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. Searching for novel alternatives for the control of the vector of diseases, *Culex pipiens* considers an important research point. Experiments were done in the laboratory to evaluate the larvicidal activity of ethanolic leaf extract of *Dracaena arborea*. The recorded mortality percentage of the treated larvae was the major indicator to evaluate the efficacy of the *Dracaena* extract. The median lethal concentrations (LC50) were (139.117 and 113.162) ppm at 24, and 48 hours respectively. Facts of histopathological alternations confirm the toxicological signs. The most distinctive disorder signs were imagined in the midgut region of treated larvae 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, economical and easy accessibility (Senthil and Kalaivani 2005).

Plants are a huge store of natural chemical ingredients produced to protect them against attack by insects. Such substances may cause significant physiological disturbances in the life stages of an insect (Rembold 1994). Extra than 30,000 secondary
metabolites have been recorded from plants (Wink 1988). Certain commercial botanical insecticides (Pyrethrum, Rynia, 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 characterized by fibrous and frequently leathery leaves and tightly crowded leaves. It is a tropical plant often used as an edging plant for the demarcation of land due to its power of renaissance when a small stem is implanted and abundant all year round. It is a woody persistent tree of the lily family, known as the Dragon tree, is an elegant decorative plant that cultivates in semi-dry areas. It is native to Africa and South Asia. The name dragon tree stems came from the fact that fluids from some species resemble dragon’s blood and two new branches tend to grow at the point where a branch is cut (Udo 2013). The plant is also applied as reptile repellents, ornamental plants for boundaries, as well as agricultural (Burkii 1985). As well it has been documented to have healing properties while there are claims of anti-fungal and anti-parasitic composure present in the plant (Okunji et al. 1996).

Dracaena species have been revealed to have insecticidal properties. Udo (2013) in his studies, testified the use of root, bark, and leaf powders in controlling two storage pests of bean (Callosobruchus maculatus) and maize (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 albopictus. Ukoroije et al. (2019) documented its biocidal properties in case applied in powder or extract forms and it was significantly active in cockroach control. Phytochemical screening discovered the existence of chemical groups like alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, and phenols in varying quantities. These chemical groups are associated with bioactivity against 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 vector of serious diseases (El-Zayyat et al. 2017). It transmits Japanese encephalitis (Chancey et al. 2015), Rift valley fever virus (Dodson et al. 2017), and West Nile virus (Bassal et al. 2017). Culex pipiens was convicted as the filarial vector in Egypt (El-Naggar et al. 2017) and has been documented by all governorates without exclusion (Abdel-Shafi et al. 2016). Larval stages of mosquitoes are attractive aim for insecticides because of mosquito
breeding in aquatic media thus, it is easy to control them. 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**

**Maintenance of Mosquito (Culex pipiens):**

The colony of Cx. pipiens were reared under laboratory conditions, at 25–30°C, 80–90% relative humidity, and 11/13 h (light/dark) photoperiod, at Vector Research and Training Center (RTC), Faculty of Science, Ain-Shams University, Cairo, Egypt. Eggs were collected in plastic cups 10X10X7 cm with clean dechlorinated water until hatching. Hatched larvae were nourished on Tetramine and used for bioassay.

**Preparation of the plant Powder:**

Fresh leaves of Dracaena arborea were bought from a florist shop. The plants were identified in the plant Department, Faculty of Science, Ain-Shams University, Cairo, Egypt. For processing and usage in the subsequent investigations.

Fresh leaves of the test plant were separated manually and washed for a short time under running tap water to remove debris, then sun-dried for seven days until hard. The leaves were ground using the hand crusher and later blended to obtain finely divided powder which was further dried in a hot air oven at 60°C for eight hours. According to Udo (2008a). 10-50g of the powdered materials were bagged and labeled separately for further usage.

**Extraction procedure of the prepared powder:**

The plant material was fully extracted at room temperature with 90% ethanol by soaking. The extract was filtered and concentrated in vacuo using a vacuum rotary evaporator, at 40°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°C until further use and diluted using 70% ethanol for the application.

**Identification of experimental sample components using the GC-MS technique**

GC–MS analysis was done for the Ethanolic extract of Dracaena arborea leaf powder using a Shimadzu GC–MS-QP 2015 plus (Kyoto, Japan). This was performed by injecting 0.5 ml of the examined extract into Hewlett Packard chromatograph model 5970, equipped with a flame ionization detector (FID) and 50 m HP capillary column (0.2 mm I.D.). The oven temperature was programmed at 3 _C/minute from 60 _C to 200 _C, then isothermally at 200 _C for 25 min. Detector and injector temperatures were 250 _C
and 200 °C, respectively. The carrier gas was helium and the gas flow rate was 1 ml/minute. Diluted samples (1% v/v) were injected with a split ratio 15:1 and the injected volume was 1 μl. The MS operative parameters were as follows: interface temperature: 280 °C, ion source temperature: 200 °C, EI mode: 70 eV, scan range: 35–500 amu. In order to identify obtained peak, this was accomplished by comparing the retention time of each peak with those of the authentic. The components' quantization was determined by comparing the area of the resulted peaks with data from the WILEY, NIST, and Tutor libraries (Beckley et al. 2014).

Bioassay of Larvicidal activity:

The larvicidal potency against the third 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 small disposable 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 LC50, LC95, slope function, and X2 according to Finney (1971).

Histopathological and Ultrastructural Studies:

The irregularities in the midgut region of the treated third larval instar with LC50 value were distinguished by light and transmission electron microscopy in comparison with control. Thin sections were performed under Reichert Supernova Ultramicrotome. Untreated and treated specimens were inspected under SEO PEM-100TEM. Electron Microscopy Unit, Faculty of Science, Ain Shams University, Abbasya, Cairo, Egypt.

For transmission electron microscopic study, 48 hrs treated midgut was fixed in glutaraldehyde (2.5%) and paraformaldehyde (4%) in phosphate buffer 0.1M (pH7.3), fixation in 1% osmium tetroxide solution takes place in the same buffer and dehydration in a series of acetone solutions, after that the specimens were embedded in epoan. Ultra-thin specimens were stained with uranyl acetate and lead citrate according to Reinbold et al. (2001).

RESULTS

Leaf Powder Ethanolic Extract of Dracaena arborea as identified by GC-MS analysis. The presence of biologically active components from ethanolic extract of Dracaena arborea was evaluated by conducting GC–MS analysis. The principal active compounds, molecular weight (g mol _ 1, M.W.), molecular formula (M.F.), retention time (R.T.), and peak area (%) are presented in Table 1.

The results of GC–MS analysis of the extracts led to the determination of three biological compounds as shown in Table 1.
**Table 1**: The GC-MS analysis of the ethanolic extract of *Dracaena arborea*

<table>
<thead>
<tr>
<th>Class</th>
<th>RT</th>
<th>Chemical formula</th>
<th>M. wt</th>
<th>Area %</th>
<th>Reported bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-Propyl 5,9,17-hexacosatrienoate</td>
<td>20.113</td>
<td>C_{20}H_{32}O_{2}</td>
<td>432.7</td>
<td>20.8201</td>
<td>Insecticidal activity not reported, catalytic activity was reported.</td>
</tr>
<tr>
<td>1H-Indene, 5-butyl-6-hexyloctahydro-</td>
<td>19.058</td>
<td>C_{16}H_{36}</td>
<td>-1.0503</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,12 Octadecadienoic acid (Z,Z)-</td>
<td>16.517</td>
<td>C_{18}H_{32}O_{2}</td>
<td>280.4</td>
<td>51.7885</td>
<td>Insecticidal activity</td>
</tr>
<tr>
<td>n-Hexadecanoic acid Fatty acid</td>
<td>15.9455</td>
<td>C_{16}H_{32}O_{2}</td>
<td>256.4241</td>
<td>8.1711</td>
<td>Insecticidal activity</td>
</tr>
</tbody>
</table>

**Larvicial bioassay:**

The larvicial efficiency of different concentrations of the ethanolic leaf extract of *Dracaena arborea* was evaluated against the newly molted 3rd instar *Cx. pipiens* larvae and illustrated in Table2. The toxicity values are diverse depending on the concentrations of the extract used and exposure time. The mortality percentage of larvae increased with the increase of the concentrations and exposure time.

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

<table>
<thead>
<tr>
<th>Concentrations (ppm)</th>
<th>Percentage mortality (%) of ethanolic leaf extract of <em>Dracaena arborea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24hrs.</td>
</tr>
<tr>
<td>untreated</td>
<td>0.0</td>
</tr>
<tr>
<td>75</td>
<td>26.6</td>
</tr>
<tr>
<td>250</td>
<td>55.5</td>
</tr>
<tr>
<td>300</td>
<td>93.3</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>LC_{50} (ppm)</td>
<td>139.117 (169.838 – 113.862)</td>
</tr>
<tr>
<td>LC_{95} (ppm)</td>
<td>503.706 (678.200 – 374.851)</td>
</tr>
<tr>
<td>Slope ± SE</td>
<td>2.943 ± 0.1518</td>
</tr>
<tr>
<td>X²</td>
<td>18.3442</td>
</tr>
</tbody>
</table>
Transmission electron microscopy:

Ultrastructure of midgut region of Control (untreated) *Cx. pipiens* 3rd instar larvae:

![TEM microphotograph of cross-sectioned midgut of the larvae of *Culex pipiens*, (a) the control larvae (1200X). (g.l.) gut lumen, (b.m.) basement membrane, (ch) chromatin, (b.b.) brush border, (n) nucleus.](image)

The section in the midgut of untreated 3rd larval instar of *Cx. pipiens* emphasized in (Figs 1). Clarified that midgut cells are formed of one layer of columnar epithelial cells situated on an intact basement membrane. The epithelial cells confined relatively large centric rounded nuclei and each nucleus was surrounded by an intact nuclear envelope and the chromatin was concentrated in the center of the nucleus. The gut lumen was crumpled with a normal adhesive brush border membrane with long microvilli.

Ultrastructure of midgut region of treated 3rd larval instar of *Cx. pipiens* treated with LC50 of *Dracaena arborea* 48 hrs post-treatment:

![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.](image)
The histopathological consequence of ethanolic leaf powder extract of *Dracaena arborea* on the midgut of 3rd larval instar 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 appeared in the cytoplasm as shown in (2c). also, it was notable that the gut lumen contained food (2b).

**DISCUSSION**

A compound extraction from plant parts depends upon the type of plant material and solvent used. 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 compounds 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. The whole extract may be more effective than a single-based active constituent due to its active ingredients’ synergisms.

The biological preparations for mosquito control comprise the use of plant essential oils and plant extracts 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, is that which is non-toxic and eco-friendly. Hence the usage of natural plant parts as biopesticides is evaluated as the best control measure which has become more applicable owing to their least toxicity to non-target organisms, economical and easy availability, and degradability (Senthil and Kalaivani 2005).

Phyto-extracts are developing as effective pest control agents, that are easy to administer, low-cost, and risk-free characters. In many countries for centuries simple crude plant extracts have been applied as insecticides (Crobsy et al. 1971). Such extracts often consist of complex mixtures of active compounds (Berenbaum 1985). The screening of local plants for mosquito larvicidal activity leads to their use as a natural product for mosquito abatement practices (Bowers et al. 1995).

Phytochemical screening by Ukoroije et al. (2019) listed the presence of chemical groups like flavonoids, tannin, saponins, terpenoids, alkaloids, and glycosides in the plant, he stated that these chemical groups are related to bioactivity against the insect
pest. Udo (2013) mentioned that the thin layer chromatography analysis for chemical compounds of ethanolic extract of D. arborea involved the alkaloids, tannins, saponins, anthraquinones, flavonoids, and terpenes. Okunji et al. (1996); Momeni et al. (2005) provoked that the significant insect mortality could be attributable to the presence of toxic secondary metabolites in D. arborea. It is recognized that some secondary metabolites may act as insecticides and antifeedants 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 fractions from the leaf extract of D. arborea achieved toxicity, repellent effects, and a reduction in the progeny of different insect species. Though, interspecific differences were noticed even closely correlated species can vary evidently in susceptibility to the same plant extract (Akhtar et al. 2012).

The most verified lowest toxic solvent is ethanol compared to acetate and acetone. Besides its insecticidal properties against insects since it is soluble, nonpolar (hydrophobic) and polar (hydrophilic) ends. The presence of Oxygen enables it to undergo hydrogen bonding plus its high electronegativity, extracting maximally the active elements present in the test plant samples (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 3rd larval instar of Cx pipiens.

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 ovicidal and larvicidal 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 in powdered form or as extracts against Periplaneta americana adults.

The histopathological effect induced by the application of 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 in ultrastructure sections of the midgut of Cx. pipiens larvae were a detachment of basement membrane, damage, and disorganization of microvilli, 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 insect midgut sections. 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).

The midgut is the chief portion of the alimentary tract of insects where food digestion and absorption occur, thus the cellular changes are more powerful here as the
midgut is the further most disposed region for the effect of external agents. The peritrophic membrane is the first barrier that opposes any constitutions before epithelial cells interact (Mohan et al. 2006). Any damage in the peritrophic membrane could permit the improvement of the efficiency of insecticide of special mediators such as viruses, bacteria, protozoans, and toxic proteins (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 detachment of cuticle from hypodermis, disintegration in the hypodermis, and destruction of the basement membrane. 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. Such processes frequently lead to the formation of electrophilic metabolites that can bind and damage DNA or enzymes in the cell thus causing a significant increase in toxicity. Randhir et al. (2004); Michalowicz 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 undergo radical reactions causing lipid peroxidation in the cell membrane. 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 risks to humans and the environment, 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 present study proved that the ethanolic leaf extract of D. arborea has insecticidal properties. 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 could be incorporated into integrated pest management strategies.

REFERENCES


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Biocidal Activity and Histopathological effect of *Dracaena Arborea* on *Culex pipiens*


المملوک العرabi

Dracaena arborea تقييم فعالية الابادة الحيوية والتأثير النسيجي للمستخلص الإيثانولي للمسحوق الورقي لـ (Culicidae) Culex pipiens L. (Asparagaceae)

شيماء، أ. أ.، أ. م.، ج. ب.، س. م.، ع. ع.، ي. ع.,
قسم علم الحشرات - كلية العلوم - جامعة عين شمس

المبيدات الحشرية النباتية غير ضارة بالبيئة والكائنات الحية. البحث عن بدائل جيدة لمكافحة ناقلات الأمراض، يعتبر نقطة بحث هامة. قد أجريت التجارب المعملية لتقييم الفعالية القاتلة للبرقات للمستخلص الإيثانولي لأوراق نبات Dracaena arborea في البرق . كانت نسبة النتائج المسجلة للبرقات المعالجة هي المؤشر الرئيسي لاقتراح فعالية مستخلص Dracaena arborea . سجلت التركيزات النصف مميزة (LC50) (117.111) جزء في المليون عند 24 و 48 ساعة على التوالي. وقد أظهرت نتائج فحص الميكروسكوب الإلكتروني لخلايا المبعث للبرق المعدة بالجرعة المقدمة للتصور بعد 48 ساعة من المعالجة علامات السمية، مثل انفصال الغشاء القاعدي، واخطاب الخلايا الظهارية، واضطراب الميكروفيلي مقارنة مع غير المعالجة. هذه النتائج تستند فعالية مستخلص مسحوق أوراق نبات D. arborea لإدارة الآفات كمستخلص نباتي صديق للبيئة.

يمكن إدراجها في الاستراتيجيات المتكاملة لإدارة الآفات كمستخلص نباتي صديق للبيئة.