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Evaluation of Biocidal Activity and Histopathological effect of Leaf Powder Ethanolic Extract of *Dracaena Arborea* (Asparagaceae) on *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Botanical insecticides are harmless to the environment and living organisms. *Culex pipiens* considers a crucial research issue as it searches for innovative solutions to control the disease-carrying vector. Experiments were done in the laboratory to evaluate the larvicidal efficience of ethanolic leaf extract of *Dracaena arborea*. The primary metric used to assess the Dracaena extract's effectiveness was the documented mortality rate of the treated larvae. The median lethal doses (LC_{50}) were (139.117and 113.162) ppm at 24, and 48 hours respectively. Facts of histopathological alternations confident the toxicological signs. The most distinctive disorder signs were imagined in the treated larvae's midgut as the detachment of basement membrane, vacuolization of epithelial cells, and disorder of microvilli. compared with a normal one. These outcomes conclude the effectiveness of *D. arborea* ethanolic leaf powder extract as bioinsecticides. Hence, should be incorporated into the integrated pest management strategies as an eco-friendly botanical extract.

INTRODUCTION

Chemical insecticides have functioned for many years in pest control, unfortunately, they have led to the development of insect resistance **(WHO 1981**). Besides, the abuse of such insecticides increases environmental pollution and causes a great effect on living organisms and the ecosystem **(Menezes 2005**). The earliest had installed various control techniques, along with the application of inorganic and organic elements **(Jitendra *et al*. 2009)**. It was necessary to find alternative biocontrol agents, hence the application of bio-pesticides as natural plant parts/products to withstand the destructive effect of synthetic chemicals. Botanical extracts had become prevalent as a pest control strategy owing to their least persistence, minimum harmfulness to plants and animals, degradability, inexpensive and easily accessible (**Senthil and Kalaivani 2005**).

Plants are a huge store of natural chemical ingredients produced to shield them from pest attacks. Such substances may cause significant physiological disturbances in the life stages of an insect (**Rembold 1994**). In excess of 30,000 secondary metabolites

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produced by plants have been identified **(Wink 1988**). Certain commercial pesticides made from plants (Pyrethrum, Ryania, and Rotenone), were significant feeding deterrents, contact toxins, and active growth inhibitors of some insects (**Akhtar *et al*. 2008**). Although pyrethrum is the best-known natural insecticide, it is not appropriate for outstanding surface handlings due to its instability towards the sunlight or strong artificial light therefore, additional synergists are required to increase its toxic effect such as piperonyl butoxide. **Akhtar *et al*. (2008, 2012**). Therefore, it is necessary to examine many botanical sources that have high biocidal efficiency to overcome the problems of commonly used plant extracts.

Dracena arborea is an abundantly spread type of the plant family; Asparagaceae **(Nwaehujor 2013 ; Okonkwo 2014)**. This family is branded by fibrous and frequently tough leaves and tightly crowded leaves. It is typically a tropical plant used as an edging plant for defining boundaries on land because of its strength of renaissance when a small stem is implanted and abundant all year round. It is a woody persistent a tree from the lily family known as dragon tree, a beautiful ornamental plant that grows in semi-dry environments is the. It is indigenous to South Asia and Africa. The moniker "dragon tree" refers to the fact that some species' secretions resemble dragon blood and that a damaged branch usually results in the growth of two new ones. (**Udo 2013**). The plant is also applied as reptile repellents, ornamental plants for boundaries, as well as agricultural **(Burkii 1985**). Additionally, it has been found to have therapeutic value, as well as claims that the plant contains components that are anti-fungal and anti-parasitic **(Okunji *et al*. 1996**).

It has been discovered that several dracaena species are insecticidal. In his research, Udo (2013) attested to the effectiveness of powdered leaves, bark, and roots in eradicating two types of stored product pests that affect maize and beans (Callosobruchus maculatus). (*Sitophilus zeamais*) and stated that aqueous and ethyl acetate fractions of leaf extract proved its insecticidal activity and existed protection to stored grains. **Prosper *et al*. (2016)** recorded its larvicidal activity against *Aedes albopictu*. **Ukoroije *et al*. (2019**) confirmed its biocidal qualities when used as an extract or powder, and it was highly effective in cockroach control. Alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, and phenols were among the chemical groups that were found through phytochemical screening in varied concentrations. The bioactivity of these chemical groups is linked to resistance to the insect pest. **(Udo 2013; Ukoroije *et al*. 2019**). Therefore, it is recommended to apply this plant in biological pest management strategies referring to its local convenience, cost-effectiveness, and ecofriendly.

In Egypt, *Culex pipiens* (Diptera: Culicidae), has been considered a dangerous disease vector **(El-Zayyat *et al*. 2017)**. It transmits Japanese encephalitis **(Chancey *et al*. 2015)**, virus of Rift valley fever **(Dodson *et al*. 2017)**, and West Nile virus **(Bassal *et al*. 2017)**. In Egypt, *Cx. pipiens* was found to be the filarial vector **(El-Naggar *et al*. 2017)**

and has been documented by all governorates without exclusion **(Abdel-Shafi *et al.* 2016)**. Due to mosquito reproduction in aquatic media, larval mosquitoes are an attractive target for insecticides, making them simple to manage. Traditional insecticides were used to control mosquitoes causing serious environmental problems, and toxicological consequences to human health (**Killeen *et al*. 2017; Bonner and Alvanja 2017**).

Numerous studies have been directed to identify new bioinsecticides, seeking active substitutes to combat vector mosquitoes. This study aims to find natural ingredients to make formulations that can be incorporated into the integrated pest management strategies as an alternative to synthetic chemical insecticides and to determine its pathological consequence on the target insect.

MATERIALS AND METHODS

Insect (*Culex pipiens*) rearing:

Cx. pipiens was raised in a lab setting at 25–30°C, 80–90% relative humidity, and an 11–13-hour light–dark cycle. Until they hatched, eggs were kept in plastic cups filled with clean, dechlorinated water. At the Research and Training Center (RTC), Faculty of Science, Ain- Shams University, Cairo, Egypt, Tetramine was fed to newly hatched larvae and then used as a bioassay.

Preparation of the plant Powder:

We purchased fresh *Dracaena arborea* leaves from a florist. The plants were identified in the faculty of science's plant department at Cairo, Egypt, Ain-Shams University. for processing and use in the studies that follow.

Fresh plant leaves were cut apart, cleaned under running water to eliminate debris, and then dried in the sun for seven days until firm. The leaves were crushed by hand to create a finely separated powder, which was then dried for eight hours at 600°C in a hot air oven. As stated by **Udo (2008a).**

Extraction procedure of the prepared powder:

The plant material was fully soaked in 90% ethanol and extracted at ambient temperature. The extract was filtered and concentrated *in vacuo* using a vacuum rotary evaporator. at 40^{o}C for 8 h (**Udo *et al*. 2004**). The concentration and percentage yield of the extract was determined. The concentrated *D. arborea* extract then was stored in a refrigerator at 4^{o}C until further use and diluted using 70% ethanol for the application.

GC-MS component identification of experimental sample materials

Gas Chromotography mass spectrometry analysis was done for the ethanol extract of *Dracaena arborea* leaf powder Using Shimadzu GC–MS-QP 2015 plus (Kyoto, Japan). Was done by inserting 0.5 ml of the assayed extract onto a Hewlett Packard model 5970 chromatograph equipped with a flame ionization detector (FID) and a 50 m HP capillary column (0.2 mm internal diameter). The temperature of the oven was preprogrammed from 60°C to 200°C at 3°C/min and then set for 25 minutes at isothermal

200 °C. Temperatures for the injector and detector were 200°C and 250°C, respectively. Helium served as the carrier gas, and there was a 1 ml/min gas flow. A 15:1 split ratio was used to inject diluted samples (1% v/v) and the injection volume was 1 µl. The following mass spectrometry parameters were employed: scan range: 35-500 amu. M., EI mode: 70 eV, interface temperature: 280 C, and ion source temperature: 200 C. By comparing each peak's retention duration with the real peak, it was possible to identify the peaks that had been acquired. By contrasting the regions of the obtained peaks with information from the Tutor, NIST, and WILEY libraries, component quantization was ascertained (**Beckley *et al.* 2014**).

Bioassay of Larvicidal activity:

The larvicidal potency towards the 3rd larval instar of *Cx. pipiens* were evaluated using the immersion procedure (**WHO 2005)**. The extract was used in four different concentrations (75, 250, 300, and 500 ppm). Groups of fifteen early 3rd instar larvae were moving by plastic droppers into test cups, each filled with 10 ml of water under laboratory conditions. For each concentration, three replicates were performed. Mortality data was documented in a probit regression line and calculated LC_{50}, LC_{95}, slope function, and X^2 according to **Finney (1971)**.

Histopathological and Ultrastructural Studies:

Irregularities in the midgut region of treated LC50 third instar larvae were distinguished by transmission electron microscopy (TEM) compared to controls. Thin sections were made under a Reichert Supernova ultramicrotome. Untreated and treated samples were examined with the SEO PEM-100TEM. Electron Microscopy Unit, Faculty of Science, Ain Shams University, Abasia, Cairo, Egypt.

For transmission electron microscopy, 48 h-treated midguts were fixed with glutaraldehyde (2.5%) and paraformaldehyde (4%) in 0.1 M phosphate buffer (pH 7.3) and fixed with 1% osmium tetroxide solution. Fix and dehydrate in the same buffer. A series of acetone solutions were performed, after which the sample was embedded in an epoane. Lead citrate with uranyl acetate were used to staine ultrathin samples according to **Reinbold *et al*. (2001)**.

RESULTS

The bioactive components from ethanolic extract of *Dracaena arborea* leaves was assessed by performing GC-MS analysis. Major active substances, molecular formulas (M.F.), retention times (R.T.), molecular weights (g mol_1, M.W.), and peak areas (%) are shown below Table 1.

The extracts' GC-MS analysis resulted in the identification of three bioactive substances. as shown in Table 1.

Table 1: The GC-MS analysis of the ethanolic extract of *Dracaena arborea*

	Class	RT	Chemical formula	M. wt	Area %	Reported bioactivity
i-Propyl 5,9,17-hexacosatrienoate		20.113	$C_{29}H_{52}O_2$	432.7	20.8201	Insecticidal activity not reported, catalytic activity was reported.
1H-Indene, 5-butyl-6-hexyloctahydro-		19.058	$C_{19}H_{36}$		-1.0503	
9,12 Octadecadienoic acid (Z,Z)-		16.5517	$C_{18}H_{32}O_2$	280.4	51.7885	Insecticidal activity
n-Hexadecanoic acid	Fatty acid	15.9455	$C_{16}H_{32}O$ ·	256.4241	8.1711	Insecticidal activity

Larvicidal bioassay:

The effectiveness of different doses of *Dracaena arborea's* ethanolic leaf extract against newly moulted 3rd instar *Cx. pipiens* larvae was assessed and is shown in Table 2. Depending on the extract concentrations and exposure duration, the toxicity values vary. As concentrations and exposure times increased, so did the percentage of dead larvae.

Table 2: Susceptibility of 3rd instars larvae *Culex pipiens* to ethanolic based leaf extract of *Dracaena arborea* at different time intervals

Concentrations (ppm)	Percentage mortality (%)	
	24hrs.	48hrs.
untreated	0.0	0.0
75	26.6	35.6
250	55.5	62.2
300	93.3	100
500	100	100
LC_{50} (ppm)	139.117 (169.838 –113.862)	113.162 (142.247 – 89.913)
LC_{95} (ppm)	503.706 (678.200 – 374.851)	434.613 (590.248 – 320.754)
Slope ± SE	2.943 ± 0.1518	2.814 ± 0.1522
X^2	18.3442	22.5640

Transmission Electron Microscopy:

Midgut ultrastructure of Control (untreated) *Cx. pipiens* 3rd instar larvae:

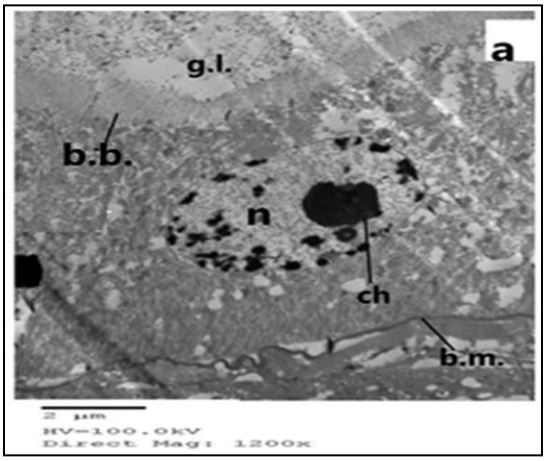

Fig. 1. TEM microphotograph of the *Culex pipiens* larvae's cross-sectioned midgut, showing (a) the control larvae (1200X). (g.l.) gut lumen, (b.m.) basement membrane, (ch) chromatin, (b.b.) brush border, (n) nucleus.

Midgut dissection of untreated third instar larvae of *Cx. pipiens* highlighted in (Fig. 1). Midgut cells have been shown to be formed from a columnar layer of epithelial cells located on an intact basement membrane. Epithelial cells surrounded a relatively large central rounded nucleus, with each nucleus surrounded by an intact nuclear envelope and chromatin concentrated in the center of the nucleus. A typical adherent brush border membrane with long microvilli was present, and the intestinal lumen was convoluted.

Midgut ultrastructure of treated third instar larvae of *Cx. pipiens* with LC_{50} of *Dracaena arborea* after 48 hrs of treatment:

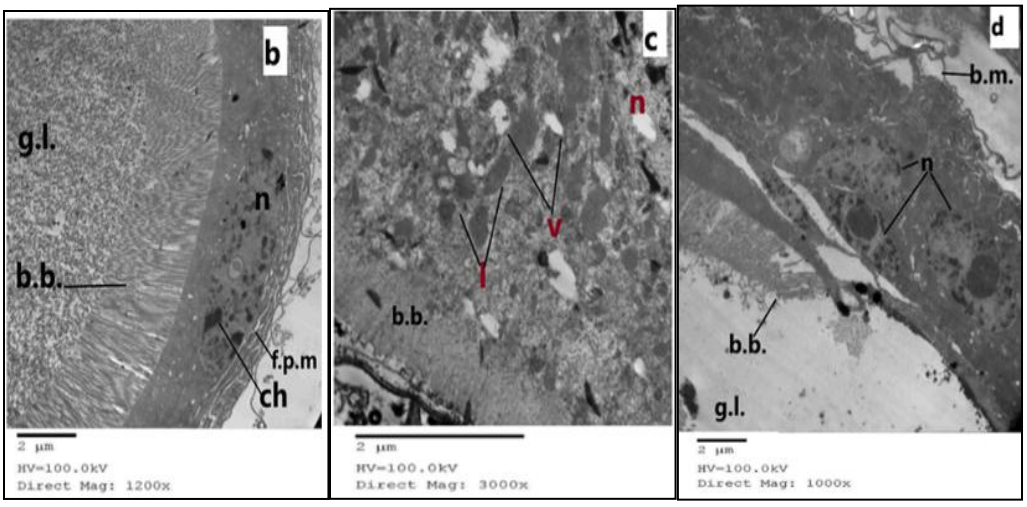

Fig. 2. TEM microphotograph of the cross-sectioned midgut of the larvae of *Culex pipiens* treated with *Dracaena arborea* 48 hrs post-treatment.(b) 1200x (c &d) 1000x . (g.l.) gut lumen, (b.m.) basement membrane, (ch) chromatin, (b.b.) brush border, (n) nucleus (f.p.m.) folded plasma membrane (v) vacuoles.

The histopathological consequence of *Dracaena arborea* leaf powder ethanolic extract on the midgut of 3rd instar larvea of *Cx. pipiens* was illuminated in Fig.2 (b, c, and d). The midgut epithelial cells showed firm elongation and magnification, with obviously elongated nuclei in comparison with the control, the chromatin was scattered in the nucleus. In addition, the epithelial cells were characterized by the folded plasma membrane. In distinct, the site microvilli were damaged, became retracted, and shorter as shown in Fig. (2d). Vacuolization seen in the cytoplasm appeared in (2c). also, it was notable that the gut lumen contained food (2b).

DISCUSSION

Depending on the type of plant material and solvent employed, a chemical can be extracted from plant components. The GC-MS analysis of *Dracaena arborea* leaf powder was done for the first time however, it afforded only three compounds in the GC-MS analysis (Table 1) as follows: i-Propyl 5,9,17- hexacosatrienoate, 9,12- Octadecadienoic acid (Z, Z)-and n-Hexadecanoic acid, the last two complexes were reported for the insecticidal activity in previous literature **(Barakat 2011**; **Zhao 2015; Abdelkader *et al*. 2018 and Kotteswari *et al*. 2020)**. While i-Propyl 5,9,17-hexacosatrienoate was reported for catalytic activity, not insecticidal activity. Due to the synergisms between its active elements, the complete extract may be superior to a single-based active component.

The biological preparations for preventing mosquitoes comprise the usage of plant extracts and essential oils as potential adulticides, larvicides, ovicides, and repellents, though, the effectiveness of plant extract differs depending on the plant species **(Shaalan *et al*. 2005**). Generally, plant EOs are documented as safe; but, some of them cause skin irritation, mostly due to the ingredients present in the plant EOs, limiting their extensive usage (**Benelli and Pavela 2018**). The most valuable pest control technique that withstands the environmental hazards induced by synthetic chemicals, and environmentally friendly. Therefore, using natural plants components as biopesticides has been evaluated as the best means of control, has the lowest toxicity to organisms other than the target, is economical, readily available and degradable, and is therefore increasingly being applied (**Senthil and Kalaivani 2005**).

Phyto-extracts are developing as effective pest control agents, that are simple to administrate, low-cost, and risk-free characters. Simple, botanical extracts used as insecticides in numerous nations for ages (**Crobsy *et al*. 1971**). Often, complex combinations of active substances make up extracts **(Berenbaum 1985**). In order to employ indigenous plants as a natural product for mosquito control measures, they must first be tested for mosquito larvicidal activity (**Bowers *et al*. 1995**).

Phytochemical screening by **Ukoroije *et al*. (2019)** listing the existence of chemical groups such as flavonoids, tannins, saponins, terpenoids, alkaloids and glycosides in plants, he explained that these chemical groups have a biological activity against insects

pest. **Udo (2013**) noted that tannins, saponins, anthraquinones, flavonoids, terpenes, alkaloids, and saponins were all examined using thin layer chromatography to determine the chemical composition of an ethanolic extract of *D. arborea*. **Okunji *et al*. (1996) ; Momeni *et al*. (2005**) provoked that the occurrence of harmful secondary metabolites in *D. arborea* may be the cause of the substantial insect death. Some secondary metabolites are known to have insecticidal and antifeedant properties, as had been observed by **Nawrot *et al*. (1988) ; Hassanali and Lwande (1989)** against *Tribolium castaneum* and some lepidopteran pests. In conclusion, some of the *D. arborea* leaf extract fractions exhibited toxicity, had repulsive effects, and decreased the number of offspring of various insect species. However, it was discovered that the susceptibility to the same plant extract might vary substantially amongst even closely related species (**Akhtar *et al*. 2012**).

Ethanol is the solvent that has proven to be the least toxic compared to acetate and acetone. In addition to its qualities that repel insects insects, it is soluble and therefore has a non-polar (hydrophobic) and a polar (hydrophilic) end. The presence of oxygen forms hydrogen bonds and high electronegativity, maximally extracting the active elements present in the test plant sample (**Khalequzzaman and Sultana 2006**). In the current study evaluation of the toxicity induced through application of the leaf powder ethanolic extract of *D. arborea* at different concentrations was estimated through recording LC_{50} and LC_{59} against the *Cx pipiens* 3rd larval instar.

As revealed from the results, 50% mortality was obtained at concentrations (113.162) ppm at 48 hrs. Such outcomes indicate its larvicidal activity which is directly proportionated with the concentration and the time of exposure to the extract. The present investigation agrees with **Udo *et al*. (2011)** who reported that *D. arborea* possess larviciding and ovicidal properties in controlling both (*C. maculatus*) and (*S. zeamais*), the extract fractions were efficient in decreasing the progeny in each of them. Also, **Prosper *et al.* (2016)** recorded its larvicidal activity against *Aedes albopictu*. **Ukoroije *et al*. (2019**) revealed its insecticidal properties in case applied as extracts or in powdered form against *Periplaneta americana* adults.

Histopathological effect induced by the median lethal concentration of *D. arborea* against *Cx. pipiens* larvae treated for 48 hrs were examined in comparison with normal larvae to illustrate the significant toxicological effect of the tested plant extract. The most distinctive signs is detached underpinning membrane, damaged microvilli, and disorganization were all present in the larval midgut sections of *Cx. Pipiens*. Epithelial cells elongation, and vacuolization. Our investigations agreed with many authors who measured the effect of the botanical extract on mosquitoes and even on different insects. For example **(Gusmao *et al*. 2002; Al-Mehmadi and Al-Khalaf 2010; Almehmadi *et al*. 2011; Mahmoud *et al.* 2019 and Farag *et al*. 2021)**.

Since food digestion and absorption in insects mostly take place in the midgut, which is also the area most susceptible to the effects of external agents, the cellular alterations in this area are more pronounced. The peritrophic membrane is the first barrier

that opposes any constitutions before epithelial cells interact **(Mohan *et al.* 2006**). Any peritrophic membrane disruption could allow the employment of certain mediators like viruses, bacteria, protozoans, and toxic proteins to increase the effectiveness of pesticide. **(Gusmão *et al.* 2002)**. Hence, botanical extracts can be incorporated into pest control management.

Alterations in the structure of the integument were induced as a result of the application of the tested botanical extract illustrated as cuticle separation from the hypodermis, hypodermal tissue breakdown, and basement membrane obliteration.Similar remarks were noticed by **Younes *et al.* (1999); Khalaf *et al.* (2009); Farag *et al.* (2021) and El Gohary *et al.* (2021)**. According to the references the presence of saponins as constituents screened in the chromatographic analysis, we can attribute the cytotoxicity to such metabolite (**Podolak *et al.* 2010)**. Plants produce phenolic complexes through the pentose phosphate and phenylpropanoid pathways as secondary metabolites. After penetrating the cell, the involvement of oxidases within cytochrome P450 leads to the active transformation of phenols. These reactions frequently result in the creation of electrophilic metabolites, which can bond with and destroy DNA or cell enzymes, greatly increasing toxicity. **Randhir *et al.* (2004) ; Michałowicz and Duda (2006)** explained the damage that occurred in the endoplasmic reticulum, nucleus, and membranes mitochondria, besides their biochemical constituents such as enzymes and nucleic acids may be due to that Phenols that experience radical reactions, which result in the cell membrane's lipid peroxidation.Such clarifications are suggested to elucidate the alteration patterns observed in both of midgut epithelium and integument of the treated larvae.

The recently used larvicides must achieve the WHO criteria. These comprise valuations of minimum environmental and human dangers, shelf-life, their storage resources, the related charges of utilization, and local vector susceptibility **(Samuel *et al.* 2016)**. Results verified that *D. arborea* leaf extract had potential larvicidal activity and histopathological effect on *Cx. pipiens* larvae. In terms of these criteria, botanical products need further investigations for their effectiveness and residual action under field conditions. So, Progressive research is ongoing to detect the active constituents and the mode of action of our tested extract as an environmental bioinsecticide**.**

CONCLUSION

The current research proved that *D. arborea's* ethanolic leaf extract contains insecticidal qualities. Its application against *Cx. Pipiens* have added to the enormous source of botanicals utilized as mosquitocidal. The plant-based pesticides are widely distributed and available round the year. Therefore, the use of *D. arborea* could be an important supplement to synthetic pesticides and possibly will be used as part of integrated pest management plans

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الملخص العربي

تقييم فعالية الاباده الحيوية والتأثير النسيجي للمستخلص الإيثانولي للمسحوق الورقي ل *Dracaena arborea* (Asparagaceae) على بعوض *Culex pipiens* L (رتبة : ثنائية الاجنحة Culicidae) .

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المبيدات الحشرية النباتية غير ضارة بالبيئة والكائنات الحية. البحث عن بدائل جديدة لمكافحة ناقلات الأمراض ، *Culex pipiens* يعتبر نقطة بحث هامه. قد أجريت التجارب المعمليه لتقييم الفعالية القاتلة لليرقات للمستخلص الإيثانولي لأوراق نبات *Dracaena arborea* . كانت نسبة النفوق المسجلة لليرقات المعالجة هي المؤشر الرئيسي لتقييم فعالية مستخلص*Dracaena arbore* . سجلت التركيزات النصف مميتة (LC_{50}) (١٣٩.١١٧ و ١١٣.١٦٢) جزء في المليون عند ٢٤ و ٤٨ ساعة على التوالي . وقد اظهرت نتائج فحص الميكروسكوب الاكترونى لخلايا المعى المتوسط لليرقات المعامله بالجرعه المميته للنصف بعد ٤٨ ساعه من المعامله علامات السمية. مثل انفصال الغشاء القاعدي ، وتفريغ الخلايا الظهارية ، واضطراب الميكروفيلي مقارنة مع غير المعامله. هذه النتائج تستنتج فعالية مستخلص مسحوق أوراق نبات *D. arborea* الإيثانولي كمبيدات حيوية. ومن ثم ، يمكن إدراجه في الاستراتيجيات المتكاملة لإدارة الآفات كمستخلص نباتي صديق للبيئة.