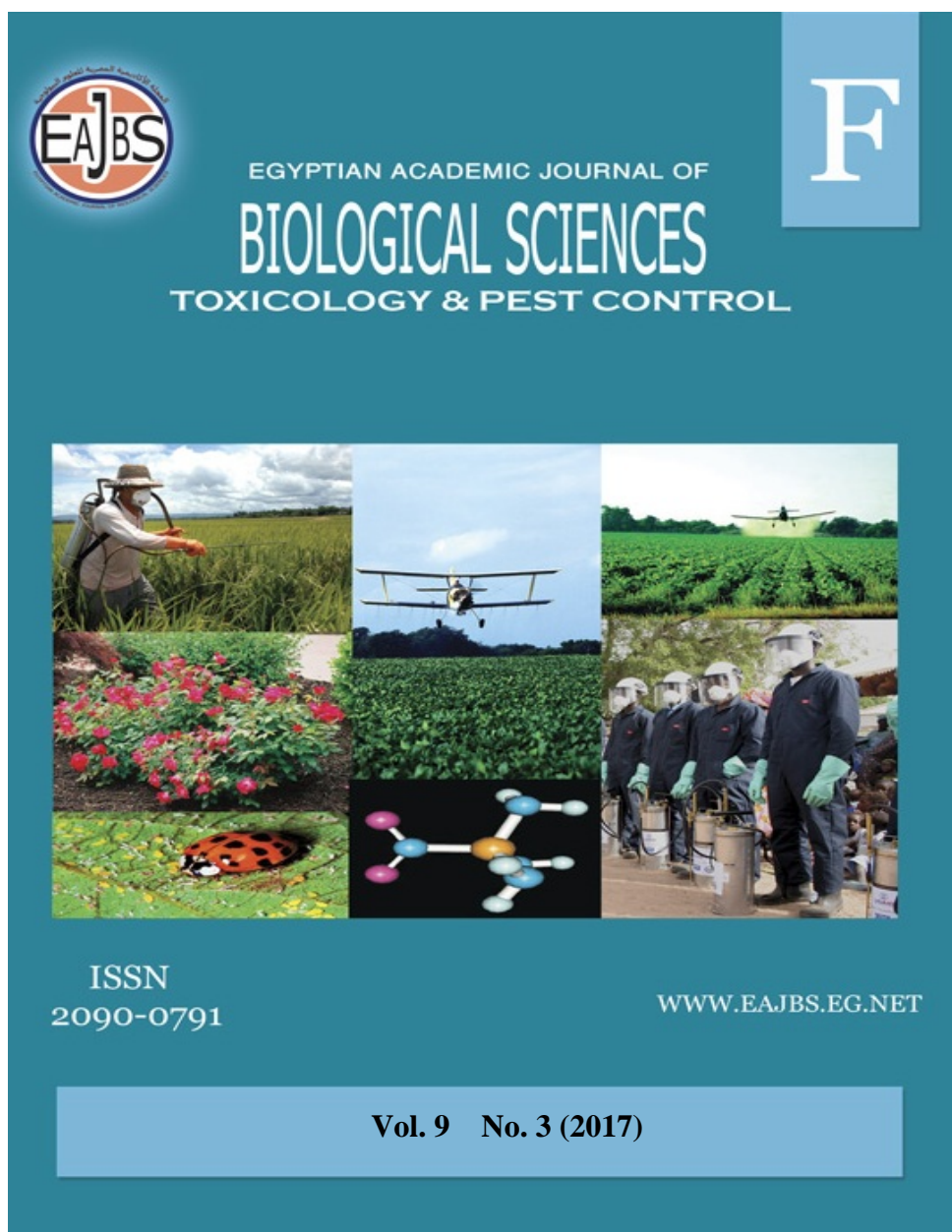


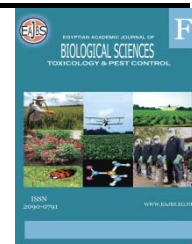
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**Impact of Neem Extracts, *Azadirachta indica* A. Juss Induced against Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) Attacking Date Palm Orchards in Egypt**

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**ABSTRACT**

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is considered to be the most devastating boring pest attacking the date palm trees, *Phoenix dactylifera* L. (Arecales: Arecaceae). During this study the date palm orchards were investigated at Ismailia governorate in Egypt to throughout 2015-2016 and results revealed the wide distribution of RPW with high infestation rates at the visited localities. Rates and degrees of infestation were detected through the attacked spots showing a direct proportional rate with the total planted date palm trees in each location recording a highest infestation rate of 91.6% and a maximum infestation degree was 3.4 hole/ tree. Least average infestation levels were 14.9% and 1.19 hole/ tree. Extracts of neem seeds, *Azadirachta indica* A. Juss (Sapindales: Meliaceae) were tested in the laboratory against the RPW stages using organic solvents in addition to the commercial Nemazal brand as a comparison treatment. Laboratory experiments revealed a potential effect of the used titers against both adult and larval *R. ferrugineus* stages resulting in high mortality rates reached 85.4 and 79.5% on average for larval and adult stages, respectively. Consequent field trials with such agents were conducted on both remedial and protective scales revealing a considerable reduction of infestation was 60.8% and 53.1% repellency on average for all applied treatments. Field trials proved the possibility of those treatments usage in the control programs of that borer in Egypt.

**INTRODUCTION**

Date palm tree, *Phoenix dactylifera* L. (Arecales: Arecaceae) is an important socioeconomic crop worldwide. Cultivars of date palm tree are considered cheap source for energy and food for mankind in addition to industrial purposes. In Egypt, date palm trees are planted all over the country and are commonly infested with many insect pests especially wood boring pests. Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is the most serious wood borer which extensively attacks date palm trees. RPW is widely distributed infesting palm orchards in many regions all over the world; in southern Asia, India, Gulf area, UAE, Saudi Arabia, Iran, Egypt, Palestine and Jordan (Cox 1993; Oehschlager 1996; Al-Saqer and Hassan, 2011; Maryam *et al.*, 2015 and Jazinizadeh *et al.*, 2017).

In Egypt, RPW is considered as the major destructive and devastating pest to date palm trees causing significant damage and its stages, larval and adult stages, existed around the year seasons and all over almost Egyptian governorates (Helal and El-Sebay, 1994; El-Sebay, 2004; Merghem, 2011; and Merghem and Bibers, 2014).

No effective method for the control of that wood boring pest control is used except chemical control trends. Attempts to control RPW with chemical insecticides got limited success associated with development of insecticide resistance and pesticide residues in nature and the extensive use of these chemicals caused environmental and economic problems such hazard effects against mammals, beneficial animals and arthropods, and worldwide pollution (Abo El-saad *et al.* 2001; Ranasinghe *et al.* 2003 and Gloria *et al.* 2008).

In response to the harmful effects of the insecticides and continuous economic loss by this borer, alternative tools grasping effective, safe, and cheap control were encouraged to solve these problems. Among these alternative measures there are the extracts of the neem tree, *Azadirachta indica* A. Juss (Sapindales: Meliaceae) and its products such as Neemazal, a brand product of neem, which proved their potential and environmentally safe to vertebrates, plant species and useful invertebrates in addition to their wide use in control many insect pests including wood borers as promising agents (Schmutterer, 1990; Chakaravarthy *et al.*, 2008; Sittichaya and Beaver, 2009; Pant and Tripathi, 2011; Kumar *et al.*, 2012; Merghem and Abd El-Ghany, 2013; and Hagstrum and Phillips, 2017).

The present study aimed to evaluate the efficacy and toxicity of the crude neem extracts, *A. indica* in addition to Nemazal brand against the

developmental stages of the red palm weevil, *R. ferrugineus* through the laboratory experiments combined with a series of field applications trials either for remedial or protective purposes securing date palm trees orchards at Ismailia governorate in Egypt.

## MATERIALS AND METHODS

### Target boring pest:

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae).

### Collection of target pest:

RPW stages were collected from infested date palm orchards in Ismailia governorate. The stages were collected from trees with no previous insecticidal treatments. Periodical and continuous survey visits were ultimately undertaken for estimation the infestation levels throughout 9 locations; Abou Khalifa, Abou Sweer, Al-Mostakbal, Al-TI Al-Kabeer, Al-Qassasin, Al-Qntra Gharb, Al-Qntra Sharq, Fayed and Ismailia localities at Ismailia governorate in Egypt during 2015-2016.

### Tested plant trees:

Date palm tree, *Phoenix dactylifera* L. (Arecales: Arecaceae) and the neem tree, *Azadirachta indica* A. Juss (Sapindales: Meliaceae).

### Preparation of the extracts:

Neem seeds, *A. indica* which contain the active compound "Azadirachtin" the well-known ingredient, were gained with the aid of Mr. Sing Galbahai (Supervisor of Al-Shrouk farm in Egypt) and ethanol and petroleum ether were used as solvents. Obtained seeds were weighed, crushed, and grinded into a powder then this powder was divided into serial groups of 6, 3, 1.5 gm. For preparation of the desired concentrations series, those groups were distributed, mixed with a definite volume of each desired solvent and skimmed of supernatant to get a

gm/ml concentration according to Ascher (1981).

#### Laboratory screening experiments:

To test the efficacy of *A. indica* extracts against RPW stages, five individuals of full grown larvae or adults were used. These individuals of each stage were put in a glass jar 15×15×20 cm<sup>3</sup> and sprayed with a series of concentrations; 6, 3 and 1.5 gm/ml of each solvent neem extraction. In a second series of experiments, a 3ml/L concentration of a commercial Nemazal brand were provided in addition to check treatments of five individuals of each developmental stage was exposed to just the solvent as control and these glass jars were incubated for 15 days; the recommended post period evaluation after insecticidal treatment for RPW control. Three replicates were made for each treatment then the readings of mortality percentages were recorded and corrected with Abbott's formula according to Abbott (1925). Mortality rates were recorded at equal time intervals and the effective titers of each treatment were chosen for the further field applications.

#### Field trials experiments:

These neem crude extracts were used separately for the field applied trials. Field experiments were designed for remedial and protective techniques tests against the *R. ferrugineus* infestations at Al-Qassasin locality in Ismailia governorate. To achieve the levels of the infestation reduction, five palm trees were chosen for the proposed trials as a replicate package for each treatment.

#### Remedial technique:

Testing the toxicity efficiency of neem extracts against RPW infestations for remedial purpose required a selection of the already infested date palm trees at the highly infested orchard to estimate their remedial effect. These chosen trees were injected with the concentrations of both the neem extracts and Nemazal

brand with the same titers applied in the laboratory experiments. Positive control checks were just tested by only the responding solvent at the same rate of treatment and water checks were for Nemazal trials. Efficacy of the all treatments was evaluated by the recovery percentage of the infested palm trees (RPIPT) which calculated according to Henderson and Tilton (1955) formula:

$$\text{RPIPT (\%)} = \frac{I - F}{F} \times 100$$

Where:

I: Initial number of infested palm trees with RPW (Pre-treatment).

F: Final number of infested palm trees with RPW (Post-treatment).

#### a. Protective technique (repellency):

To study the repellent effect of these extracts, two selected intact non-infested groups of date palm trees were chosen for this purpose; one for the applied treatments and the other group just as control checks with the used solvents. Protective experiments were evaluated by the repellency against RPW poles indicated by the weevil attacks. Thus the rates of infestation were observed for all the treated replicates as the repellency was achieved.

#### Statistical analysis:

Obtained data were statistically analyzed according to Finney (1992) and Gomez and Gomez (1984). Observed mortality rates were corrected by Abbott formula (1925) and lethal toxicity lines were estimated by the log-time probit model using the LdP Line software after Ehab-software.

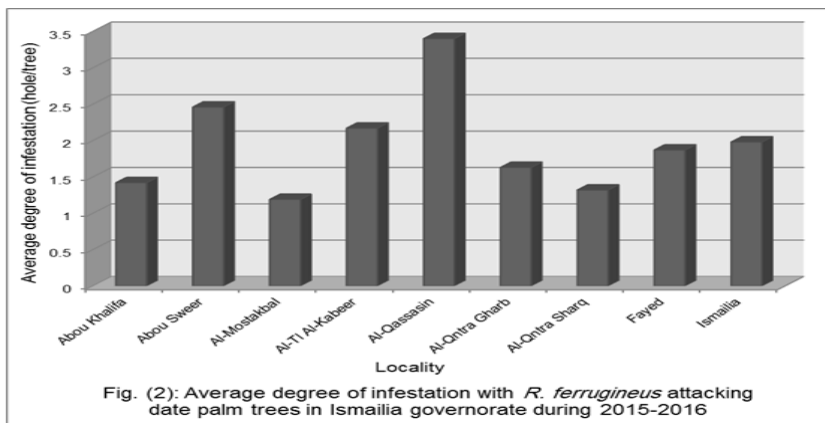
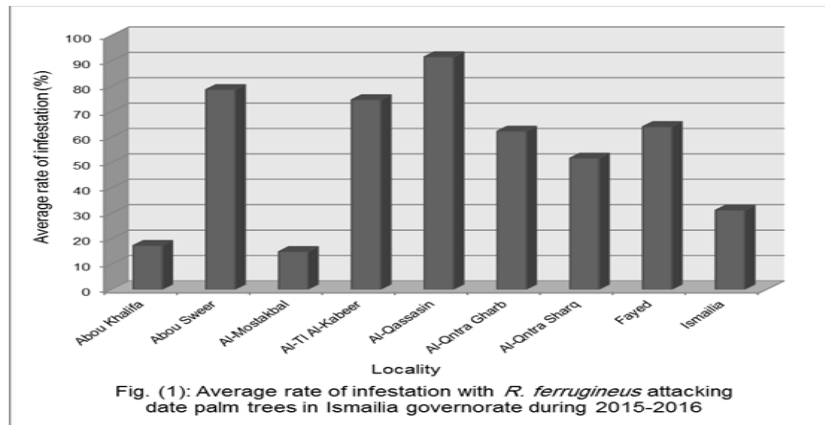
## RESULTS AND DISCUSSION

### Infestation levels with *R. ferrugineus*:

Infestation levels with the curculionid borer, *R. ferrugineus* are shown in Figs. 1&2 represented by the rates and degrees of infestations observed throughout the attacked palm tree orchards at the stated localities in Ismailia during 2015-2016. The gained

results revealed that the Al-Qassasin locality is the broadest attacked locality with RPW recording a maximum average infestation rate reached 91.6 % a highest infestation degree was 3.4 hole/tree. On the other hand, Al-Mostakbal locality was found to show the lowest infestation levels with *R. ferrugineus* showing a least average rate of infestation was 14.9 % and a degree of infestation scored 1.19

hole/tree on average. RPW was significantly recorded in high average level at  $P>0.05$  according to Duncan's multiple range test through the Al-Qassasin location different from the existence of RPW all over the rest localities of Ismailia governorate wherefore this locality was chosen for the incoming field control trials against that boring pest.



### Laboratory screening experiments:

Fig. (3) Shows the average mortality percentages achieved due to the laboratory applications of the different concentrations of Nemazal and crude neem extracts against the adult and larval *R. ferrugineus* stages.

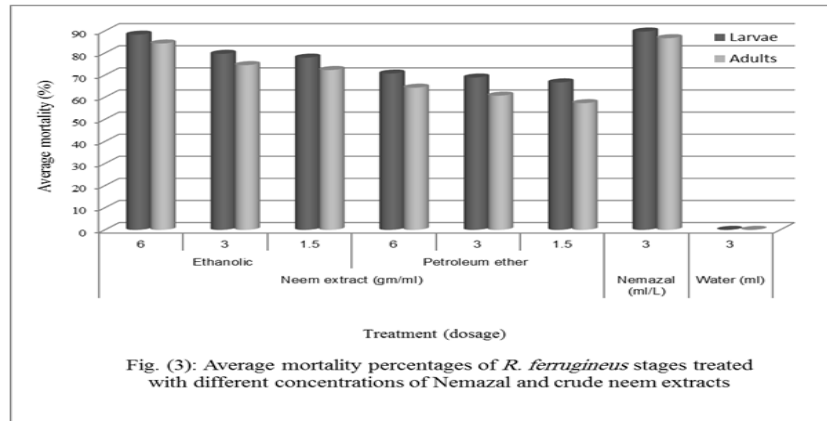
Laboratory toxicity tests revealed average mortality rates as  $88.3\pm 1.3$  and  $84.3\pm 0.4$  % for larval and adult stages at 6 gm/ml concentration, respectively whereas mortality rates were  $79.6\pm 1.2$  and  $74.5\pm 0.7$  % on average for larval and adult stages, respectively in the case of 3

gm/ml concentration. The least average mortality rates were  $77.9\pm 1.3$  and  $72.3\pm 0.9$  % for larval and adult stages, respectively with the treatment of the concentration 1.5 gm/ml. whereas the lowest larval and adult mortalities (66.7 and 57.3 %) were gained by the neem petroleum ether extract.

Through the laboratory screening tests larvae of RPW showed a higher susceptibility to the applied treatments more than adult stages. Nemazal treatments recorded a not significant mortality rates (at  $p>0.5$ ) in relation with

those of 6 gm/ml ethanolic neem extracts scoring  $89.7 \pm 2.3$  and  $86.7 \pm 1.1$  % on average for larval and adults stages, respectively meanwhile its levels were

significantly different at  $p > 0.5$  with the two other ethanolic extracts concentrations for both *R. ferrugineus* stages.



Bioassay experiments indicated that the mortality levels of *R. ferrugineus* stages subjected to the petroleum ether neem extracts were significantly different at  $p > 0.5$  from the gained mortality rates of the Nemazal brand titers. Ethanolic neem extracts concentrations especially with the titer of 6 gm/ml proved to be more virulent against RPW stages than those of the petroleum ether neem extracts. These obtained results were in accordance with Nassar and Abdulah (2005) who reported the efficacy of the plant extracts against RPW; and matching to Pant and Tripathi (2011) findings who addressed the quite efficiency of the neem treatments against the destroying lyctid borers.

Table (1) elucidates the lethal concentrations of the used treatments against *R. ferrugineus* stages with titers of Nemazal and neem extracts. Results reveal that Nemazal brand and ethanolic neem extraction concentrations induced the high mortalities accompanied with the effective  $LC_{50}$ ,  $LC_{90}$  and  $LC_{99}$  values for both larval and adult stages.

These lethal titers of the Nemazal brand were 0.826, 3.273 and 6.854 respectively for the larval stages whereas these figures scored 1.264, 3.741 and 7.426 for the adult stage. On the other

hand, ethanolic neem extracts had  $LC_{50}$ ,  $LC_{90}$  and  $LC_{99}$  values of 1.792, 7.026 and 9.566 respectively for the larval stages meanwhile the responding values for the adult stages were 1.895, 8.956 and 12.023.

From the forgoing laboratory results the experiments revealed a potential effect of the used titers of Nemazal brand and neem crude extracts against both adult and larval *R. ferrugineus* stages thus a completion with these tools was recommended for the further field applications.

#### Field trials experiments:

Field trials were conducted to examine the efficacy of the tested neem extracts and Nemazal against *R. ferrugineus* infestations attacking date palm trees at Al-Qassasin in Ismailia governorate as shown in Fig. (4). The applied experiments were undertaken on both protective and remedial scales.

Remedial experiments revealed mortality levels of *R. ferrugineus* represented by the recovery percentage of the infested palm trees (RPIPT) reaching  $61.3 \pm 1.4$  and  $47.8 \pm 1.7$  % on average for the tested neem extracts; ethanolic and petroleum ether extracts concentrations, respectively.

Table 1: Lethal concentrations of neem extracts and Nemazal against *R. ferrugineus* stages.

Treatment	Stage*	LC**	Titer	Fiducial limits		Slope	Chi <sup>2</sup>	P
				Lower	Upper			
Ethanol extract	L	50	1.792	0.665	4.143	2.132	2.751	0.2311
		90	7.026	5.044	8.771	2.458	4.992	0.2619
		99	9.566	8.992	11.521	5.874	9.643	0.3745
	A	50	1.895	0.989	4.289	2.026	4.137	0.3012
		90	8.956	7.618	10.927	3.872	7.273	0.3401
		99	12.023	9.096	14.976	5.967	9.945	0.3947
Petroleum ether extract	L	50	2.405	1.812	4.728	3.732	4.613	0.3701
		90	8.022	6.362	9.908	3.892	4.733	0.3986
		99	9.914	8.997	12.783	5.948	9.749	0.3994
	A	50	2.316	1.268	4.798	3.032	6.523	0.3581
		90	9.614	9.027	12.351	3.944	7.696	0.3792
		99	13.026	10.122	15.736	6.308	9.984	0.3836
Nemazal	L	50	0.826	0.584	1.693	2.033	2.456	0.1523
		90	3.273	2.851	3.945	4.783	4.831	0.1736
		99	6.854	4.986	9.367	5.324	9.855	0.2392
	A	50	1.264	0.872	1.963	3.142	2.548	0.1641
		90	3.741	3.104	4.176	3.541	6.912	0.1837
		99	7.426	5.067	9.987	5.779	9.894	0.2718

\*Symbols (L) and (A) indicate the larval and adult stage of *R. ferrugineus*.

\*\* Symbol (LC) indicates the lethal concentration of the applied titer.

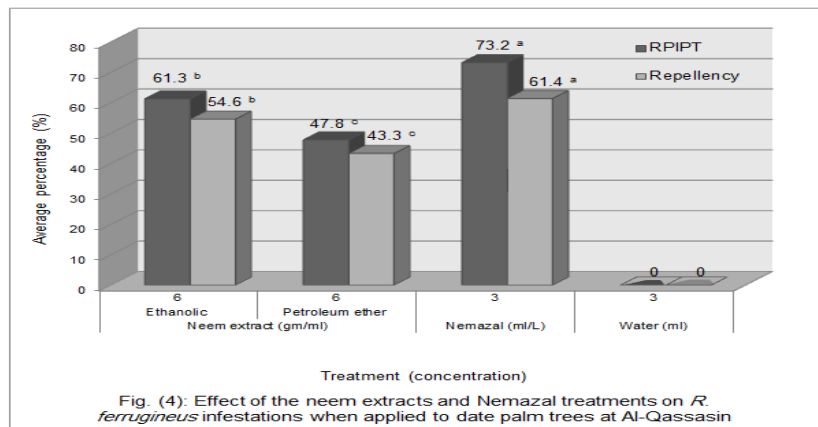


Fig. (4): Effect of the neem extracts and Nemazal treatments on *R. ferrugineus* infestations when applied to date palm trees at Al-Qassasin

Whereas Nemazal treatments resulted in an average RPIPT was  $73.2 \pm 2.3$  %. For neem extracts, RPIPT recorded its maximum level (61.3%) when infested palm trees treated with 6 mg/ml concentration of the neem ethanolic extract meanwhile RPIPT scored its minimal values (47.8%) with the same titer (6 mg/ml) concentrations of neem petroleum ether extracts. Remedial outputs due to Nemazal treatments were significantly different at  $P > 0.05$  from those ones for both the used neem extracts. Through field applications for remedial purposes, the mortality rates

were markedly less than those resulting from laboratory experiments; this may be attributed to the natural field and palm trees conditions. As in laboratory bioassay experiments, the 3 ml/L Nemazal concentration showed the highest RPIPT levels indicating the mortality percentages attained.

As illustrated in Fig. (4), average repellency rates against *R. ferrugineus* attacks were  $54.6 \pm 2.1$ ,  $43.3 \pm 2.7$  and  $61.4 \pm 1.8$  % for the applied titers of ethanolic and petroleum ether neem extracts and Nemazal treatments, respectively. Non-infested date palm



trees got a significant protection level against the attacks of *R. ferrugineus* at  $P > 0.05$  when treated with Nemazal titers differs from the responding titers of the used neem extracts. Ethanolic neem extracts had a noticeable significant different levels of repellency at  $P > 0.05$  against the attacks of RPW from the petroleum ether extracts. Provided results are matching with those of Jaglan *et al.* (1997) who reported the efficacy of methanol neem extract concentrations. These findings with the applied treatments are concordant with those of Roy and Joy (2014) who confirmed the possibility to control wood boring pests efficiently using ethanolic neem extracts.

From the overall results obtained from the laboratory and field experiments through this study, a target to represent the broad figure for the occurrence and infestation levels of RPW the most devastating curculionid borer attacking the date palm trees was conducted. This was coupled with a series of applied control experiments using Nemazal brand and neem crude extracts aiming to suppress its prompt and steady damaging attacks providing healthy palm trees. Results confirmed that Nemazal and ethanolic neem extracts have a considerable significant remedial efficacy altogether with an effective repellency against *R. ferrugineus* infestations. This may encourage their recommended usage as a component in integrated pest management (IPM) programs against the *R. ferrugineus* attacks in Egypt.

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## ARABIC SUMMERY

تأثير مستخلصات نبات النيم *Azadirachta indica* A. Juss المستحثة ضد سوسة النخيل الحمراء *Rhynchophorus ferrugineus* (Olivier) المصبية لبساتين نخيل البلح في مصر

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- جيزة - مصر

٢- المعمل المركزى لأبحاث و تطوير نخيل البلح- مركز البحوث الزراعية- جيزة - مصر

تعتبر سوسة النخيل الحمراء (Coleoptera: Curculionidae) من أشد الحفارات المهلكة التى تصيب أشجار نخيل البلح *Phoenix dactylifera* L. (Arecales: Arecaceae) ، ومن خلال هذه الدراسة تم فحص أشجار نخيل البلح بمحافظة الإسماعيلية فى جمهورية مصر العربية وذلك للوقوف على إصابتها بسوسة النخيل الحمراء و مدى إنتشار و كثافة الإصابة بهذه الآفة خلال عامى ٢٠١٥ و ٢٠١٦ ، وقد أظهرت نتائج زيارات المسح عن إنتشار هذا الحفار بكثافة و معدلات إصابة مرتفعة فى مزارع نخيل البلح بالمحافظة ، كما أوضحت النتائج أن المواقع التى تم فحصها كانت جميعها مصابة بهذه الآفة ، كما لوحظ أن تواجد هذا الحفار متناسبا طرديا مع عدد أشجار نخيل البلح المنزرعة بكل موقع ، ولقد تم تقدير معدلات و درجات الإصابة بسوسة النخيل الحمراء فى هذه المواقع ، و قد وجد أن أعلى متوسط لمعدل الإصابة بهذه الآفة وصل الى ٩١,٦ ٪ بينما كان أقل متوسط لمعدل الإصابة هو ١٤,٩ ٪ ، كما تراوح متوسط قيم درجات الإصابة بسوسة النخيل الحمراء ما بين ١,٩ و ٣,٤ ثقب لكل شجرة ، و لقد تم تجريب المستخلصات النباتية الخام لنبات النيم معمليا ضد أطوار سوسة النخيل الحمراء الكاملة و غير الكاملة بإستخدام بعض المذيبات العضوية بالإضافة لإستخدام مركب النيمازال التجارى كمعاملات مقارنة، وقد أظهرت التجارب العملية فعالية هذه المستخلصات و مركب النيمازال ضد أطوار سوسة النخيل الحمراء محققة مستويات نسب موت مرتفعة بلغت ٨٥,٤ و ٧٩,٥ ٪ فى المتوسط لكل من الطور اليرقى و الطور الكامل على الترتيب ، و بناء على هذه النتائج تم تطبيق المعاملات الحقلية ضد هذه السوسة فى مزارع النخيل على مستويين أحدهما وقائى و الاخر علاجى ، و لقد أسفرت نتائج هذه التجارب عن معدلات جيدة فى خفض الإصابة بلغت ٦٠,٨ ٪ و نسبة طرد للإصابة وصلت ٥٣,١ ٪ فى المتوسط ، المعاملات الحقلية أعطت إمكانية لطرح مثل هذه المستخلصات و مركباتها للدمج فى برامج مكافحة المتكاملة لسوسة النخيل الحمراء فى مصر. **الكلمات المفتاحية:** سوسة النخيل الحمراء ، أشجار نخيل البلح ، محافظة الإسماعيلية ، مستويات الإصابة ، نبات النيم ، المستخلصات النباتية ، النيمازال.