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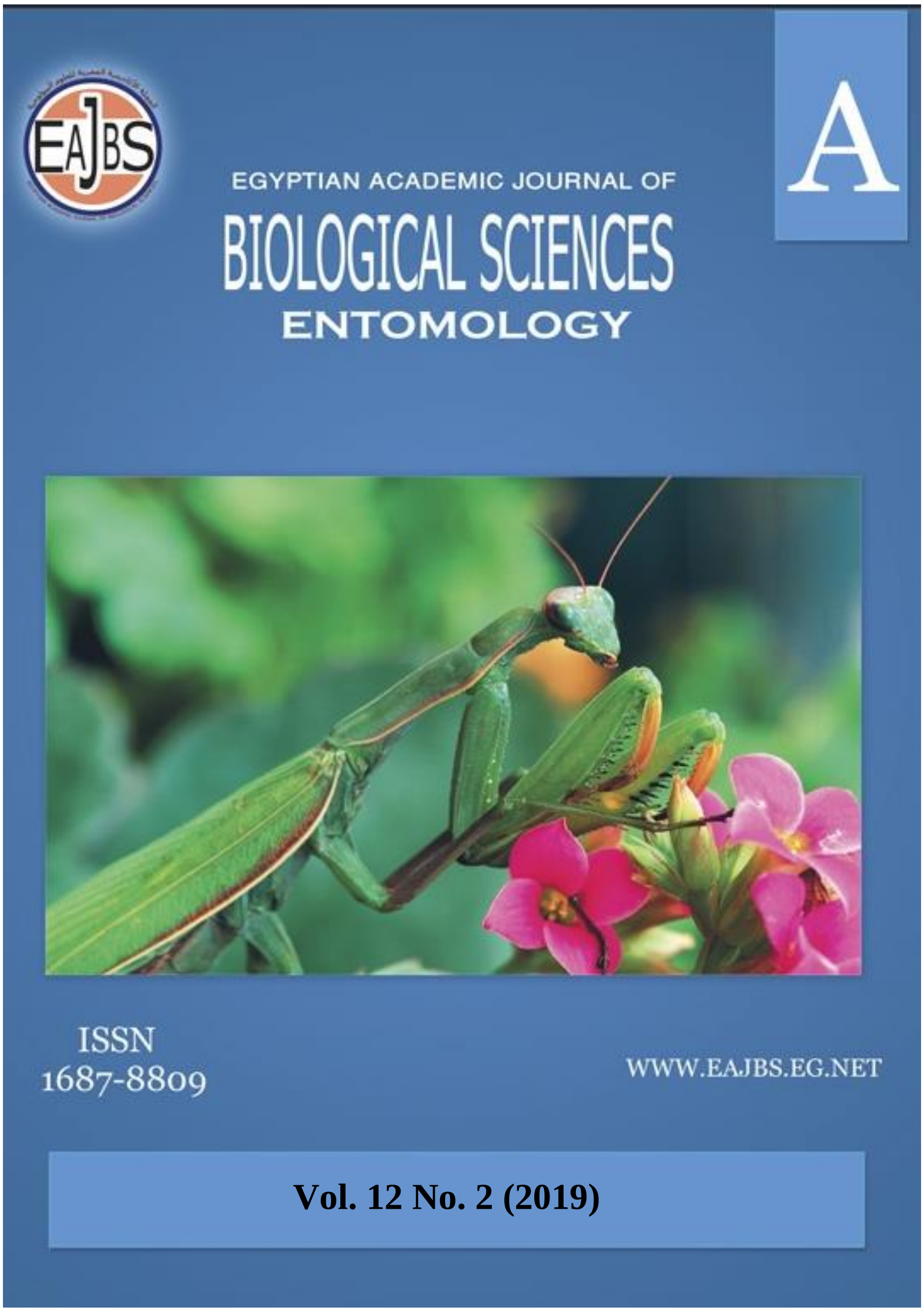

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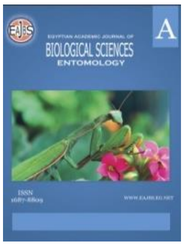

Comparative Assessment of Certain Insecticidal Compounds on the Development and Adult Performance of Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae).

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ABSTRACT

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of date palm in different regions of the Middle East. The present study was carried out to evaluate comparatively the effects of pyriproxyfen (insect growth regulator), neemazal (a plant product) and spinetoram (a bacterial product) on growth, development and adult performance of this insect pest. The early 5th instar larvae were treated with five sublethal concentrations of each compound. The most important results can be summarized as follows. Both larval duration and weight were considerably reduced by Spinetoram. Pyriproxyfen significantly reduced the larval duration only at 4000 ppm (7.2±2.97, compared to 11.7±1.6 days control insects) but Neemazal could not exhibit a remarkable effect. The strongest reducing effect on larval weight was recorded for Pyriproxyfen, since the growth inhibition% was determined as 49.10, 48.58 and 38.04, at 4000, 2000 and 1000 ppm, respectively. Pupation was inhibited by all tested insecticidal compounds and the pupal duration was significantly shortened, except at the lower three concentrations of Neemazal. Some deformations had been recorded only in adults, irrespective of the tested compound. The adult longevity was significantly shortened at the higher concentrations of each compound.

INTRODUCTION

The red palm weevil (RPW) *Rhynchophorus ferrugineus* Olivier is a dangerous insect pest of date palm in the Arabian Gulf region since the mid-1980s and Egypt since 1992 (Saleh, 1992; Cox, 1993). In almost all Egyptian governorates, the larval and adult stages existed around the year seasons (Helal and El-Sebay, 1994; El-Sebay, 2004; Merghem, 2011; Merghem and Bibers, 2014). Serious damage has been recorded for the larval stage of this weevil in some palm genera and, thus, control operations should be carried out (EPPO 2008). The chemical control of RPW with conventional insecticides usually exhibits a limited success in addition to the insecticide resistance of palms as well as their chemical residues accumulated in nature as pollutants. In addition, the extensive use of these insecticides caused various hazards to the environment, mammals, beneficial animals and arthropods (Abo El-Saad *et al.*, 2001; Ranasinghe *et al.*, 2003; Gloria *et al.*, 2008; Koul and Walia, 2009).

Therefore, different insecticide alternatives should be taken in consideration. Some of these alternatives are insect growth regulators (IGRs), like Pyriproxyfen, plant extracts, like neem formulations, and microbial products, like Spinetoram. Pyriproxyfen (IGR) exerts its toxic effect through the disturbance of the normal endocrine functions in insects (Smet et al., 1990; Oberlander *et al*., 1997). Plant extracts, such as neem seed and leaf extracts, as well as, azadirachtin formulations, like neemazal, are effective agents as antifeedant, repellents, growth disruptors, moulting inhibitors and oogenesis suppressors (Rembold and Sieber, 1981; Garcia and Rembold, 1984; Dorn *et al*. 1986; Richer *et al.*, 1997; Ghoneim *et al*., 2000). In a previous study, pyriproxyfen, neemazal and spinetoram were recorded as active agents causing mortality in RPW (Hamadah and Tanani, 2014). Also, Hamadah and Tanani (2017) determined the deleterious effects of these compounds on haemogram of the same weevil. Spinetoram (microbial product) has been derived from soil-dwelling bacteria *Saccharopolyspora spinosa* and exerts its toxic action by contact or ingestion (Sparks *et al.*, 1998). It targeting a nicotinic acetylcholine receptor as well as ɤ-aminobutyric acid (GABA) gated chloride channels causes insect paralysis (Watson 2001; Sparks, 2004; Sarfraz *et al*., 2005). The objective of the current study was to evaluate the disruptive effects of certain insecticide alternatives (pyriproxyfen, neemazal and spinetoram) on the growth, development and adult performance of *Rh. ferrugineus*.

MATERIALS AND METHODS

1. Experimental Insect:

A culture of the red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) was initiated by a sample of field-collected pupae. The culture was established at Faculty of Science, Al-Azhar University, Cairo, Egypt, on sugarcane stem under constant laboratory conditions (29oC±2, 60-70 % RH, the light intensity of about 30 foot-candles)(Rahalkar *et al*., 1972; Rananvare *et al*., 1975).

2. Tested Compounds and Larval Treatments:

Spinetoram 5% (radiant), Pyriproxyfen 10% (admiral) and Neemazal (azadirachtin 20%) were kindly obtained from the Central Laboratory of Insecticides, Agricultural Research Centre, Doqqi, Giza, Egypt. Each compound was dissolved in distilled water for preparing five concentration levels: 40000, 20000, 10000, 5000 and 2500 ppm for Pyriproxyfen; 4000, 2000, 1000, 500 and 250 ppm for Neemazal and 200, 100, 50, 25 and 12.5 ppm for Spinetoram.

The newly moulted last instar larvae (5^{th}) of *Rh. ferrugineus* were fed (for 24h) on fresh pieces of sugarcane stem after dipping for ten minutes in different concentrations of each compound but the control congeners were dipped in water only for the equal time period. All sugarcane pieces were allowed to dry before offering to the larvae. A day after treatment, all larvae (treated and control) were provided with an untreated piece of sugarcane. Three replicates of 10 individual of larvae per each were used for each concentration. Each individual larva was isolated in a suitable glass vial. All vials tightly cover and located in a large cage supplied with a suitable electric bulb. At one day after feeding, the larvae were carefully weighted every day and also examined for mortality or other criteria.

3. Criteria of Study:

Body Weight: The fresh body weight was obtained every day using a digital balance. **Growth Inhibition:** It was calculated as follows: [a-A/A] ×100, Where: a: mean weight of treated larvae. A: mean weight of control larvae.

Pupation rate: It was calculated according to Jimenez-Peydro *et al.* (1995) as follows: PR= [No. pupated larvae/No. treated larvae] ×100

Developmental Duration and Rate: The developmental durations (in days) were estimated using Dempster's equation (1957). The developmental rate was calculated using

Richard's equation (1957).

Adult Emergence: The number of metamorphosed adults was expressed in % according to Jimenez-Peydro et al. (1995) as follows: [No. of completely emerged adults / No. of pupae] × 100

Morphogenic Efficiency: It was calculated according to Vargas and Sehnal (1973) as follows: [No. of deformed adults/No. of emerged adults] × 100

Adult Longevity: was determined in days, from the emergence until death.

4. Statistical analysis of data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means±SD.

RESULTS

As clearly shown in the data of Table 1, Spinetoram considerably shortened the larval duration of the red palm weevil *Rhynchophorus ferrugineus* larvae, irrespective of the concentration. Larval duration was shortened to 7.77±2.2, 8.41±2.0, 9.12±2.4, at 100, 50, 25 and 12.5 ppm, compared to 11.7±1.6 days of control larvae. Only the highest concentration (4000 ppm) of Pyriproxyfen significantly shortened the larval duration in a developmental rate of 13.89, in comparison of 8.54% for controls. Moreover, Neemazal had no effect on the larval duration or developmental rate.

With regard to the larval body weight, it generally decreased, regardless of the tested compound. Amongst all tested compounds, the strongest reducing effect on larval body weight was exhibited by Pyriproxyfen, at its higher concentration levels (1.98±0.63, 2.00±0.49 and 2.41±0.38 mg, at 4000, 2000 and 1000 ppm, respectively, *vs*. 3.89±0.74 mg of control larvae with growth inhibition 49.1%.

Data of pupation and pupal duration had been arranged in Table (2). Depending on these data, pupation % decreased after treatment with all compounds. Treatment with the highest concentration led to the reduction of pupation to 40, 50 and 10%, by Pyriproxyfen, Neemazal and Spinetoram, respectively. The pupal duration was shortened at all concentrations of the tested compounds, with the exception of lower concentrations of Neemazal. As for example, the pupal duration was considerably shortened by Spinetoram to 8.46±1.09, 12.14±3.01 and 12.33±3.66 days, at 50, 25 and 12.5 ppm, compared to 18.21±1.62 days of control pupae. The developmental rate increased to 11.82, 8.24 and 8.11, at these concentration levels, compared to 5.49% of controls.

Table (3) contains the results of the most important parameters of the adult performance. The adult emergence was detrimentally inhibited by Spinetoram (10.00, 50.00, 71.40 and 88.90% of adult emergence, at 100, 50, 25, and 12.5ppm, respectively, *vs*. 90.00% of control adults). Also, Pyriproxyfen prohibited the adult emergence at all concentrations and the highest reduction was determined as 50.00% at 4000ppm. On the other hand, Neemazal reduced the adult emergence at all concentrations, except at the lower two levels.

All insecticidal compounds caused some adult deformities. Fifteen percent of adult deformation had been caused by pyriproxyfen (at 4000, 2000, and 1000ppm) and neemazal (at 40000 ppm). Also, spinetoram exhibited adult deformation, to some extent, at 50 and 12.5 ppm. However, the deteriorated adult morphogenesis was observed in atrophied wings and legs as well as expanded wings (Fig. 1).

The tested compounds exhibited some shortening effects on adult longevity. Adult longevity was remarkably shortened to 41.62±3.56 and 44.67±5.86 days, at 2000, 1000 ppm, respectively, of Pyriproxyfen; to 55.34±7.81 days, at 20000 ppm of neemazal; 54.79±6.98 days, at 25 ppm of spinetoram, compared to 62.45±5.67 days of control adults.

Table 1. Larval growth and development of *Rh. ferrugineus* after treatment of early 5th instar larvae with certain insecticidal compounds.

Compound	Conc. (ppm)	Duration (mean days±SD)	Develop. Rate (%)	Weight (mean mg±SD)	Growth inhibition (%)
Pyriproxyfen	4000	7.2 ± 2.97 c	13.89	1.98 ± 0.63 d	49.10
	2000	10.8 ± 1.03 a	9.26	2.00 ± 0.49 d	48.58
	1000	11.0 ± 1.16 a	9.09	2.41 ± 0.38 d	38.04
	500	11.9 ± 0.73 a	8.40	3.44 ± 0.42 a	11.58
	250	11.3 ± 0.94 a	8.85	3.54 ± 0.47 a	8.99
Neemazal	40000	9.9 ± 1.37 a	10.10	2.53 ± 0.49 c	34.96
	20000	10.22 ± 1.71 a	9.78	2.91 ± .051 b	25.19
	10000	11.3 ± 1.33 a	8.85	2.77 ± 0.37 c	28.79
	5000	11.5 ± 1.34 a	8.70	2.97 ± 0.46 c	23.65
	2500	11.2 ± 0.66 a	8.93	3.53 ± 0.42 a	9.25
Spinetoram	100	5*	25.77	2.36	39.33
	50	7.77 ± 2.2 c	17.33	2.5 ± 0.75 c	35.73
	25	8.41 ± 2.0 c	12.87	2.89 ± 0.81 b	25.70
	12.5	9.12 ± 2.4 b	10.96	2.86 ± 0.49 c	26.47
Control		11.7 ± 1.6	8.54	3.89 ± 0.74	----

Conc.: Concentration; mean±SD followed with the letter (a): is not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). *: one insect.

Table 2. Pupation and developmental duration of pupae of *Rh. ferrugineus* after treatment of early 5th instar larvae with certain insecticidal compounds.

Compound	Conc. (ppm)	Pupation (%)	Pupal duration (mean days±SD)	Develop. rate
Pyriproxyfen	4000	40	10.09 ± 6.26 c	9.91
	2000	60	13.0 ± 3.74 c	7.69
	1000	70	14.37 ± 2.50 c	6.96
	500	100	14.61 ± 2.41 c	6.84
	250	90	15.4 ± 1.64 c	6.49
Neemazal	40000	50	11.0 ± 6.04 c	9.09
	20000	50	11.5 ± 4.32 c	8.7
	10000	70	16.0 ± 4.32 a	6.25
	5000	90	18.77 ± 3.52 a	5.33
	2500	100	18.0 ± 1.48 a	5.56
Spinetoram	100	10	---	---
	50	40	8.46 ± 1.09 d	11.82
	25	70	12.14 ± 3.01 d	8.24
	12.5	90	12.33 ± 3.66 d	8.11
Control		100	18.21 ± 1.62	5.49

Conc., a, c, d: see footnote of Table 1.

Table 3. Adult performance of *Rh. ferrugineus* after treatment of early 5th instar larvae with certain insecticidal compounds.

Compound	Conc. (ppm)	Emergence %	Deformation %	adult longevity (mean days±SD)
Pyriproxyfen	4000	50.00	50.00	---
	2000	66.66	50.00	41.62 ± 3.56 d
	1000	57.71	50.00	44.67 ± 5.86 d
	500	40.00	14.28	58.44 ± 4.33 a
	250	88.88	---	64.51 ± 5.58 a
Neemazal	40000	40.00	50.00	---
	20000	80.00	25.00	55.34 ± 7.81 b
	10000	71.42	40.00	62.56 ± 5.66 a
	5000	90.00	28.50	61.78 ± 5.87 a
	2500	90.00	11.11	60. 56 ± 5.89 a
Spinetoram	100	10.00	---	---
	50	50.00	25.00	55.62*
	25	71.40	---	54.79 ± 6.98 b
	12.5	88.90	12.50	59.52 ± 5.66 a
Control		90.00	---	62.45 ± 5.67

Conc., a, c, d: see footnote of Table 1.

Fig. 1. The most important malformations of *Rh. ferrugineus* adults after treatment the early 5th instar larvae with certain insecticidal compounds. a): normal adult, b): pupal-adult intermediate, c): atrophied wings and legs, d): expanded wings.

DISCUSSION

1. Reduced Body Weight And Growth:

The larval body weight of the red palm weevil *Rhynchophorus ferrugineus*, in the current investigation, was significantly reduced after treatment with all insecticidal compounds (pyriproxyfen, neemazal and spinetoram). Similar results had been reported for prepupae and pupae of the same weevil by the plants products, Azadirachtin, jojoba oil, and the insect growth regulators (IGRs), Lufenuron and Diofenolan (Tanani, 2001). The present result was in accordance to some reducing effects different extracts and compounds on the body weight in different insects, such as the Egyptian cotton leafworm *Spodoptera littoralis* after treatment with Neemazal (Ghoneim *et al.,* 2000), the false stable fly Muscina stabulans after treatment with Margosan-O (Al-Dali *et al*., 2003) and the house fly *Musca domestica* after treatment with Margosan-O (Amer *et al*., 2004), and the desert locust *Schistocerca gregaria* after treatment with neemazal (Hamadah, 2009). Reduction of the body weight, or weight gain, or the inhibition of growth, in general, by some IGRs, had been reported for several insect species, such as *M. domestica* (Bakr *et al.,* 1991); the African armyworm *Spodoptera exempta*, the beet armyworm *Spodoptera exigua*, the cabbage moth *Mamestra brassicae* and the greater wax moth *Galleria mellonella* (Smagghe and Degheele, 1994), *S. littoralis* (Ghoneim *et al.,* 1998), and the house mosquito *Culex pipiens* (Djeghader *et al.,* 2013, Djeghader *et al.,* 2014) by Novaluron, *C. pipiens* by Kinoprene (Hamaidia and Soltani, 2014) and the black cutworm *Agrotis ipsilon* by Methoprene and Flufenoxuron (Khatter, 2014), as well as, *P. unionalis* by Novaluron (Ghoneim *et al.,* 2017). However, such suppressing action of the tested insecticidal compounds, in the present study, might be due to an ecdysonergic activity, as suggested by Smagghe and Degheele (1994) after using tebufenozide (an ecdysone agonist).

2. Affected Developmental Durations:

In the current study on *Rh. ferrugineus*, duration of the treated larvae was considerably shortened by all concentrations of Spinetoram but at the highest concentration of pyriproxyfen. To some extent, this result agreed with some reported results for other plant extracts and IGRs, such as *M. domestica* by Margosan-O (Ghoneim *et al.,* 2004), *S. gregaria* by Neemazal (Hamadah, 2009), *S. littoralis* (Ghoneim *et al.,* 2015) and olive leaf moth *Palpita unionalis* (Ghoneim *et al.,* 2017) by novaluron, and *M. stabulans* by pyriproxyfen (Hamadah, 2018). On the other hand, the pupal duration of *Rh. ferrugineus* was adversely affected by the tested insecticidal compounds in the present investigation. However, similar results on the same weevil had been reported for different compounds, such as neem and Flufenoxuron (El-Bokl *et al.,* 2010), lufenuron and diofenolan (Tanani, 2001), as well as the affected pupal duration of *M. stabulans* by pyriproxyfen (Hamadah, 2018). In contrast to our result, some authors reported prolongations in the pupal period in different insects by some IGRs, such as the vinegar fly *Drosophila melanogaster* by Pyriproxyfen (Bensebaa *et al.,* 2015), the mosquitoes *Culex quinquefasciatus* and *Aedes albopictus* after larval treatments with pyriproxyfen or methoprene (Khan *et al.,* 2016) and the pink bollworm *Pectinophora gossypiella* after treatment of newly hatched or full-grown larvae with Novaluron (Hassan *et al.,* 2017). To understand the shortening or prolongation of the developmental periods, it may be ascribed to the effects of IGRs on the release of ecdysteroids indirectly, by interfering with the neuroendocrine sites responsible for the release of tropic hormones (especially the prothoracicotropic hormone) (Schluter *et al*., 1985; Subrahmanyam *et al*., 1989).

3. Inhibited Pupation and Adult Emergence:

In the present study on *Rh. ferrugineus*, the tested compounds significantly inhibited both pupation and adult emergence. This result was, to a great extent, corroborated with those reported results for various compounds against different insects, since the adult emergence of *D. melanogaster* was inhibited by pyriproxyfen (Bensebaa *et al.,* 2015), both pupation and adult emergence percentages were reduced in the stable fly *Stomoxys calcitrans* by pyriproxyfen (Liu *et al.,* 2012). On the other hand, Tanani (2001) recorded no effect on pupation% of RPW by Azadirachtin and jojoba oil. Also, pyriproxyfen suppressed the adult emergence in Asian citrus psyllid (Boina *et al.,* 2009), the mosquito *Aedes aegypti* (Sihuincha *et al.,* 2005; Harburger *et al.,* 2011), the mosquitos *Anopheles subpictus*, *Anopheles culicifacies* (Yapabandara and Curtis, 2004), the mosquito

Anopheles gambiae (Mbare *et al*., 2013), dark-winged fungus gnat *Bradysia coprophila* (Ludwig and Oetting, 2001), the fly *Lycoriella ingenue* (Erler *et al.*, 2011) and *Cx. pipiens* (Al-Sarar *et al.*, 2011). In addition, Singh and Kumar (2015) reported inhibition of adult emergence in the flesh fly *Sarcophaga ruficornis* by pyriproxyfen. Pupal treatment of the parasitic wasp *Encarsia formosa* with pyriproxyfen resulted in blockage of adult emergence (Wang and Liu, 2016). Adult emergence of *P. unionalis* was blocked by methoxyfenozide (Hamadah *et al*., 2017). Moreover, adult emergence was completely blocked in the rice meal moth *Corcyra cephalonica* after treatment of 4th instar larvae with fenoxycarb (Singh and Tiwari, 2016). In general, inhibition of pupation and blockage of adult emergence might result by the reduction of eclosion hormone production or release, since this hormone is responsible for some prerequisite process of the completion of moulting in insects (Ghoneim *et al*., 1998).

4. Influenced Adult Longevity:

The adult longevity of *Rh. ferrugineus* was shortened in the current study, irrespective of the tested compounds. Our result was in corroboration with those reported shortening effects on the adult longevity in different insects by various botanicals and IGRs, such as *S. littoralis* by the methanolic extract of *M. azedarach* (Schmidt *et al.,* 1997); *M. stabulans* by Margosan-O (Ghoneim and Al-Dali, 2002); *M. domestica* by lufenuron or diofenolan (Ghoneim *et al.*, 2004) or Margosan-O (Amer *et al.*, 2004); *A. ipsilon* by pyriproxyfen, IKI-7899 or flufenoxuron (Shaurub *et al.*, 1999; El-Shiekh, 2002) and *M. stabulans* by Pyriproxyfen (Hamadah, 2018). On the contrary, El-Sayed (1982) observed no effect of a neem seed suspension on the adult longevity of *S. littoralis*; and Hassan (2002) recorded only a weak effect of the neem *Melia azedarach* extracts on the adult longevity of the same lepidopteran. The shortened adult longevity can be explained by the accumulation of toxic xenobiotics in the insect body which disturbs a complicated balance of factors such as absorption, excretion and detoxification (Abdel-Al, 1996).

5. Deteriorated Adult Morphogenesis:

In the present study, disrupted morphogenesis was observed only in adults of *Rh. ferrugineus*, since some deformed adults had been recorded, irrespective of the tested compound. To some extent, the present finding was in partial resemblance with the reported results for various insect pests after using different compounds, such as the tsetse fly *Glossina morsitans* (Jordan *et al.*, 1979), *C. pipiens* (Bakr *et al.*, 1989), and *M. stabulans* (Basiouny, 2000) after treatment with diflubenzuron; the confused flour beetle *Tribolium confusum* (El-Sayed *et al.*, 1984), *M. domestica* (Miller and Schmidtman, 1985), *M. stabulans* (Ghoneim *et al*., 1992) after treatment with Bay SIR-8514; *P. argyrostoma* (Ghoneim and Ismail, 1995) and *M. stabulans* (Basiouny, 2000) after treatment with IKI-7899 as well as *Rh. ferrugineus* after treatment with Azadirachtin, jojoba oil, lufenuron or diofenolan (Tanani, 2001); and *M. domestica* after treatment with lufenuron or diofenolan (Ghoneim *et al*., 2004) or with Jojoba (Hamadah, 2003). Recently, Hamadah (2018) recorded some adult deformities of *M. stabulans* after treatment with pyriproxyfen. The adult deformation might be owing to the absence of necessary titer of ecdysteroids, as a response to the action of botanicals and/or IGRs (Jagannadh and Nair, 1992).

In **conclusion**, the recorded disturbing effects of the tested insecticide alternatives (pyriproxyfen, neemazal, and spinetoram), in the present study, on growth, development, and adult performance of the red palm weevil, *Rhynchophorus ferrugineus* revealed that the bioinsecticide spinetoram was the most potent for controlling this dangerous weevil.

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