THE DISRUPTIVE EFFECTS OF AZADIRACHTIN AND JOJOBA ON DEVELOPMENT AND MORPHOGENESIS OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* (CURCULIONIDAE: COLEOPTERA).

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ABSTRACT

Nine dose-levels of Jojoba oil (Joj) or six ones of Azadirachtin (Azt) were topically applied onto the prepupae of *Rh. ferrugineous*. The dose range of Joj was 20.000-0.001 µg/insect and of Azt was 0.500-0.001 µg/insect. The lethal action of Joj had not appeared clearly in the early-aged pupae, but in the late-aged ones. With regard to Azt, the lethal action increased by the age of pupae. By each extract, increased water loss may act as one of the importance causes of pupal death. Prepupal maximal body weights decreased, irrespective of the extract, and were reflected on small growth index of prepupae and pupae. On the other hand, the two higher dose levels of each extract shortened the pupal durations and hence their developmental rates increased. Joj had no effect on the pupation rate in spite of its influence on the pupation program because various malformations were observed increasingly by increasing dose-level. Similar results, almostly, had been obtained by Azt. Adult emergence was blocked in different percents by Joj and was reversely correlated with the dose value of Azt. Also, various adult deformations were observed by Joj and by Azt.

Additional Index Words: *Rhynchophorus ferrugineus,* Azadirachtin, Jojoba oil, lethal effects, growth, development, morphogenesis, pupation, adult emergence, deformation.

INTRODUCTION

Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by neem seed extracts and azadirachtin (*cf*. Schmutterer and Ascher, 1984). Neem seed and leaf extracts, as well as, the purified compound azadirachtin, are powerful insect antifeedant and repellents (Butterworth and Morgan, 1968; Zanno *et al.*, 1975). They may also disrupt growth, inhibit moulting (Koul, 1984; Garcia and Rembold, 1984;

Dorn *et al.*, 1986) and oogenesis (Steets, 1976; Rembold and Sieber, 1981).

Red palm weevil *Rhynchophorus ferrugineus* oliv. is a devastating insect pest of date palm in the Arabian Gulf region. It was reported on date palm, for the first time, from the United Arab Emirates in the mid-1980s, then its reported distributed expanded its range westwards until it reached Egypt in 1992 (Saleh, 1992; Cox, 1993). The objective of the present work was mainly to determine the efficacy, in the laboratory, of azadirachtin and Jojoba oil for disrupting growth and development of *Rh. ferrugineus*.

MATERIALS AND METHODS

1) The Experimental Insect:

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the plam in its prime of life The hatched grubs burrow into the trunk and feed on tissue of the stem. The pupation and adult emergence within the same stem allow successive generations. In the present study, prepupae were collected for every experiment from large cavities of infested date trees especialized for this purpose; i.e. received no chemicals such as insecticides. No laboratory culture of *Rh. ferrugineus* could be established because of the legislative regulation preventing the transfer of it outside the infestation region (Ismailia and Sharqia Governorates, during the experimental period of the practical work of the present study, 2000).

2) Administration of Plant extracts:

Azadirachtin (Azt) and Jojoba oil (Joj) were bioassayed against *Rh. ferrugineus*. The purified compound of Azt (a tetranortriterpenoid) was purchased from "Sigma Chemical Company". Joy (oily extract of jojoba bean *Simmondsia chinensis*) was favourly obtained from Lab. of Pesticides, Agric. Res. Center, Doqqi, Giza. Nine dose levels were prepared from Joj: 20.00, 10.00, 1.000, 0.50, 0.10, 0.050, 0.01, 0.005, and 0.001 μg/insect; and six dose levels from Azad: 0.50, 0.10, 0.05, 0.01, 0.005 and 0.001 μg/insect. Eight replicates for each experiment were topically treated with 1 μl aceton containing the plant extract. Twelve replicates of controls were topically treated with 1 μl aceton only. All treated and control insects were kept at 27 ± 2 °C and 70 ± 5% RH.

3) Criteria and calculations:

Pupal mortalities were observed during the pupal period, especially of the early-, mid- and late-aged pupae. Also, adult mortalities were calculated basing on the successfully emerged individuals All mortalities were counted and expressed in %s.

In addition, pupation and adult emergence percentages were calculated as suggested by Jimenez-Peydro *et al.* (1995). Morphogenic aberrations were recorded and expressed in %s. For calculating the developmental duration, Dumpster's equation (1957) was used, and for calculating the developmental rate, Richard's equation (1957) was used. Growth index was determined according to Saxena and Sumittra (1985). Water loss % was calculated basing on the data of initial and final weights of pupae.

4) Statistical analysis of data:

Data obtained were analysed by the Student's *t*-distribution and refined by Bessel correction (Moroney, 1956).

RESULTS AND DISCUSSION

1) Lethal effects:

As shown in Table (1), the lethal action of Joj had not appear clearly in the early-aged pupae, but in the late-aged pupae and, to some extent, in the mid-aged pupae. According to the data of the same table, a parallel course was obviously seen, almostly, between the mortalities and the dose levels. Good evidence was obtained by the results of total mortalities, where it was 87.5% at the highest dose of the extract and 12.5% at the dose level 0.005 μg/insect; while at the lowest dose level, the extract did not exhibit a lethal action.

Water loss may be the principal factor for causing death of pupae, where Table (1) indicated that the increase in water loss (%) paralleled to the ascending of mortality % and increasing dose level.

Referring to Table (1), adult mortalities ranged from 12.5 to 37.3%. However, no certain trend was appreciated for this effect. It is noteworthy to mention that the lowest dose (which did not cause pupal mortalities) caused adult mortality, but in the most little %.

As similar to be found in Table (1), the results of Table (2) prevailed a latent lethal action of Azt, because the mortality of newly formed pupae was found in the most little % allover the pupal stage. Generally, the calculated mortality % s for mid- and late-aged pupae were consecutively correlated with the dose level. Also, the same trend was seen to the total pupal mortality (25.0% at 0.005 μg/insect and 50.0% at 0.5 μg/insect).

Regarding to the results arranged in Table (1), water loss could be largely considered as one of the important reasons of pupal death, since it increased by ascending dose value of Azt and increasing mortality %.

The counted mortalities among adult weevils ensured the parallel interrelation of potency of Azt to the dose value (ranging from 37.5%, at the highest dose, to 12.5%, at the lowest one).

2) Effects on growth and development:

Concerning with prepupae treated topically with Joj, maximum weights (Table 3) depleted significantly, especially at the higher five dose-levels. The most reducing effect of this extract on maximum body weights was estimated by using the highest dose level (2.57±0.45 mg vs 4.53±1.45 mg of controls), while the lower four dose-levels (0.05, 0.01, 0.005 & 0.001 μg/insect) slightly suppressed the maximum body weights.

In addition, this was reflected on the growth index, where its smallest value was calculated after applying the highest dose level and its largest value was recorded after applying the lowest dose-level until it approximately reached the control index (6.50 and 7.20 at the lower two dose levels vs 7.34 of control congeners).

On the other hand, topical application with Joj induced the developmental rate and shortened the durations of prepupae. This shortening was statistically significant at the higher two dose-levels (5.40±1.30 and 5.70±1.00 days after using 20.0 and 10.0 μg/insect, respectively, vs 7.50, 1.92 days of controls). Data of the same table evidently indicated the same trend of effect on pupae. This reducing effect was remarkably detected after using the higher two doses (4.50±0.20 days at 20.0 or 10.0 μg/insect vs 6.80±1.85 days of controls).

The body weights of newly formed pupae and late-aged pupae were recorded. A great reduction of these weights were found after using the majority of dose levels of Joj (For details, see the same table).

As obviously demonstrated in Table (4), Azt prohibited the prepupae to be only with reduced body weights. This reduction in prepupal weights was statistically significant at the higher three levels (2.14±0.77, 2.95±0.57, 3.01±0.49 mg at 0.50, 1.00 and 0.05 μg/insect, respectively, vs 4.53±1.45 mg of controls. Furthermore, the growth index decreased but in no certain trend.

Also, the treatment with this extract shortened the duration of prepupae and enhanced their developmental rates reaching 19.49 (vs 13.33 of control congeners) at the highest dose (0.5 μg/insect). At the same dose level, the prepupal duration significantly shortened (5.13±0.55 vs 7.50±0.92 days of control congeners).

Considering the resulted pupae from these treatments, the same effect was noticed for duration pronouncedly at the higher two doses (4.0±0.10, 5.2±0.65 days, at 0.50 and 0.10 μg/insect, respectively, vs 6.8±1.85 days of controls). An acceleration in their development was recorded by increasing dose level (for more details, see Table 4). The data of the same table reflected a reduction in the body weights of newly formed and late-aged pupae. This reduction was consecutively correlated with the dose value.

3) Metamorphic and morphogenic effects:

It is clearly concluded from the data of Table (5) that Joj has no effect on the pupation rate, in spite of its influence on the pupation program because some different malformations were observed increasingly by ascending dose-level.

On the contrary, adult emergence was blocked in different percents, where the emergence decreased by increasing dose-level (75.0% emergence blockage was calculated at the highest dose 20.0 μg/insect, but no blockage was found at the lowest dose, 0.001 μg/insect). In regard to the effect of this plant extract on the adult morphogenesis, such effect decreases greatly by the decreasing dose level, until no deformed weevil were seen at the lower four doses.

To clarify the metamorphic and morphogenic effects of Azt, Table (6) showed no effect on the pupation rate at any dose-level, while the pupal deformation percents increased by increasing the dose level (with the exception of dose 0.05 μg/insect). The adult emergence was reversely correlated with the dose-level, and adult deformation increased in this direction.

Different categories of deformations were observed among pupae as a response to the morphogenic activity of Joj. These deformed forms varied between dorso-ventrally-compressed body collapsed external appendages and pale coloured pupae. These deformed pupae failed to metamorphose into adult weevils (Fig. 1). Azt treatments caused almostly similar deformations among pupae in addition to blackish body with slightly charred wing pads (Fig. 3). Dealing with the adult weevils, Joj treatments caused various degrees of deformation, such as: permanently expanded membranous wings, remained pupal skin, failure of wing formation and remained wing pads of pupae (Fig. 2). Azt treatment resulted in such deformations in addition to other features such as collapsed antennae, mouthparts and legs, formation of pitted elytra or appearance of some protrusions on mouthparts (Fig. 4).

All parts of the neem tree (*Azadirachta indica* A. Juss) are insecticidal although the seeds possess the largest concentrations of **azadirachtin**, (Azt) a steroid-like tetranortriterpenoid. Neem seed extracts have been tested against a large number of insects (e.g.: Ladd *et al.*, 1978; Larew *et al.*, 1985; Saxena and Khan, 1985; Prabhaker *et al.*, 1986; Jilani *et al.*, 1988; Larew, 1988; Zehnder and Warthen, 1988; Stark *et al.*, 1990; Lowery *et al.*, 1993; Naumann *et al.*, 1994; AliNiazee *et al.*, 1997; Ghoneim *et al.*, 1998; Ghoneim *et al.*, 2000). However, Schmutterer and Singh (1995), as for example, listed 413 insect pest species as sensitive to neem extracts. These extracts have wide ranging biological activities against insects (Isman *et al.*, 1990; Schmutterer, 1990) including feeding and oviposition deterrence (Rice *et al.*, 1985), impairing the development (Sieber and Rembold, 1983; Barnby and Klocke, 1990), as well as inhibiting growth, mimicing the juvenile hormone (Parakash and Rao, 1997). It is noteworthy to mention that, the structural analysis of Azt indicates that it could act as a genotoxic carcinogen (Rozencrantz and Klopman, 1995) and a study of Cohen *et al.* (1996) suggests that the limonoids in the neem extracts could be cytotoxic (Guerrini, 2000).

In the present study, Azt and Jojoba oil (Joj) have been used against the red palm weevil, *Rhynchophorus ferrugineus*, comparatively, to recognize and clarify some possible effects on different biological criteria and physiological phenomena. The obtained results can be discussed and comprehensively interpreted as arranged herein.

1) Lethality of Joj and Azt on *Rh. ferrugineus.*

Azt is a famous neem seed kernel extract, so it does not need to be structurally shown now. On the other hand, Joj is a vegetable oil obtained from the Jojoba bean. After the topical application of Joj (in a dose range of: 20.0, 10.0, 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 μg/insect) onto the prepupae of the present insect species, its lethal action did not exhibit clearly in the early-aged pupae, but in the late-aged ones. A parallel course, was obviously detected, to a large extent, between the mortalities and the dose-levels. In respect to Azt treatments (with one of these dose -levels: 0.5, 0.1, 0.05, 0.01, 0.005 or 0.001 µg/insect), a latent lethal action was recorded since the mortality of newly formed pupae was found in the least percent and then almostly increased by the age. After Joj treatments, adult mortalities ranged from 12.5 to 37.3% with, however, no certain trend of the effect was detected while Azt exhibited a mortal potency on the adults in a dose-dependent manner.

Toxicity of neem extracts, such as Azt or different neem preparations, had been reported by many authors against various insect species. El-Sayed (1983) observed complete mortality at 0.2-0.5% of a neem extract in the majority of larval instars of *Spodoptera littoralis*. Osman (1993) observed some different mortalities *of Pieris brassicae* after treatment of 1-day old 5th instar larvae with 5.0 and 2.5% Azt On the otherhand, Jagannadh and Nair (1992) reported an acute toxic effect of Azt applied against 5th larvae of *Spodoptera mauritia*.

Margosan-O (a neem preparation with 0.3% Azt content) strongly affected the European corn borer, *Ostrinia nubilalis*, by feeding larvae on 0.25%-treated corn seedlings (Meisner *et al.*, 1981) and caused mortalities ranging from zero to 70 or 94% in the spiny boll worm *Earias insulana* (Meisner and Nemny, 1992). The same neem preparation caused complete larval mortality of the European leaf roller *Archips rosanus*, within 48 h of the treatment (AliNiazee *et al.*, 1997).

Using another neem preparation, NeemAzal (with 20% of Azt content), Ghoneim *et al.* (2000) recorded various mortality percents among larvae, pupae and adults of the Egyptian cotton leafworm *S. littoralis*. The latter neem preparation exhibited various degrees of lethality on the house fly *Musca domestica* which decreased if the concentration decreased below 2000 ppm in the artificial diet of larvae (Mohamed *et al.*, 2000). However, so many results had been reported by several authors for Azt or Azt preparations against different species (e.g.: Meisner *et al.*, 1981; Dorn *et al.*, 1986; Osman, 1993; Osman and Bradly, 1993; Linton *et al.*, 1997).

Death of treated insects may be due to the inability of the moulting bodies to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis, or to a metamorphosis inhibiting effect of the plant extract, which is possibly based on the disturbance of the hormonal regulation (Al-Sharook *et al.* (1991).

On the other hand, prevention of ecdysis, and subsequently death, could be attributed to the reduction in ecdysteroid peak or interference with the release of eclosion hormone (Sieber and Rembold, 1983; Dorn *et al.*, 1986). For Joj only, El-Defrawi *et al.* (1965) suggested a possible action of the vegetable oils that penetrate the integument of the insect to affect presumably the nervous or respiratory system to exert the lethal effect.

In addition, the present work may provide another factor and possibility to explain the lethal action of Azt or Joj, since water loss of pupae increased parallely to the increasing dose-level and increasing mortality %. Such adverse process resulted in a degree of desiccation and subsequently impaired some vital physiological events leading to death of pupae, in particular.

2) Influence of Joj and Azt on growth and development of *Rh. ferrugineus*:

The growth and development regulatory effects of Azt on insects are well known. Treatment of insects, or their food with Azt causes growth inhibition and increasing doses of Azt in larval instars result in different forms of effect, one of them is extending the life period of larvae which remain as ‘over-aged” larvae of a wide variety of insects, such as: Lepidoptera (Arnason *et al.*, 1985; Schluter *et al.*, 1985; Barnby and Klocke, 1987; Koul *et al.*, 1987), Diptera (Zebitz, 1987; Miller and Chamberlain, 1989), Orthoptera (Sieber and Rembold, 1983; Mordue (Luntz) *et al.*, 1985; Rao and Subrahmanyam, 1986; Ascher *et al.*, 1989; Champagne *et al.*, 1989; Ghoneim and Ismail, 1995 a,b), Hemiptera (Redfern *et al.*, 1981; Koul, 1984; Garcia and Rembold, 1984; Dorn *et al.*, 1986), Coleoptera (Ladd *et al.*, 1984; Schluter, 1985) and Hymenoptera (Rembold *et al.*, 1982, 1984).

In the present study, topical application of Joj onto the prepupae led to pronounced suppression in the maximal body weights especially at the higher five dose-levels. This was reflected on the growth because its smallest index was calculated by the highest dose-level and *vice versa*.. Also, Joj enhanced the development because the prepupal duration was significantly shortened especially at the higher two dose-levels. To a great extent, similar results on growth and development of prepupae were obtained by Azt

The present available data distinctly show a reducing effect of Joj and Azt, generally, on the pupal body weight and stimulating action of both extracts upon the development because the pupal duration was significantly shortened. Unfortunately, earlier instars of larvae had not undergone to Joj or Azt application in the present work and only the prepupae were the available larval shape for investigating the plant extracts. However, the obtained results for prepupae in the present study may be indicative for the effects which could possibly be exerted on larvae.

Referring to the results obtained by various authors for different insect species, as affected by Azt and other plant extracts, Jagannadh and Nair (1992) recorded a prolongation of 5^{th} or 6^{th} larval instars of *S. mauritia* after Azt treatments. Amr *et al.* (1995) observed a significant prolongation in the larval duration of *S. littoralis* by 3.0% concentration of chloroform or ethanolic extract of *Nerium oleander*. Dissimilarly, Darvas *et al.* (1996) recorded a shortening in the period required for larval/puparial intermediate development of the sarcophagid *Neobellieria bullata* by extracts of *Ajuga reptans reptans*. Another dipterous insect (*Muscina stabulans*) was affected by ethanolic extracts of *N. oleander* in remarkably prolonged larval and pupal durations (El-Shazly *et al.*, 1996). Such effect was recorded for the same species, also, by Khalaf and Hussein (1997) after using the oils of *C. citratus* and *Rosmarinus officinalis*.

Pronouncedly longer larval duration in the hemipteran, *Spilostethus pandurus* was caused by Azt (El-Sherif, 1998), in the orthopteran *Euprepocnemis plorans* was caused by Margosan-O (Mohamed, 1998). Mohamed *et al.* (2000) observed remarkedly depleted maximal body weights in *M. domestica* larvae by feeding on the neem preparation (NeemAzal)-treated diet, irrespective of the concentration level or the starting instar. Also, they recorded conspeciously retarded larval development at 2000, 1000 and 500 ppm of NeemAzal. In addition, Ghoneim *et al.* (2000) observed tremendously depleted larval maximal weights and body weight gain of *S. littoralis* by the treatment of 2^{nd} or 4^{th} instar larvae with NeemAzal.

On the contrary, Osman (1993) reported no significant effect of a neem extract on the weight gain of *P. brassicae* larvae. Moreover, Azt causes considerable delay (i.e. prolongation of the developmental periods) or even complete inhibition of ecdysis (Sieber and Rembold, 1983; Gaaboub and Hayes, 1984; Dorn *et al.*, 1986; Pener and Shalom, 1987). Also, injection of 1 μg Azt into *Tenebrio molitor* pupae induced a delayed and reduced ecdysteroid peak, which inhibited the imaginal moult (Marco *et al.*, 1990).

Anyhow, the suppressing action of Azt or Joj, in the present study, as reflected in drastically reduced weights in both prepupae and pupae, as well as decreased growth index of prepupae (which can be considered as representative to the larvae in the present work) may be attributed to the increased energy expenditure in order to detoxify the extracts within the insect body (Schoonhoven and Meerman, 1978; Dowd *et al.*, 1983; Al-Sharook *et al.*, 1991).

On the other hand, growth inhibition in insects, by the action of Azt or other plant extracts are thought to result from a blocked release of morphogenic peptides, causing alterations in ecdysteroid and juvenoid titers (Sieber and Rembold, 1983; Barnby and Klocke, 1990; Linton *et al.*, 1997). Also, some possible direct effects of Azt and Joj on tissues and cells undergoing mitosis may have occurred (Nasiruddin and Mordue, 1994).

With regard to the hastening of development which have been evidently conceived by the shortening effect of Azt or Joj on prepupae and pupae of *Rh. ferrugineus,* in the present study, be explicated by a specific physiological elasticity in the insect body enabling it to overcome the adverse condition (penetrating extract) by shortening the time interval into a period during which the insect would be more tolerant. We have no more than this rationally conceivable interpretation of the hastened development during a shortened duration, right now.

3) Metamorphosis and morphogenesis of *Rh. ferrugineus* as affected by Joj and Azt

Moulting inhibition had been reported for neem and neem derivatives (Schmutterer, 1990). Azt exhibits several morphogenic effects in a number of insect species which can be due to delayed or suppressed ecdysteroid titers (Rembold and Sieber, 1981; Sieber and Rembold, 1983; Rembold, 1984; Schluter *et al.*, 1985; Mordue (Luntz) *et al.*, 1986; Zhang and Chiu, 1987; Smith and Mitchell, 1988; Jagannadh and Nair, 1992).

Neither Joj nor Azt affected the pupation rate of *Rh. ferrugineus* -in the present study- in spite of their influence on the pupal program because various pupal malformations were observed increasingly as the dose-level of these botanicals increased. Pupal deformities varied between dorso-ventrally compressed body and collapsed appendages and failed to metamorphose into adult weevils. Although the pupation rate had not significantly influenced, adult emergence was blocked in different percents, in the case of Joj, and reversely correlated with the dose-value, in the case of Azt Adult deformities varied between permanently expanded membranous wings, failure to form elytra, collapsed external appendages and appearance of pits on elytra.

The present results of unaffected pupation rate disagreed with the pupation inhibition recorded by many authors for different insect species due to Azt and various plant extracts (Jagannadh and Nair, 1992; Abou El-Ela *et al.*, 1995; El-Shazly *et al.*, 1996; Khalaf and Hussein, 1997; Youssef, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000). Inhibition of adult eclosion by both Joj and Azt seemed to be in accordance with several findings of many authors (El-Sayed, 1983; Al-Sharook *et al.*, 1991; Khalaf and Hussein, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000).

Whereas the present study reported no larval pupal or pupal-adult intermediates, various pupal and adult deformities had been observed as previously mentioned. To a great extent, similar results had been obtained in *Bombyx mori* by Azt (Koul *et al.*, 1987), in *Spodoptera litura* by Azt (Gujar and Mehrota, 1983), in *Aedes aegypti* by Azt (Naqvi, 1986), in *M. stabulans* by some plants extracts and oils (El-Shazly *et al.*, 1996; Khalaf and Hussein, 1997); in *M. domestica* by Azt (Wilps, 1989).

In spite of the great variety of pupal deformations in different insect species by different plant extracts, such deranged or halted program of pupation in the present study may be attributed to the absence of necessary titer of ecdysteroids needed for achieving the larval-pupal transformation normally (Jagannadh and Nair, 1992). The appearance of deformed pupae by the action of Azt or Joj may be, also, due to the alterations in ecdysteroid and juvenoid titers (Kauser and Koolman, 1984; Schluter *et al.*, 1985; Barnby and Klocke, 1990). Also, the suggestion of hormonal influence by Azt was explained by the production of malformed pupae (Smith and Mitchell, 1988).

Another conceivable suggestion is that Azt or Joj may indirectly affect the prepupal release of ecdysteroid, by interfering with the neuroendoerine sites of release of tropic hormones, especially the prothoracicotropic hormone. Such effect of Azt on the neurohormones was reported in a few species (Dorn *et al.*, 1986; Jagannadh and Nair, 1992).

Effect of Azt or Joj on the adult morphogenesis of *Rh. ferrugineus* in the present study was suggested by almostly similar finding in different insect species by various plant extracts (El-Sayed, 1983; Schmutterer, 1989; Al-Sharook *et al.*, 1991; Khalaf and Hussein, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000). The impaired pupal-adult transformation resulting in adult deformities may suggest a persistent metamorphic and morphogenic actions of Azt (or Joj) vis its effect on the hormonal events (Schluter *et al.*, 1985; Schmutterer, 1990; Ali Niazee *et al.*, 1997; Ghoneim *et al.*, 2000).

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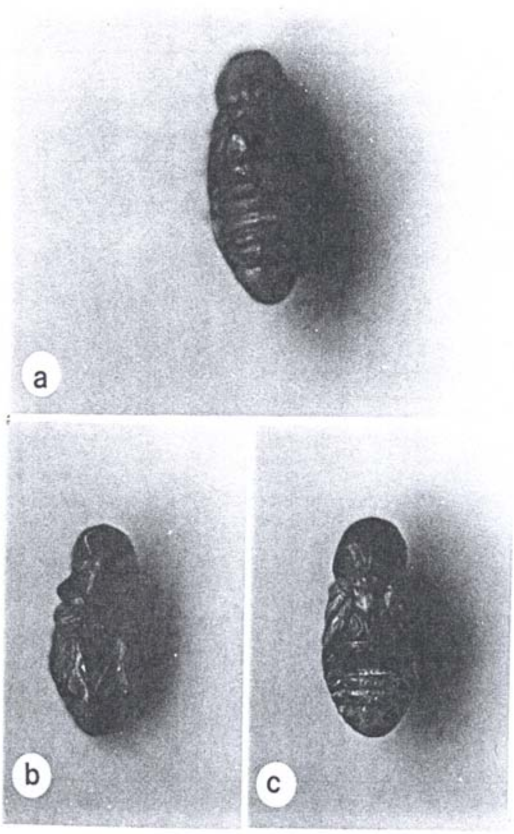

Fig. (1): Topical application of 20.0, 10.0, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 μg/insect of Jojoba onto prepupae of Rh. ferrugineus resulted in different categories of deformed pupae as follows: a) Dorso-ventrally compressed pupae. b) Pupae had collapsed antennae, mouth parts and legs. c) Pale yellowish pupae. All pupae failed to metamorphose into adult weevils and died as pupae.

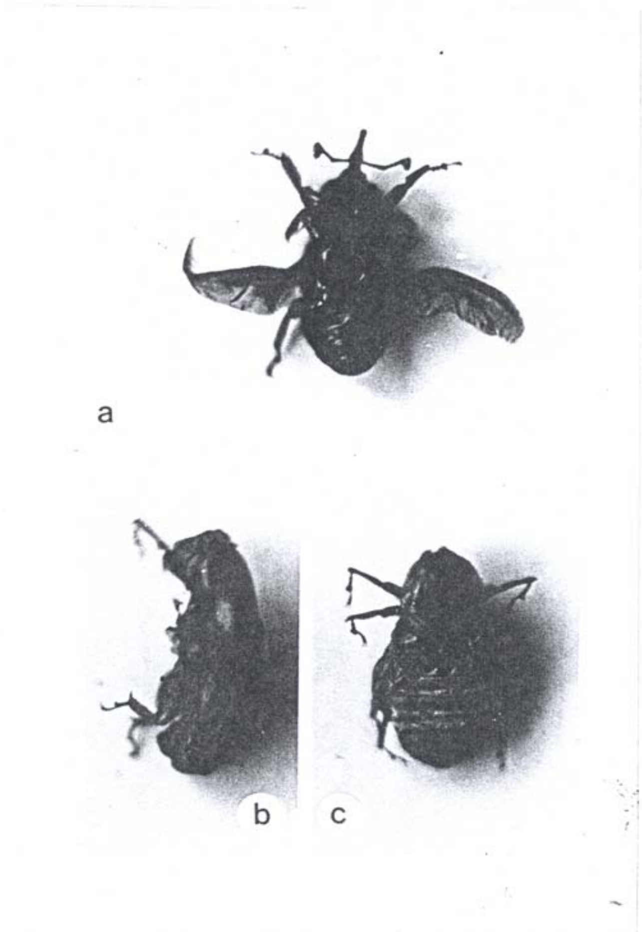

Fig. (2): Topical application of the doses 20.0, 10.0, 1.0, 0.5 and 0.1 µg/insect of Jojoba onto prepupae of Rh. ferrugineus resulted in the following categories of deformed adult weevils: a) Adult had permanently expanded membranous wings. b) Lateral side of adult which could not to emerge completely from the pupal skin. c) Adult faild to form wings and the wing pads of pupae still occurred. The upper photo shows some normally formed adult weevils.

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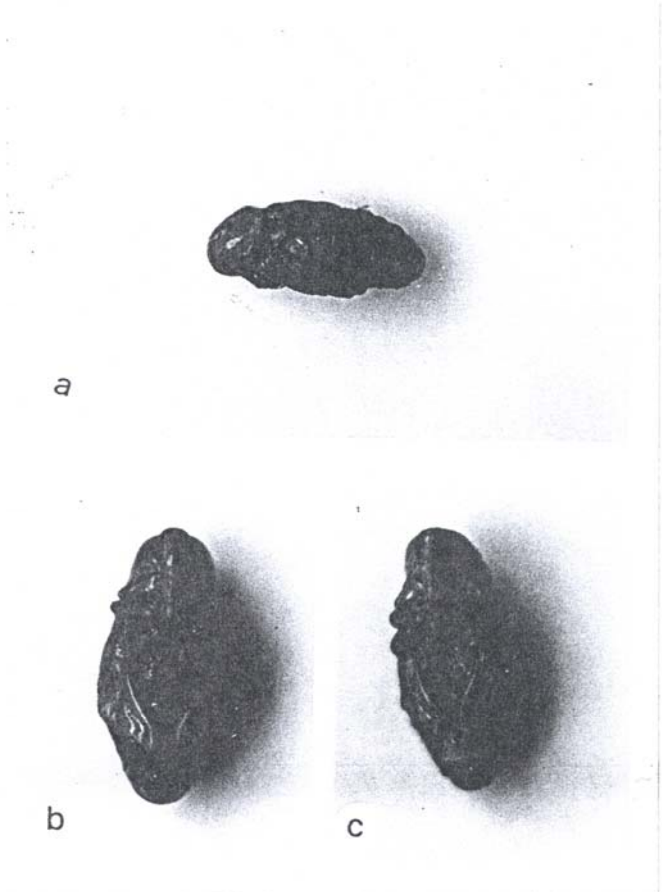

Fig. (3): Topical application of the doses 0.5, 0.1, 0.05, and 0.001 μg/insect Azadirachtin onto prepupae of Rh. ferrugineus resulted in the following categories of deformed pupae: a) Blackish pupae with slightly burned wing pards. b) and c) Pupae had collapsed antennae, mouth parts and legs. The right photo shows a normally formed pupa.

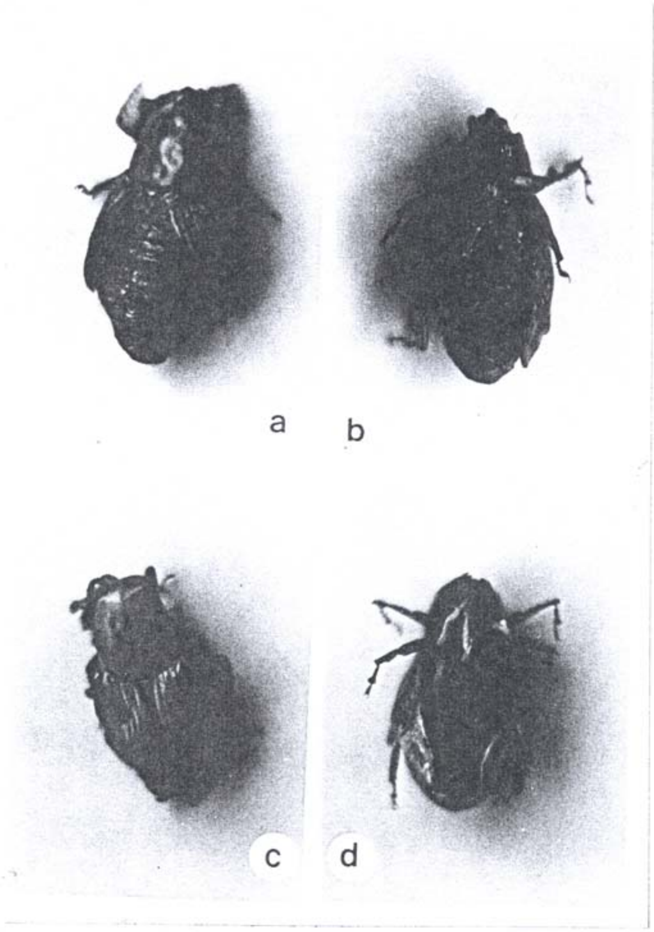

Fig. (4): Topical application of the previously mentioned dose levels of Azadirachtin onto prepupae of Rh. ferrugineus resulted in the following categories of deformed adult weevils: a) Adult failed to form wings and abdomen attached to the pupal exuvium. b) Ventral side of an adult to show collapsed mouth parts and curved antennae. c) Impaired left elytron with deep pit and right membranous wing exposed outside the elytron. d) Ventral side of an adult to show some protrusions of mouth parts and abnormal antennae.