupae (Figure 5). Concentrations ranging from 100 to 500 ppm caused progressive mortality and therefore no adult emerged from treated cocoon at 500 ppm after treatment. LC_{50} and LC_{95} against cocoons of RPW were 65.1 and 709.7 ppm respectively with fiducial limits of 43.3-110.9 and 533.4-1019.9. This interesting finding sheds light on the possibility of targeting RPW in the pupal stage before adult emergence using a proper concentration of ABM.

In summary, the red palm weevil is one of the most invasive and destructive insect pests causing immense damage to date palms across the world. Of the most important methods used to achieve this goal is integrated pest management, which in turn involves the application of insecticides as a preventative or curative key element in such program. Therefore, an effective insecticide, which is nontoxic to mammals and safe for the environment, must be chosen. According to the results of this study, ABM proves to be an effective contact bio-insecticide against all developmental stages of RPW. ABM a natural insecticide can be a possible candidate to

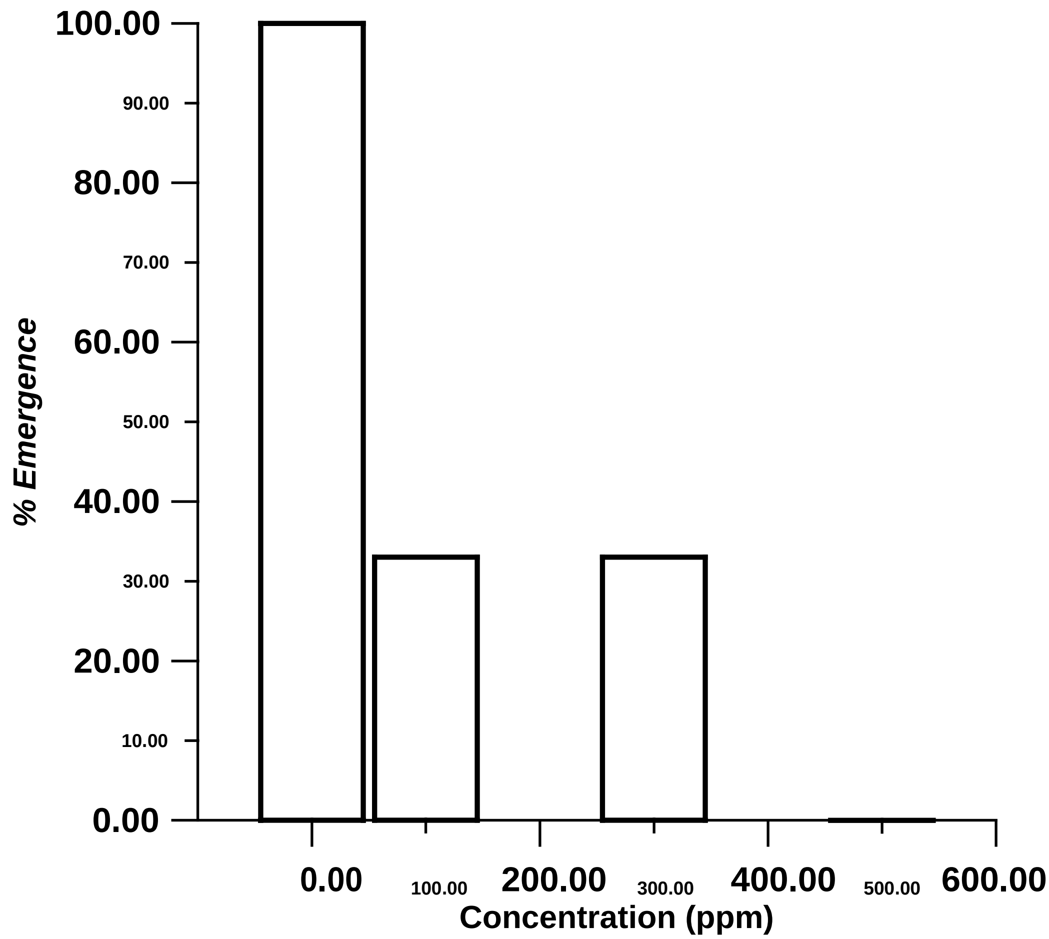

Figure 5. Percent emergence of adults of RPW, *Rhynchophorus ferrugineus*, from cocoons after exposed to ABM.

be applied on date palm by the Ministry of Agriculture after successful field experiments.

ACKNOWLEDGEMENTS

The authors would like to thank the Deanship of Scientific Research, King Faisal University, Saudi Arabia for their kindly provision of financial support of project No. 120082.

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