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The Insecticidal and Antifeedant Activity of *Calotropis procera* Latex and Foliar Extracts Against the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (Coleopter: Curculionidae), is one of the most threatening and most important pests that infect date palms in Egypt. Many strategies have been employed to control this pest, among them, the employment of botanical extracts. The present study aimed to evaluate the insecticidal and antifeedant activity of *Calotropis procera* latex and foliar extracts against the 3rd and 5th instar larvae of *R. ferrugineus*. Results showed high insecticidal activity against both larval instars, however, the latex extract was more toxic than the leaves extract. Results also showed that both leaves and latex extracts have a high antifeedant index which was confirmed by the decreased mean larval weight. Finally, we can conclude that C. *procera* either used as leaves extract or latex extract represents an excellent alternative for conventional management procedures. More studies are considered on the effect of this plant on the different biological and biochemical parameters.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (Coleopter: Curculionidae), is one of the most threatening and most important pests that infect date palms (Faleiro, 2006; Rochat *et al.*, 1991). This is due to the insect's capability to develop and feed causing damage inside the palm stem tissues (El-Juhany, 2010; Giblin-Davis *et al.*, 1996; Gonzalez *et al.*, 2019; Milosavljević *et al.*, 2019). Managing this pest has always represented a challenge since the 1990s, as its control is based on insecticide spraying and injection, combined with the removal of infected palms (Gonzalez *et al.*, 2019). Red palm weevil has been evidenced as a major and serious pest in many locations in the Mediterranean; Egypt, Saudi Arabia, and the United Arab of Emirates (Azmi *et al.*, 2017; Ishak *et al.*, 2020; Orfali *et al.*, 2020). All red palm weevil' stages are found in the same host palm. The female lays about 200–300 eggs in an isolation cavity. Formed eggs crosshatch in 2–5 days, and larvae holes in the inner host palm nourishing at soft succulent tissues, abandoning total fibrous materials (Muhammad *et al.*, 2019). Infested date palms exhibit several symptoms depending on the stage of the attack, such as producing of brown fluid with a fermented odor that is mixed with palm tissue exerted by feeding larvae, tunneling of palm tissue, presence of adults and pupae at the base of fronds, dried of infested offshoots, pupae around the palm base, dried outer leaves, and topping off the trunk in cases of severe and extensive tissue

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damage (Al Dawsari, 2020). Several strategies have been employed to control and manage the red palm weevil and its damage, of these strategies is the use of insecticides. The extensive application of conventional insecticides led to the development of insect resistance to these chemical compounds besides the harm hazards of these compounds to the environment and ecosystem leading to the need to develop new control approaches safe for the environment and effective against this pest (Ferry *et al.,* 2004). Screening of plant extracts for deleterious effects on insects is one of the approaches in the search for novel biological insecticides (Carlini & Grossi-De-Sá, 2002). Insecticidal activity of many plants against several insects has been demonstrated (Carlini & Grossi-De-Sá, 2002; Khatter & Abuldahab, 2012). *Calotropis procera* is a soft-wooded, perennial shrub of the family Apocynaceae and subfamily Asclepiadaceae (the milkweed family). The plant is erect, tall, large, branched and perennial with milky latex throughout. It is an evergreen xerophytic plant, generally found in arid and semi-arid habitats (Al-Rowaily *et al.,* 2020; Kaur *et al.,* 2021). It is known by various common names such as apple of Sodom, calotrope, giant milkweed, Indian milkweed, wild cotton, rubber tree, ushar, etc., in different parts of the world. Its subspecies, *C. procera* subsp. *procera* and *C. procera* subsp. *hamiltonii*, vary from each other in fruit morphology (Dhileepan, 2014). It is a multipurpose plant, which provides a wide range of provisioning ecosystem services. It has been widely used in traditional medicinal systems in North Africa, Middle East Asia, South Asia, and South-East Asia (Al Sulaibi *et al.*, 2020). It has also been utilized for fiber, fuel, fodder, and timber purposes since antiquity (Batool et al., 2020). *C. procera* has many alkaloids, flavonoids, terpenes, terpenoids, enzymes, and other inorganic elements (Quazi *et al.,* 2013). These compounds also exhibit insecticidal properties apart from other bioactivities. Numerous reports are claiming the insecticidal properties of plant extract and essential oils of *C. procera* (de Morais *et al.*, 2007; Esmaeily *et al.*, 2012; Nighat *et al.*, 2013). The latex of *C. procera* has shown larvicidal efficacy against all three important vector species, viz. *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinqefasciatus*, are vectors of dengue, malaria, and *lymphatic filariasis*, respectively (Conti *et al.*, 2010; Shahi *et al.*, 2010). Latex of this plant has complex chemical constituents such as N-acetyl-β-d-glucosamines (Ramos *et al.*, 2006). Although the insecticidal activities of *C. procera* have been extensively studied on various pests, mosquitoes, larvae, stored grain pests, etc., several authors have studied various parts such as latex, flowers, and stem, roots, and barks for insecticidal properties (Kumar *et al.,* 2022). Given the vast usage and importance of the plant as insecticidal activities, it is highly required to bring the majority of the available data to one platform (Kumar *et al.*, 2022). In this context, the present study aimed to evaluate the insecticidal and antifeedant effect of *Calotropis procera* latex and leaves extract against the young larval instars of red palm weevil, *Rhynchophorus ferrugineus*, under laboratory conditions as a new strategy for managing and controlling this pest.

MATERIALS AND METHODS

Tested Insects:

Adults of red palm weevil, *R. ferrugineus*, were collected from infected date palms in Al Qasasin Center, Sharkia Governorate. They were then transferred to the laboratory in plastic containers (10×15×30 cm) that were perforated from the top and provided with sugarcane cuttings (6 cm long) (supplied from the local market) for feeding. Insects were reared at 27 ± 1 °C and 75±5% RH, and L:D cycle of 10:14 h, at the Wood and tree scavenger research department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Adults were fed until egg oviposition. Larvae were reared until the 3rd and 5th instar larvae for further investigation.

Plant Source:

Plant parts of *C. procera* were collected from areas surrounding Cairo Airport region and from Ain Sokhna Road, Red Sea Governorate. The collected plant parts were then identified at the department of botany, Faculty of Science, Cairo University. Latex was collected freshly by cutting the tip-stem junction and allowing the latex to flow into dark glass bottles containing ethanol 95%. Leaves, branches, and flowers were also collected and transferred to the laboratory for further analysis.

Extraction of Collected Plant Parts:

The crude extract either from leaves or latex was prepared according to the procedure described by Seiber *et al*. (1982) with few modifications. The leaves were washed with distilled water and left in the shadows to dry separately at room temperature for one week. The dried leaves were ground finely to powder using an electrical mill. The fine leaves powder (400 gm) was extracted by the addition of 2000 ml of 50% ethanol for 48 h. at 40-50° C and then filtered using a Buchner funnel. The obtained filtrate was then treated with lead acetate ($(CH_3COO)_2Pb$) to help precipitate all undesirable materials except for the cardenolide glycosides. The resulting solution was filtered, and the filtrate was treated with hydrogen sulphide gas (H_2S) to precipitate the excess of $(CH_3COO)_2Pb$ as lead sulphide (PbS), then filtered and the obtained filtrate was concentrated to about one-third of its volume at 40-50° C using a rotary evaporator.

The obtained filtrate was then soaked in 1L of ethanol 95% for five days, followed by sonication for 30 min. and centrifugation for 10 min. at 5000 r.p.m. the supernatant was then collected and evaporated to dryness using a rotary evaporator with high pressure at a temperature ranging from 45-50° C. The obtained crude ethanol extract was kept in the refrigerator until use (Al-Rajhy *et al.,* 2003; Al-Sarar *et al.*, 2012; Seiber *et al.,* 1982). 150 ml of collected latex was coagulated with 150 ml of 95% ethanol and then filtered on a Buchner funnel. The coagulate was then shaken with 150 ml of 50% ethanol and filtrated. The obtained mixture of the two filtrates was treated with an excess concentrated solution of $(CH_3COO)_2Pb$ and filtered on a Buchner funnel. The formed filtrate was then treated with H_2S gas to precipitate the excess of $(CH_3COO)_2Pb$ as lead sulphide (PbS), then filtered and the obtained filtrate was concentrated to about one-third of its volume at 40-50° C using a rotary evaporator. The stock solution of either leaf extract or latex extract was prepared by dissolving 5 gm of the extract in 10 ml of distilled water and in the addition of 5 ml of Tween 80. The formed suspension was then completed to 100 ml with distilled water. The stock solution was used for further dilution.

Larvicidal Activity of Leaves and Latex Extracts:

Newly molted 3rd and 5th instar larvae were selected for investigating the larvicidal activity of latex and leaves extracts. Sugarcane stems were cut into cuttings of 6 cm long and 2.5 cm in diameter, then a longitudinal chamber was made (3 cm long × 1.5 cm diameter). Four suspensions were prepared from the stock solution (described in the previous section), 1, 2, 4, and 6%, in 100 ml of distilled water. A solution of tween 80 in distilled water was prepared as the control. Five replicates were set for each concentration, each replicate had five larvae. The sugarcane cuttings were then soaked in the prepared suspensions for 5 min. and then left on clean filter papers and left to air dry. The treated sugarcane cuttings were offered to larvae for 48 h. then replaced with clean cuttings. The larval mortality was recorded on daily basis until the death of all larvae. The mortality percentage was corrected according to Abbott's formula (Abbott, 1925).

The Antifeedant Activity of Leaves and Latex Extracts:

To study the antifeedant effect of latex and leaves extracts against the 3rd and 5th instar larvae, the sugarcane cuttings were weighed before being offered to larvae and after larvae been fed on them both in treated and untreated. Sugarcane cuttings have emerged for

5 min. in 6% suspension prepared from the stock solution. The larvae were fed on the treated cuttings for 48h. then the cuttings were replaced by fresh clean ones. In addition, the mean larval weight before treatment and after treatment was also recorded daily. The percent of the antifeedant index was calculated using the following formula (Ben Jannet et al., 2000):

$$Antifeedant\ Index = \frac{C-T}{C+T} \times 100 \qquad \text{Eq. (1)}$$

where, C and T represent the amount of food eaten by the larvae on control and treated cuttings, respectively.

Statistical Analysis:

The LC_{50} values for latex and leaves extracts were determined according to Finney (1971) using "LdPLine®" software. [http://embakr.tripod.com/ldpline/ldpline.htm]. The obtained data were presented as mean ± S.E. One-way analysis of variance (ANOVA) and significant differences between treatments were determined by Tukey's multiple range tests (Snedecor & Cochran, 1980) ($P \leq 0.05$) using SPSS statistics 23.0 release 23 software.

RESULTS AND DISCUSSION

Larvicidal Activity of Leaves and Latex Extracts of *C. Procera* Against Red Palm Weevil Larvae:

The toxicity of leaves and latex extracts of *C. procera* were represented in the LC_{50} values and shown in the Figures (1-4). Results showed that the LC_{50} values for leaves extract against 3rd and 5th instar larvae were 1.406, and 3.696 gm/ml, respectively. Furthermore, the 2.132, and LC_{50} values for latex extract against 3rd instar larvae were 2.132 gm/ml and for 5th instar larvae were 2.648 gm/ml. The obtained results confirmed the toxic effect of *C. procera* against the larval stage of red palm weevil either treated with leaves or latex extract. Results also revealed that the larval mortality increased as the concentrations increased. *C. procera* is known to contain some chemical constituents which proved to exhibit insecticidal activity such as Calotropin and Calotoxin (Dubey *et al.*, 2007; Kumar *et al.*, 2022; Shahi *et al.*, 2010) and they are the main cause of larval mortality. The larvicidal activity of both latex and leaves extracts was previously recorded against mosquito larvae (Nighat *et al.*, 2013; Ramos *et al.*, 2006; Shahi *et al.*, 2010; Singh *et al.*, 2005) and red palm weevil (Orfali *et al.*, 2020).

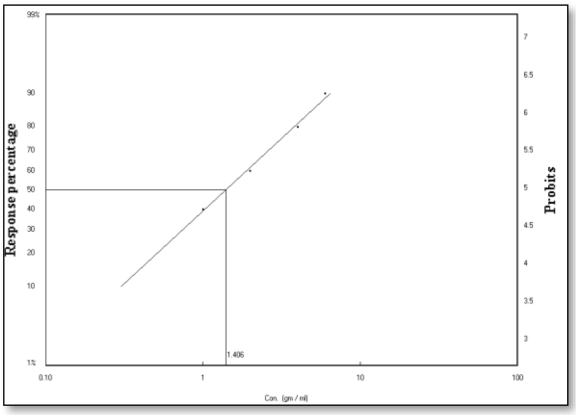

Fig. 1: LC_{50} value of *Calotropis procera* leaves extract against the 3rd instar larvae of red palm weevil, *Rhynchophorus ferrugineus*

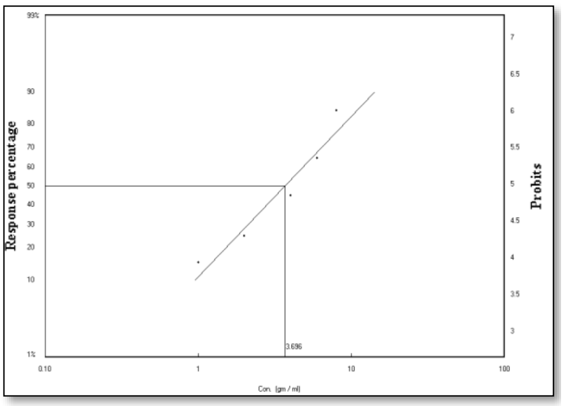

Fig. 2: LC_{50} value of *Calotropis procera* leaves extract against the 5th instar larvae of red palm weevil, *Rhynchophorus ferrugineus*

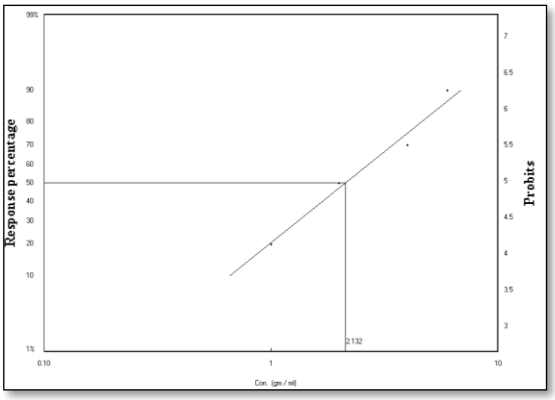

Fig. 3: LC_{50} value of *Calotropis procera* latex extract against the 3rd instar larvae of red palm weevil, *Rhynchophorus ferrugineus*

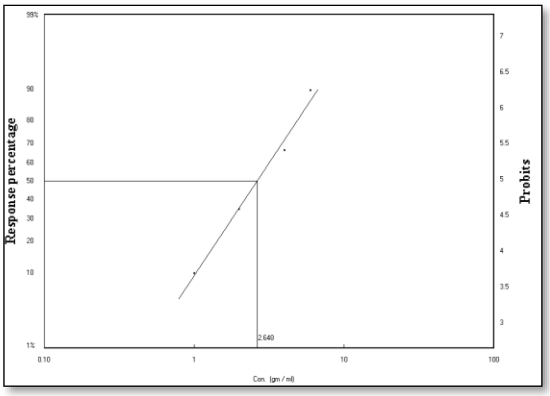

Fig. 4: LC_{50} value of *Calotropis procera* latex extract against the 5th instar larvae of red palm weevil, *Rhynchophorus ferrugineus*

The Antifeedant Activity of Leaves and Latex Extracts of C. Procera Against Red Palm Weevil Larvae:

Results in Table (1) showed the antifeedant index of leaves and latex extracts of *C. procera* against the 3rd and 5th instar larvae of *R. ferrugineus* after feeding larvae on treated sugarcane cuttings for 48h. Results showed both leaves and latex extracts had a high antifeedant index for the 3rd instar larvae; 80.3 and 98.3 %, respectively. This may be due to the high toxicity of both extracts against the 3rd instar larvae. Furthermore, the antifeedant index for leaves and latex extracts for the 5th instar larvae was less than of the 3rd instar but still high (78.3 and 80.6 %, respectively). This could be related to the that the 3rd instar larvae are more susceptible than the 5th ones. Both the extracts showed antifeedant activity leading to weight loss and reduced size of the treated larvae as compared to control ones (Table 2). The antifeedant property of *C. procera* may be due to the different compounds present in the extract possessing different bioactivities (Nighat *et al.,* 2013). The deleterious effect of plant extracts or pure natural/ synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior, and reduction of fecundity and fertility. The antifeedant effect of a plant is related to the phytochemicals produced by this plant. These phytochemicals help in the protection of plants from insect-pests attacks (Ahmad, 2007; Prabhu *et al.,* 2018). The production of phytochemicals is stimulated as a response to insect attacks (Ahmad, 2007; Prabhu *et al.,* 2018) leading to disturbed feeding habits and fertility issues (Ahmad, 2007).

Table 1: The antifeedant index (%) of leaves and latex extracts of *Calotropis procera* against the 3rd and 5th instar larvae of *Rhynchophorus ferrugineus* (Mean ± S. E.).

Treatment	3rd instar larvae	5th instar larvae
Leaves extract	80.3 ± 0.13^b	78.3 ± 0.01^a
Latex extract	89.3 ± 0.01^a	80.6 ± 0.02^b

Means followed by the same letters are insignificantly different at $P < 0.05$

Table 2: Mean larval weight of the 3rd and 5th instar larvae of *Rhynchophorus ferrugineus* during the experiment period after treatment with leaves and latex extracts at 6% concertation (Mean ± S. E.).

Treatment	3rd instar larvae (gm)	5th instar larvae (gm)
Leaves extract	0.75±0.11a	1.60±0.01a
Latex extract	0.66±0.10a	1.45±0.02a
Control	2.23±0.03b	5.48±0.01b

Means followed by the same letters are insignificantly different at $P < 0.05$

Conclusion:

In the present study, we evaluated the insecticidal activity of *Calotropis procera* leaves and latex extracts against the young larval instars of *Rhynchophorus ferrugineus* under laboratory conditions. The present study proved the high larvicidal property of the extracts. In the same context, results also confirmed the antifeedant characteristic of *C. procera* against larvae and this led to weight loss for treated larvae. *C. procera* either used as leaves extract or latex extract represents an excellent alternative for conventional management procedures. More studies are considered on the effect of this plant on the different biological and biochemical parameters.

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ARABIC SUMMARY

النشاط الابادي والمضاد للتغذية للسائل اللبني والمستخلصات الورقية لنبات *كالوتوربس بروسيرا* ضد حشرة سوسة النخيل الحمراء

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تعتبر سوسة النخيل الحمراء من أهم وأخطر الآفات التي تصيب نخيل التمر في مصر. تم استخدام العديد من الاستراتيجيات للسيطرة على هذه الآفة، من بينها توظيف المستخلصات النباتية. هدفت الدراسة الحالية إلى تقييم فعالية الإبادة ومضادات التغذية لمستخلصات الأوراق والسائل اللبني لنبات العشار ضد يرقات العمرين الثالث والخامس لسوسة النخيل الحمراء. أظهرت النتائج سمية عالية ضد الطورين اليرقيين، إلا أن مستخلص السائل اللبني كان أكثر سمية من مستخلص الأوراق. كما أوضحت النتائج أن كلا من الأوراق ومستخلصات السائل اللبني لهما معامل مضاد للتغذية مرتفع وهو ما أكده انخفاض متوسط وزن اليرقات. أخيرًا، يمكننا أن نستنتج أن نبات العشار سواء المستخدمة كمستخلص أوراق أو مستخلص اللبني يمثل بديلاً ممتازًا للوسائل التقليدية في مكافحة سوسة النخيل الحمراء.