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Seagrasses extracts as potential mosquito larvicides in Saudi Arabia

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ABSTRACT

The present study aimed to evaluate the toxic and biological effects of some extracts of seagrasses (*Cymodocea rotundata*; *Halophila ovata* & *Thalassia hemprichii*) against *Aedes aegypti*, which transmits dengue fever, and *Culex pipiens*, which is the dominant species of mosquitoes in the Kingdom of Saudi Arabia, as a safe method for its control. The cumulative death rate during larval development into pupae and adults was used as a criterion for evaluating tested seaweed extracts against *Ae. aegypti*, *Cx. pipiens*. According to the obtained IC_{50} values (the concentration that inhibits the exit of 50 % of adult mosquitoes), the results showed that *C. rotundata* extract (70.78 & 77.47 ppm) was more effective against *A. aegypti* and *Cx. pipiens* in comparison with *H. ovata* (86.98 & 95.87 ppm) and *T. hemprichii* (83.94 & 88.82) extracts by (1.186, 1.229, 1.146 & 1.237) fold, respectively. The results showed that the treatment with marine plant extracts against mosquito larvae of *Cx. pipiens* and *Ae. aegypti* gave different biological effects similar to those of other insect growth regulators (IGRs). The results also revealed the presence of morphological abnormalities in larvae that were treated with all seaweed extracts and these effects extended to all stages of growth, which caused damage to the insect without completing its life cycle. Generally, the results indicate the importance of carrying out bio-assessment tests for the pesticides that are used against mosquitoes and establishing a database to be referenced when planning control programs and making the right decision about the pesticide used.

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1. Introduction

Worldwide, the mosquito is still relevant to the most important epidemic diseases, where the diseases they transmit pose a fatal threat to millions of people around the world (Govindarajan et al. (2016). Mosquito Females transmit a lot of diseases to humans, such as; Malaria which is caused by the transmission of the Plasmodium parasite, it is one of the most dangerous pathogens transmitted by female mosquitoes, with a child dying every 30 s globally and killing more than one million people annually. Also, they transmit the nematode worms that cause elephantiasis, which is a non-fatal disease but causes social hardship and disability. It

comes in the second rank after leprosy as a cause of lifelong disability. In addition, they transmit the virus that causes dengue fever and which is considered one of the most important viral diseases transmitted by mosquitoes at the present time and the most widespread, the indicators confirm that this disease is growing steadily, both in terms of the number of cases of infection or the number of countries in which the disease is endemic (Govindarajan and Benelli, 2016).

Although the traditional chemical pesticides have been used in pest control for more than 100 years; however, the danger still exists, in addition, to the damage of pesticide residues in the food chains, especially since some of them are not easily degradable. Not to mention the many harmful effects of these pesticides on humans, pets, wildlife and the environment (Matsumura, 1985).

On the other hand, the indiscriminate and extensive use of traditional chemical pesticides led to the recording of resistance against many insecticides in 504 species of arthropods all over the world, including vectors of epidemic diseases, especially mosquitoes (Georghiu, 1991).

It recorded the phenomenon of resistance against all major types of insecticides, including organochlorine compounds and

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organophosphorus compounds; carbamates; and synthetic pyrethroids (Beach et al., 1989).

With the development of chemical analysis devices for pesticide residues, many environmental risks have emerged from the indiscriminate use of chemical pesticides, whether it is to control public health pests or agricultural pests. In addition to the emergence of resistance against many of them (Benelli, 2015).

To solve this problem, efforts have been directed towards the search for new and effective compounds that do not have harmful effects on the non-target population and organisms in the breeding sites and have a water basis that is easily degradable; the active substances extracted from plants are considered the best alternative source for mosquito control because they contain biologically active compounds that reduce mosquito reproduction (Sukumar et al., 1991; Wink, 1993).

A number of plant extracts have been reported to possess biological activities as larvicides against different types of mosquitoes (Goellner et al., 2018).

In the early part of history, humans used some plants and compounds extracted from them to control some insects that transmit diseases to humans and their domesticated animals. There is a tendency to search for these compounds on a large scale because the use of readily biodegradable plant compounds is one of the safest methods for controlling insect pests and disease vectors (Alkofahi et al., 1989).

Some studies also indicated the advantages of using medicinal plant extracts in mosquito control, being effective and significantly reducing the risks of harmful environmental effects from the use of chemical pesticides, in addition to the fact that mosquitoes did not acquire resistance against them. Recently, interest in research regarding the potential use of plant extracts as alternatives to synthetic insecticides has increased, with many researchers around the world beginning to test some medicinal and herbal plants for mosquito control (Choochote et al., 2004).

The current study aims to keep pace with recent trends in mosquito control by evaluating the biological activity; toxic and delayed effects of some marine grasses growing on the Saudi Red Sea coast against larvae of *Ae. aegypti* which is the main vector of dengue virus types and larvae of *Cx. pipiens* is prevalent species in Saudi Arabia.

2. Materials and methods

2.1. Definition of tested species

2.1.1. Eggs stage

Females of the species *Culex pipiens* lay eggs in boat-like clumps where the bottom side touching the water is convex and the upper side is concave and the eggs are perpendicular to the surface of the water, and they do not have the ability to withstand drought (Fig. 1A), While females of the species *Ae. aegypti* lay oval eggs, single, spindle-shaped and it is black color at the edges of the water or in wet places. These eggs are characterized by resistance to drought for several months (Fig. 1B).

2.1.2. Larvae stage:

The larva is characterized by a long siphon with more than one tuft of hair branching from its root in the form of a ray in *Cx. pipiens* (Fig. 2A), while the siphon is short, barrel-shaped and contains a single hair tuft in *Ae. aegypti* (Fig. 2B).

2.1.3. Pupa stage

The pupae of mosquitoes are difficult to distinguish, nevertheless, pupae of the genus *Culex* are tubular and in *Cx. pipiens* respi-

ratory horns are long (Fig. 3A), while in *Ae. aegypti* the respiratory horns are short as in (Fig. 3B).

2.1.4. Adult stage

The adult insect of *Cx. pipiens* is light brown in color and the end of the abdomen is rounded and the scales of its are regular section (Fig. 4A), while in *Ae. aegypti* the insect is dark brown or blackish in color and the end of the abdomen is tapering with white scales distributed on the body, especially dorsally from the thorax with a lyre shape (Fig. 4B).

2.2. Laboratory breeding of tested mosquitoes

The eggs were obtained from the Dengue Research Unit of the Department of Biological Sciences at King Abdul-Aziz University. The breeding of mosquitoes was at a laboratory dedicated to breeding mosquito strains in the unit, where the egg blocks of *Cx. pipiens* were placed in porcelain dishes with white background of 30 × 20 depth 7 cm and filled to the middle with tap water. Whereas the species *Ae. aegypti* the filter papers must be placed in breeding dishes and immersed in water.

Mosquito larvae were fed food, which is a mixture of yeast and fish food flakes in a 1:1 ratio, with daily follow-up until the pupal stage. When the larvae turn into pupae, they are collected using a transparent plastic dropper to be placed in cups containing water and then inserted into square cages with dimensions of 30 × 30 × 30 cm designated for breeding mosquitoes to follow the exit of the adult insect.

After the adult emergence, males and females are fed on a 10 % sugar solution for three days. To obtain eggs, the females are starved by keeping the sugar solution away for 12–24 h, then females were fed on a blood meal using the Mosquito Membrane Feeder.

After two to three days of blood-feeding, the breeding cages are provided with dishes of water to receive the egg laid by the blood-fed females. After laying eggs, the previous steps are repeated in order to obtain a sufficient number of larvae to carry out all research experiments.

2.3. Bioassay experiments

The extracts were obtained and their insecticidal activity was measured against mosquito larvae in the Natural Products Laboratory of the Dengue Mosquito Research Unit and Vector Control, Department of Biological Sciences KAU.

2.3.1. Seagrasses tested

Three types of seagrasses (*Cymodocea rotundata*; *Halophila ovata* & *Thalassia hemprichii*) that grow in the Gulf of Salman area on the Saudi Red Sea coast were collected as shown in (Fig. 5).

2.3.2. Preparation of extracts

The three samples were washed well with water to get rid of sediments, algae and suspended dust, then the washing process was repeated using distilled water after that samples were dried at room temperature to keep the compounds from breaking down. The leaves of each sample were crushed separately with an electric grinder and placed inside a sealed plastic box until the start of extraction.

Cold extraction was carried out using a mixture of seagrasses powder and methanol alcohol in a ratio of 1:3 taking into account the shaking of the samples by Vortex Shakers (The solvent was removed for each extract separately using a Rotary Vacuum Evaporator at a temperature of 45 °C to obtain the crude extract of the

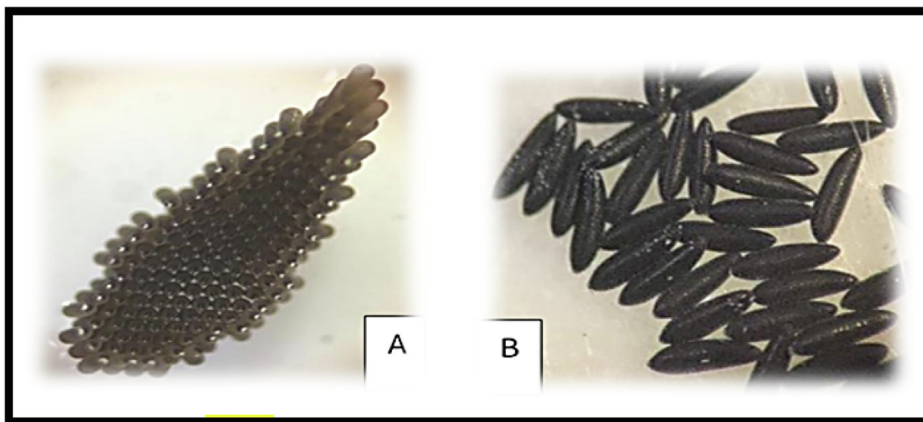


Fig. 1. The eggs (A) *Cx. pipiens* (B) *Ae. Aegypti*.

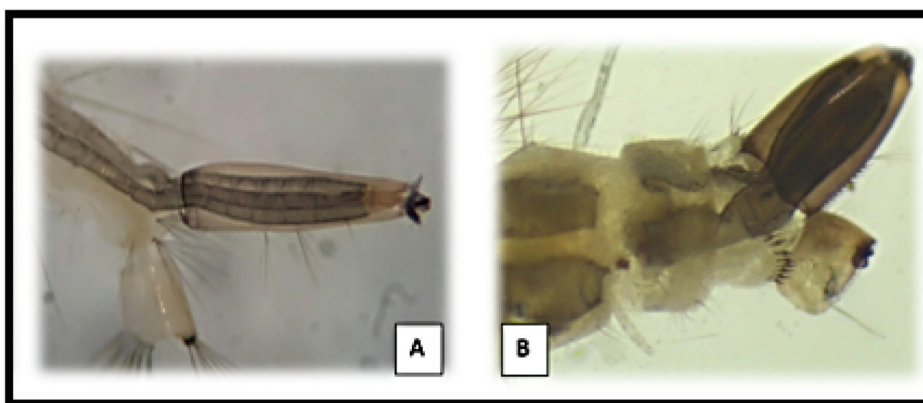


Fig. 2. The larva (A) *Cx. pipiens* (B) *Ae. Aegypti*.

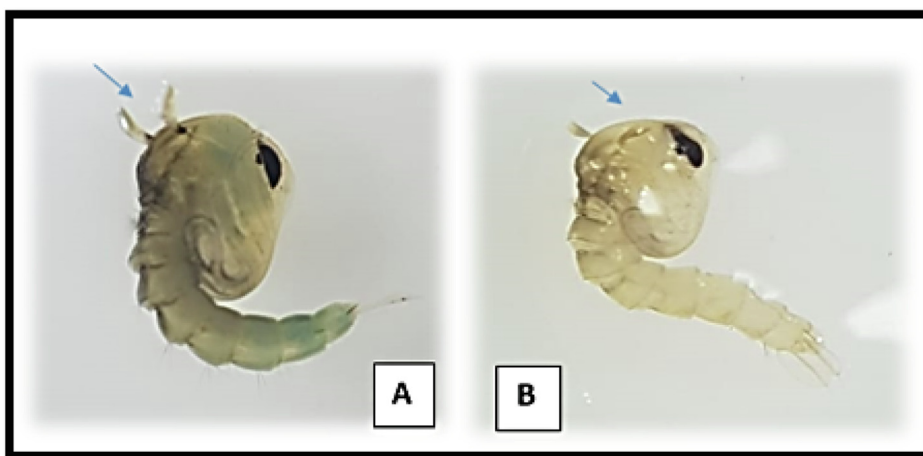


Fig. 3. The pupa (A) *Cx. pipiens* (B) *Ae. Aegypti*.

sample completely dry according to the method (Mahyoub, 2021; Al-Hakimi et al., 2022a).

2.3.3. Preparation of stock solutions

Standard solutions of the tested extracts were prepared by taking 1 g of each extract and dissolving it in 98.5 ml distilled water in a 250-standard flask. 0.5 ml Triton X-100 was added as an emulsi-

fier to ensure a homogeneous distribution of the extract with the extracts completely dissolved using the Ultrasound Sonicator.

2.3.4. Test trials

The tests were carried out in small white plastic dishes (11 cm diameter, 4 cm depth) containing 100 ml of water as a test environment. The larvae at the beginning of the fourth instar were

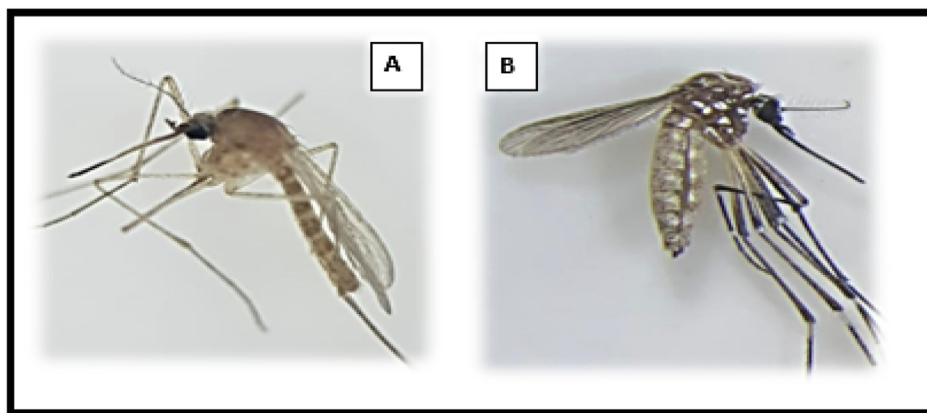


Fig. 4. The adult (A) *Cx. pipiens* (B) *Ae. Aegypti*.

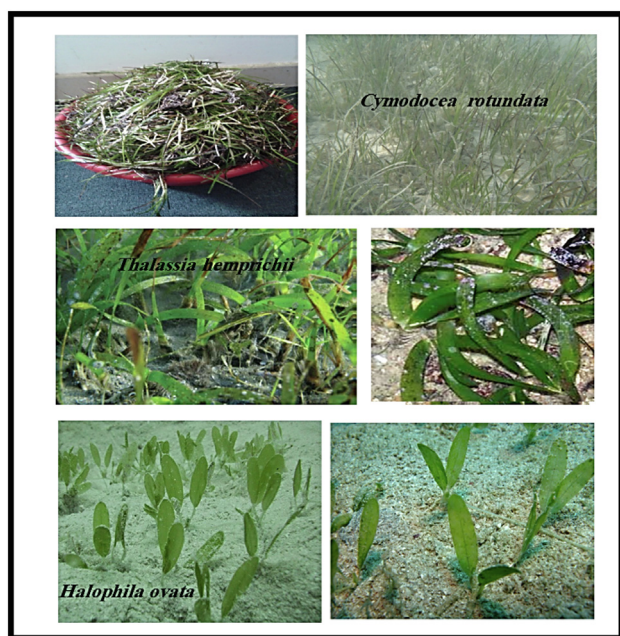


Fig. 5. Seagrasses tested.

exposed to a series of different concentrations of each extract. Five replicates were used for each concentration, where every replicate contained 20 larvae, in addition to five replicates for the control, taking into account the feeding of larvae during the treatment.

The number of dead larvae, as well as the dying larvae, were recorded on a daily basis, taking into account the transfer of live pupae to cups containing clean water to follow up on the late effects of these extracts and recording the number of dead pupae daily and calculating the number of insects that succeed in reaching the adult stage for each replicate separately. Dead larvae and pupae were maintained in 70 % ethyl alcohol to study them under an anatomical microscope to determine the morphological abnormalities in different instars as physiological effects of these extracts.

Where these extracts cause a physiological imbalance during the stages of the development of the insect, similar to the effect of growth hormone, which can lead to the appearance of abnormal molts since these distortions have been taken into account when evaluating this type of compounds. The evaluation was also done on the basis of recording the percentage emergence of adults for

each concentration and calculating the average percentage of inhibition in the exit of adults resulting from larvae treated for each concentration separately, given that the extracts were from compounds with delayed effects, the evaluation was done on the basis of calculating the percentage of cumulative mortality of larvae, pupae and adults.

2.4. Statistical analysis

Abbott's equation was used to correct the percentage of death in the treatments, according to its counterparts in the control, in the experiments in which the death rate at the control exceeded 5 %, and it was less than 20 % Abbott (1925). The results were analyzed using a specialized statistical program IC-p lines. Toxicity curves were drawn that show the relationship between the concentrations used and the percentage of inhibition of the exit of adults resulting from larval treatments by tested extracts and recording all Statistical parameters such as Chi -squared values; LC50; IC50 values; Fiducial limits; Slope and other statistical constants that help to interpret the results and compare between the tested compounds, according to the method of (Litchfield & Wilcoxon 1949).

3. The results

The sensitivity level of the fourth-instar larvae of *Ae. aegypti* and *Cx. pipiens* were measured for some marine grasses extracts (*H. ovata*, *T. hemprichii*, *C. rotundata*.) that grow on the Saudi Red Sea coast. The insecticidal annihilation effect of selected extracts against mosquito larvae was evaluated, whereas the death rates of larvae were recorded, with daily follow-up of experiments until they became pupae, as well as until the exit of the adults.

With the registration of the cumulative mortality ratios in larvae, pupae and adults of insects, as well as the calculation of the percentages of inhibition of the exit of adults and adults of larvae treated with different concentrations of extracts. The IC indicator was used because these compounds are not toxic or have rapid effects, as is the case with conventional chemical pesticides, but are compounds that act with delayed action as an inhibitor of chitin synthesis in insects or a similar effect of juvenile hormone.

The results in Table 1 showed the effective concentrations of *C. rotundata* ranged from 50 – 130 ppm, the death rates of the fourth-instar mosquito larvae of *Ae. aegypti* and *Cx. pipiens* were in the range of (20–74) % and (16–70) %, respectively. Percent inhibition of exit of adult insects varied from (27–92) for *Ae. aegypti* and from (22–87) % for *Cx. pipiens* at the concentrations tested ranging from

Table 1The biological effects of the Seagrasses extracts *C. rotundata*, *T. hemprichii* and *H. ovata* on the developmental stages of *Ae. aegypti* and *Cx. pipiens*.

Seagrasses extracts	Mosquito Species	Effective Concentrations (ppm)	Larval mortality* (%)	Pupation (%)	Adultemergence (%)	Adult emergence Inhibition (%)
<i>C. rotundata</i>	<i>Ae. aegypti</i>	50–130	20–74	80–26	73–8	27–92
	<i>Cx. pipiens</i>	50–130	16–70	84–30	78–13	22–87
<i>T. hemprichii</i>	<i>Ae. aegypti</i>	50–170	10–80	90–20	78–7	22–93
	<i>Cx. pipiens</i>	50–170	6–76	94–24	83–12	17–88
<i>H. ovata</i>	<i>Ae. aegypti</i>	50–170	18–88	82–12	78–3	22–97
	<i>Cx. pipiens</i>	50–170	14–84	86–16	83–8	17–92

* Five replicates were used, with 20 larvae/repeat; control adult emergence Inhibition ranged from 0.0 – 2%.

(50–130) ppm, with a mean death recorded in the control treatment of 2 %.

The values of IC₅₀ and IC₉₀ were (70.78 & 128.4) ppm for *Ae. aegypti* and (77.47 & 141.5) ppm for *Cx. pipiens*. The (RR) values showed that *C. rotundata* extract was more effective against *Ae. aegypti* by 1.09 times than its effect on *Cx. pipiens* as shown in Table 2 and Fig. 6.

The effective concentrations of *T. hemprichii* extract ranged between (50–170) ppm, while the percent inhibition of adult insect emergence resulting from the treated larvae ranged between (22–93 %) for *Ae. aegypti* and (17–88) % for *Cx. pipiens* Table 1.

The IC₅₀ and IC₉₀ values were (86.98 and 178.9) ppm for *Ae. aegypti* and (95.86 and 198.08) ppm from *Cx. pipiens*, respectively. According to the resistance ratio values obtained, the fourth instar larvae of *Cx. pipiens* were more tolerant to the extract concentrations of *T. hemprichii* than those of *Ae. aegypti* by 1,102 times as shown in Table 3 and Fig. 7.

The level of sensitivity of fourth-instar larvae for *Ae. aegypti* and *Cx. pipiens* to the *H. ovata* extract were evaluated, with the results as shown in Table 1 and Fig. 8 showing that the percentages of larval death ranged from (18–88) and (14–84) % when treated at concentrations between (50–170) ppm for both species. While the percent inhibition of emergence of adults resulting from larvae that were treated with *H. ovata* extract varied from (22–97) % for *Ae. aegypti* and (17–92) % for *Cx. pipiens* at the same concentrations.

The IC₅₀ and IC₉₀ values were between (83.9 & 162.8) ppm for *Ae. aegypti* and (88.8 & 165.5) ppm for *Cx. pipiens* respectively. The results also indicate that fourth-instar larvae of *Cx. pipiens* were more tolerant when treated with *H. ovata* extract compared to *Ae. aegypti* with a value of 1,058 times, corresponding to the resistance ratio values in the Table 4.

The results of this study show that there are differences in the sensitivity level of mosquito larvae to the tested extracts. *Ae. aegypti* is more susceptible to *C. rotundata* extract compared to *H.*

Table 2The values of inhibitory concentrations for the emergence of 50 & 90% of adult insects resulting from the treatment of fourth instar larvae and the different stages of *Ae. aegypti* and the *Cx. pipiens* by the extract of *C. rotundata*.

Statistical parameters	Mosquito Species		Resistance Ratio (RR)
	<i>Ae. aegypti</i>	<i>Cx. pipiens</i>	
IC ₅₀ (ppm) 95 % (F. L.)	70.78 66.09–75.10	77.47 72.77–82.03	1.095
IC ₉₀ (ppm) 95 % (F. L.)	128.4 117.6–144.4	141.5 128.7–161.2	
Slope	4.9533	4.8954	
Tabulated (Chi) ²	7.81	7.81	
calculate (Chi) ²	3.4305	2.0527	

(Chi)² Tabulated is larger than calculated, at a 0.05 level of significance which indicates the homogeneity of results.

Five replicates, 20 larvae for each.

The mortality rate in the control ranged from% (2–0).

ovata and *T. hemprichii* extracts by (1,186 & 1,229) fold, respectively, Table 5 and Fig. 9, while *Cx. pipiens* larvae were approximately (1,14&1.23) times more susceptible to the *C. rotundata* extract compared to the *H. ovata* and *T. hemprichii* extracts respectively, as shown in Table 6 and Fig. 10.

On the other hand, the results obtained confirmed that the seagrasses extracts tested had biological effects against the different developmental stages of both *Ae. aegypti*- as well as *Cx. pipiens* species, and these abnormalities resembled those induced by insect growth regulators (IGR).

Through Figs. 11–12, the results of this study demonstrated the failure of some mosquito larvae to moult to the pupal stage, where the extract-treated larvae gave intermediate stages combining characteristics of larva and pupa or between pupa and adult insect, in addition to the appearance of dwarfism and shrinkage of the body segment of the larvae, where all the intermediate stages die-off. The study also showed that some of the larvae, which appeared to have developed into a natural pupa, either died before reaching the adult stage or died as albino pupae immediately after moulting. In addition, some of the adults that emerged from the extract-treated larvae had wings that were folded out of the pupa or attached to the skin during moulting.

4. Discussion

There is no doubt that the random and wasteful use of chemically manufactured pesticides has caused serious damage to the environment and to human and animal public health, that often outweighs the desired benefits of their use, in addition to the fact that many species of mosquitoes have it acquired resistance to many of them; therefore, the need arose to look for safer alternatives that would help control mosquitoes and reduce their harm. Using plant-based insecticides is one of the safest ways to overcome the problems associated with using synthetic compounds in mosquito control. In this study, the results showed that seagrasses extracts tested under laboratory conditions were effective against two species of mosquitoes; *Ae. aegypti* which causes dengue fever and *Cx. pipiens*, which is prevalent species in Saudi Arabia.

The results showed late efficacy of the sea-grasses extract in the pupation process and exit of adults, therefore IC₅₀ was used as a standard to assess the effectiveness of the extracts tested against selected mosquito species, and results of this study are consistent with many studies on the effect of plant extracts on various species of mosquito (Elimam and Ombabi, 2007; Elimam et al., 2009; Zahran & Abdelgaleil, 2011).

The results also showed that the sensitivity of the mosquito larvae and the resulting stages of the larvae treated with the tested sea-grasses extracts vary, which may be due to the levels of active substances contained in these plants, therefore differ in their effect, or maybe because to the active substances contained in the tested-

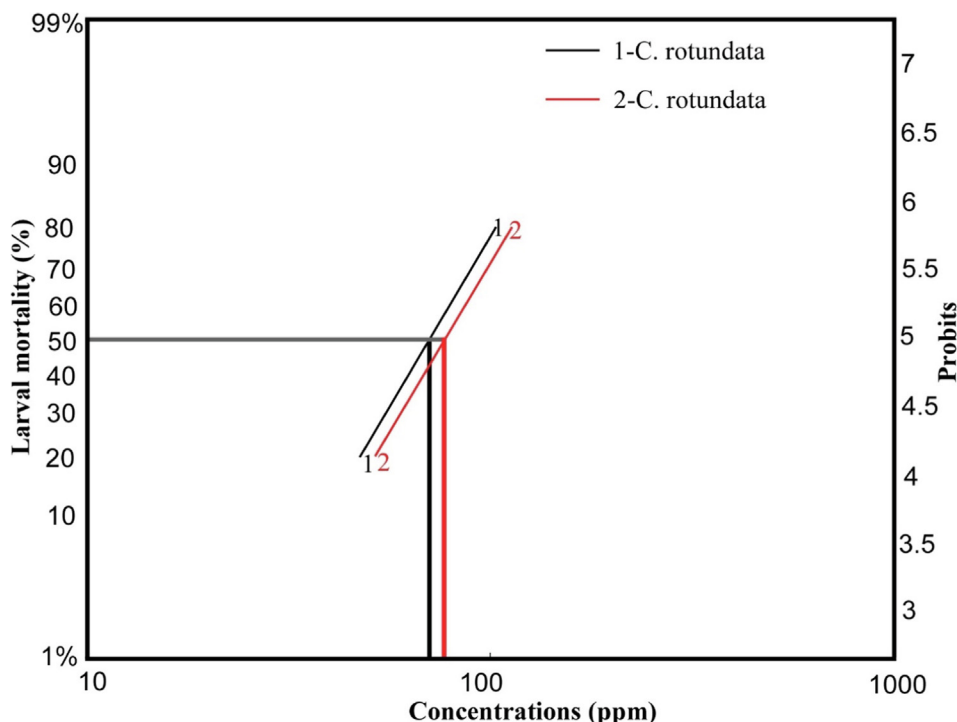


Fig. 6. The relationship between the tested concentrations of *C. rotundata* extract and the percentage of adult emergence inhibition resulting from the treatment of the fourth instar larvae of *Ae. aegypti* & *Cx. pipiens*.

Table 3

The values of inhibitory concentrations for the emergence of 50 & 90% of adult insects resulting from the treatment of fourth instar larvae and the different stages of *Ae. aegypti* and the *Cx. pipiens* by the extract of *T. hemprichii*.

Statistical parameters	Mosquito Species		Resistance Ratio (R.R)
	<i>Ae. aegypti</i>	<i>Cx. pipiens</i>	
IC ₅₀ (ppm)	86.98	95.87	1.102
95 % (F. L.)	80.46–93.29	89.10–102.6	
IC ₉₀ (ppm)	178.9	198.08	
95 % (F. L.)	160.5– 206.8	198.08–232.05	
Slope	4.0903	4.0661	
Tabulated (Chi) ²	7.81	7.81	
calculated (Chi) ²	5.3592	2.6408	

(Chi)² Tabulated is larger than calculated, at a 0.05 level of significance which indicates the homogeneity of results.

Five replicates, 20 larvae for each.

The mortality rate in the control 1%.

extracts, which need to be separated and isolated in subsequent studies to determine their chemical composition and mode of action (Ghosh et al., 2012; Al-Hakimi et al., 2022b).

By analyzing the results, it was observed that there was a positive relationship between the concentrations tested and larval mortality rates, as well as the percentage of inhibition of adult insect emergence. The reason for this may lie in the increased binding of active ingredients to sensitive sites in the insect’s body with increasing concentration, and possibly the reason lies in a decrease in the enzymes that decompose toxic substances released, which are released by insects as a defense method for species preservation, at the low concentration) Mansour et al., 2010).

On the other hand, *Cx. pipiens* larvae are more tolerant to the tested marine extracts and this may be due to cross-resistance, as marine extracts have a mode of action quite similar to the group of insect growth regulators, that is the most common and used to control mosquito larvae in swamps, which are suitable environ-

ments for this species of mosquito, unlike the *Ae. aegypti* that prefers shaded water and indoors.

It is worth noting that there are many indicators confirming the breadth of mosquito resistance to all commonly used organophosphorus groups and pyrethroid compounds, as well as many evidence of emerging resistance against some of the IGRs according to (WHO, 1990).

Overall, the current study was consistent with many scientific researches conducted in most countries around the world, to confirm the effectiveness of many plants and sea-grasses extracts against different species of mosquitoes. These scientific studies showed the importance of using plant extracts as alternative methods in the field of mosquito control, which transmits many pathogens, to reducing the problems of traditional chemical pesticides causing the death of natural enemies, which alters the biotic balance in the environment. In addition to the emergence of a resistance mosquito strains to these pesticides. (Mahyoub et al., 2016a, 2016b, 2016c; Murugan et al., 2017; Barnawi et al., 2019; Mahyoub, 2021).

The results of this study showed that treatment of fourth-instar larvae of *Cx. pipiens* and *Ae.aegypti* with marine extracts resulted in intermediate instars having larval and pupa characteristics or between pupa and adult insect characteristics and some larvae evolved into albino pupae. In addition to that, some of the adult insects that evolved from the extract-treated larvae were related to the moulting skin of the pupae, and these results are consistent with much previous research (Bridges et al., 1977; Saleh & Aly, 1987; Al-Sharook et al., 1991).

The action of these extracts in all growth stages may be due to the fact that they contain compounds whose effect on mosquito larvae is similar to that of the mode of action of insect growth regulators, such that these compounds interfere with the physiological processes of the insect during its metamorphosis, or there may be an imbalance between stimulating or inhibiting the secretion of Ecdysone hormone or Juvenile hormone. or due to that

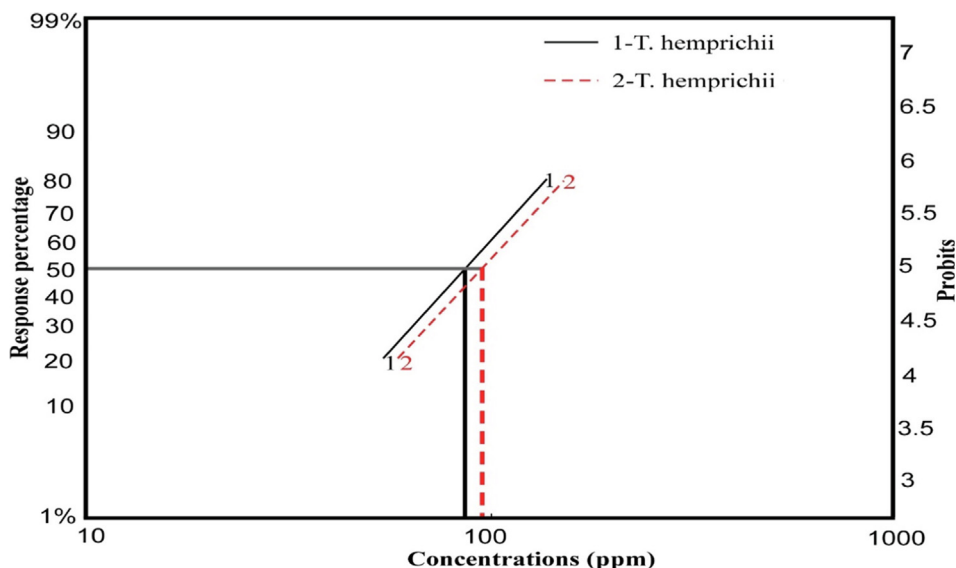


Fig. 7. The relationship between the tested concentrations of *T. hemprichii* extract and the percentage of adult emergence inhibition resulting from the treatment of the fourth instar larvae of *Ae. aegypti* & *Cx. pipiens*.

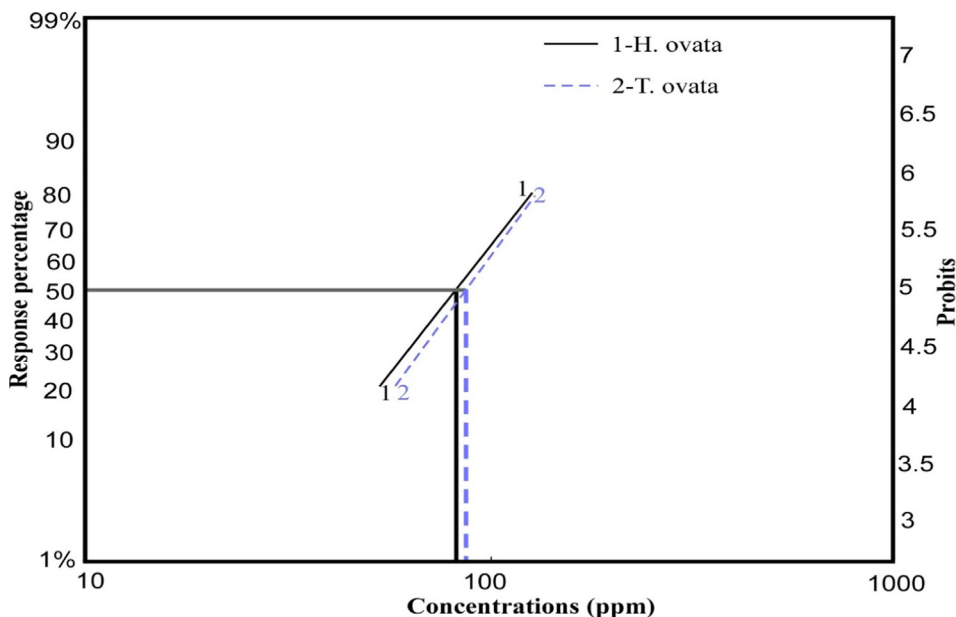


Fig. 8. The relationship between the tested concentrations of *H. ovata* extract and the percentage of adult emergence inhibition resulting from the treatment of the fourth instar larvae of *Ae. aegypti* & *Cx. pipiens*.

Table 4

The values of inhibitory concentrations for the emergence of 50 & 90% of adult insects resulting from the treatment of fourth instar larvae and the different stages of *Ae. aegypti* and the *Cx. pipiens* by the extract of *H. ovata*.

Statistical parameters	Mosquito Species		Resistance Ratio (RR)
	<i>Ae. aegypti</i>	<i>Cx. pipiens</i>	
IC ₅₀ (ppm) 95 % (F. L.)	83.94 77.76–0.117	88.82 82.98–94.52	1.058
IC ₉₀ (ppm) 95 % (F. L.)	162.83	165.52	
Slope	4.4534	4.741	
Tabulated (Chi) ²	7.81	7.81	
calculated (Chi) ²	6.8805	3.9269	

(Chi)² Tabulated is larger than calculated, at a 0.05 level of significance which indicates the homogeneity of results.

Five replicates, 20 larvae for each. The mortality rate in the control 0.0%.

Table 5

Comparison of the sensitivity level of fourth instar larvae of *Ae. aegypti* for tested seagrasses *H. ovata*, *T. hemprichii*, *C. rotundata*.

No	Line name	IC ₅₀ (ppm)	RR
1	<i>Cymodocea rotundata</i>	70.78	1
2	<i>Halophila ovata</i>	83.94	1.186
3	<i>Thalassia hemprichii</i>	86.98	1.229

these compounds can disrupt the work of hormones secreted by the endocrine glands, which leads to a defect in the growth process and death of the insect. These abnormalities arising from this study are similar to those resulting from the effect of growth regulators on mosquito larvae observed by many researchers (Silva & Mendes.2007; Arivoli & Tennyson 2011).

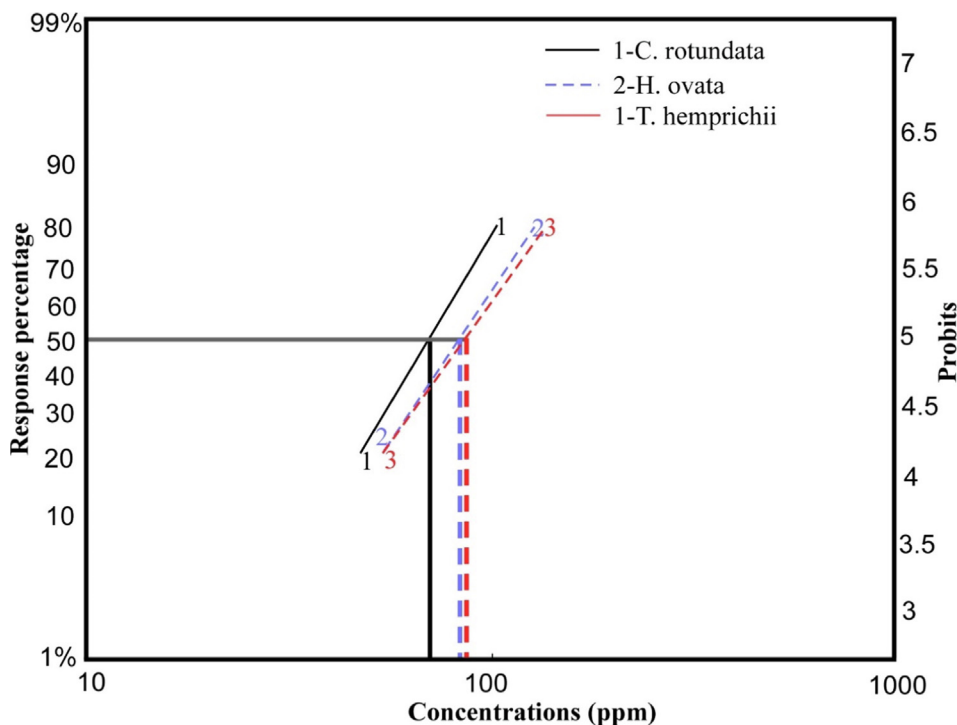


Fig. 9. IC-p lines that show the relationship between the concentrations of the tested extracts (C. rotundata, T. hemprichii & H. ovata) and the percentage of mortality of the fourth instar larvae of Ae. aegypti after continuous exposure to the extract for several days.

Table 6
Comparison of the sensitivity level of fourth instar larvae of Cx. pipiens for tested seagrasses H. ovata, T. hemprichii, C. rotundata.

No	Line name	IC ₅₀ (ppm)	RR
1	Cymodocea rotundata	77.48	1
2	Halophila ovata	88.825	1.146
3	Thalassia hemprichii	95.865	1.237

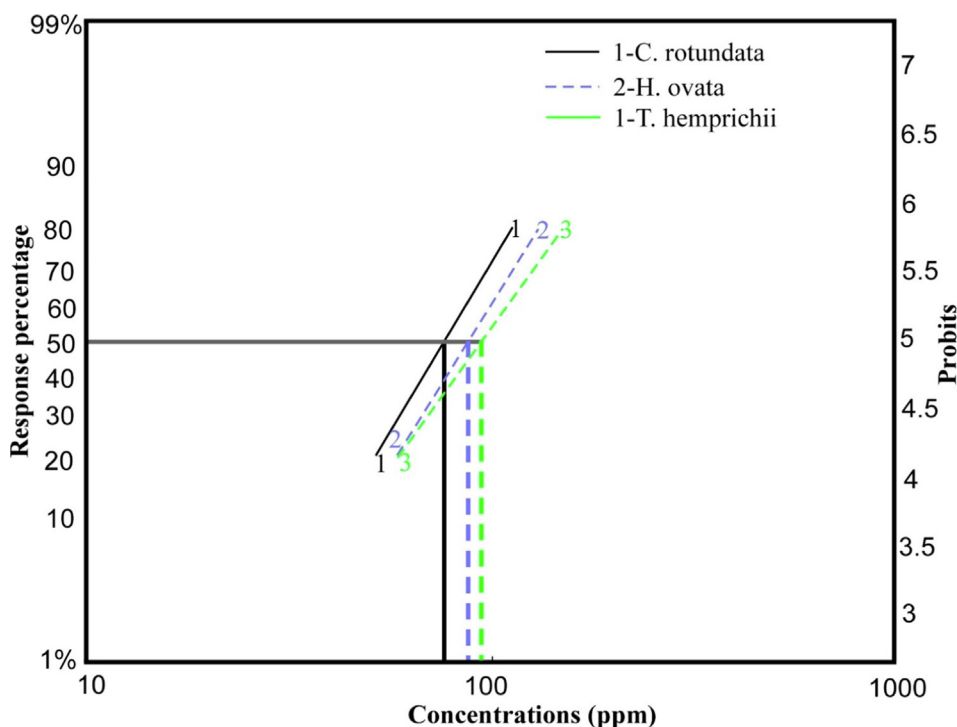


Fig. 10. IC-p lines that show the relationship between the concentrations of the tested extracts (C. rotundata, T. hemprichii & H. ovata) and the percentage of mortality of the fourth instar larvae of Cx. pipiens after continuous exposure to the extract for several days.

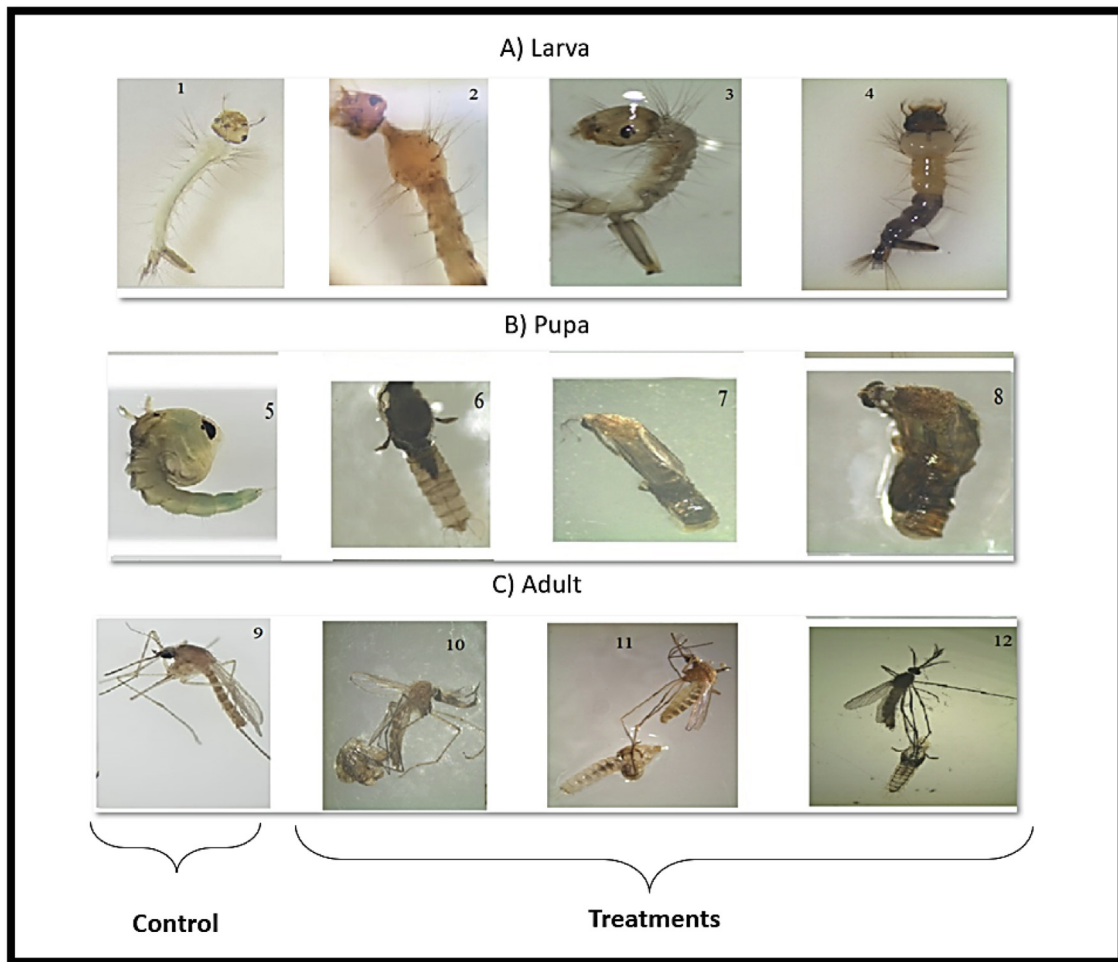


Fig. 11. Morphological effects of the tested extracts against different stages of *Cx.pipiens*. A: (1) untreated larva; (2) Elongation in the neck; (3) Shrinking of the abdominal rings; (4) pigmentation B): (5) Untreated pupa; (6) An intermediate stage between adults and pupa; (7) An intermediate stage; (8) Failed to develop in to an adult insect C): (9) Untreated adult mosquitoes; (10–12) Failed to get the adult insect out of moulting skin.

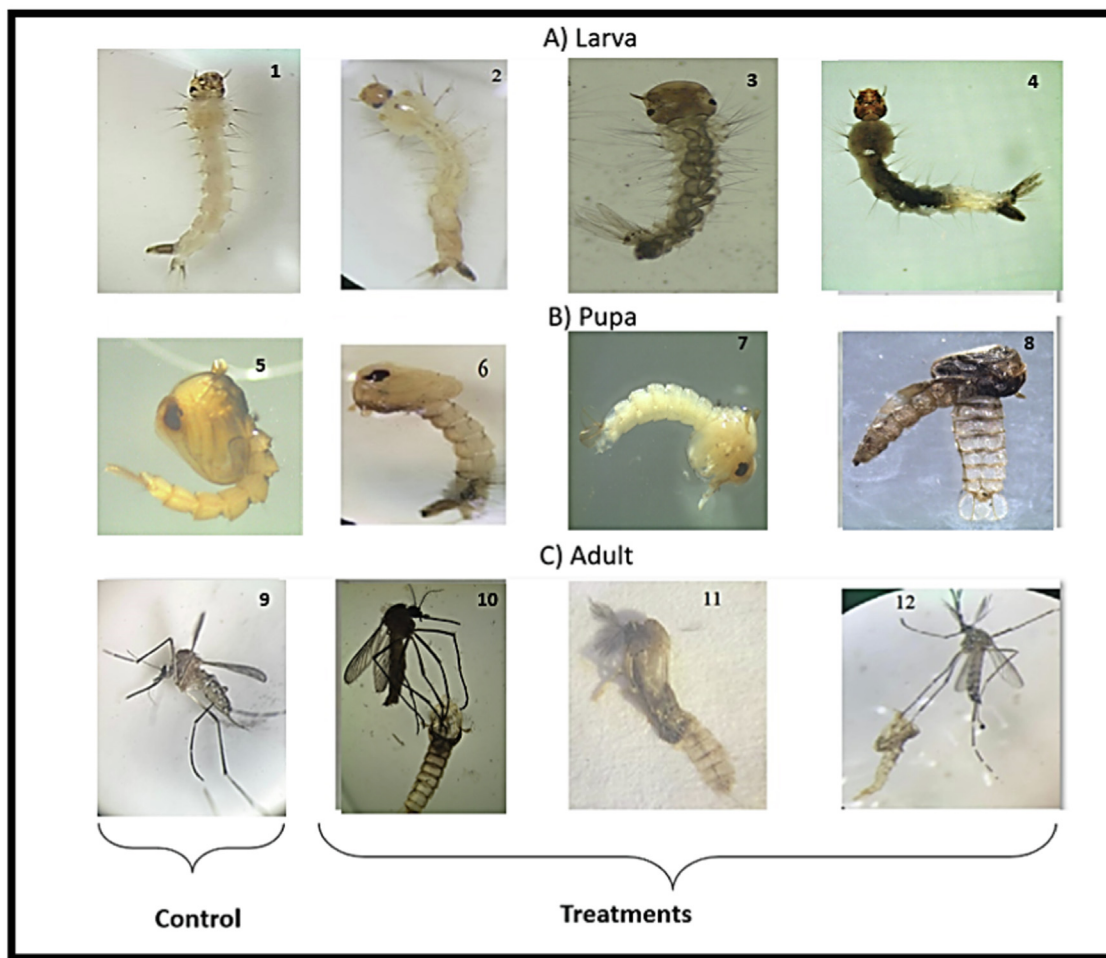


Fig. 12. Morphological effects of the tested extracts against different stages of *Ae. aegypti* A: (1) untreated larva; (2) Elongation in the neck; (3) Shrinking of the abdominal rings; (4) pigmentation B): (5) Untreated pupa; (6) An intermediate stage between adults and pupa; (7) Albino pupa; (8) Failed to develop into an adult insect C): (9) Untreated adult mosquitoes; (10–12) Failed to get the adult insect out of moulting skin.

From the above, it can be said that conducting bioassay experiments against the prevalent mosquitoes in an area will provide baseline data on their level of sensitivity to compounds used in that area and will understand the nature of mosquito resistance to them. This is undoubted of paramount importance when planning mosquito control programs and making the right decisions about the best use of pesticides (Paeporn et al., 2005).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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