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# Larvicidal and Inhibition Activities of Marine Algae *Ulva lactuca* Extracts on *Culex pipiens* Mosquito

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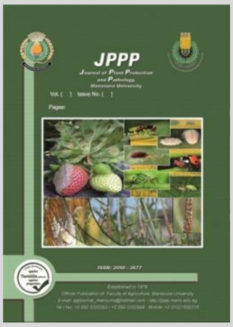


## ABSTRACT

This study aimed to investigate the larvicidal activity and inhibition effect of Sea lettuce *Ulva lactuca* extracts using various organic solvents (acetone, ethanol, and petroleum ether), against mosquito larvae of *Culex pipiens* (L.). The results revealed that all the extracts showed varied levels of larvicidal activity and inhibition effect against *C. pipiens*. Among them, the maximum efficacy was observed in the acetone extract which resulted in the strongest inhibition effect in the adult emergence (95%) and showed larvicidal activity ($LC_{50}$=5.004 and $LC_{90}$=9.76 mg/ml). Moreover, the three extracts of *U. lactuca* caused a reduction in the egg-laying capacity of mosquito females. Besides, the acetone extract caused the highest decrease ($P < 0.05$) on the mean number and hatchability of eggs produced by mosquito females. Thus, the present study suggested that using acetone, ethanol, and petroleum ether extracts of *U. lactuca* could be an effective method, somewhat easier, safe, and less expensive for controlling *C. pipiens* mosquito.

***Keywords:*** Mosquito larvae, Eggs production, Sea lettuce, solvent extracts.

## INTRODUCTION

Mosquito is the most important vectors transmitting a variety of diseases for humans and animals. Among them, *Culex pipiens* (L.) (Diptera: Culicidae) is the common and widely distributed mosquito in Egypt which causing nuisance and transmitting many dangerous diseases such as Rift Valley Fever, West Nile Fever viruses, St Louis encephalitis virus and periodic lymphatic filariasis (Southgate 1979, Farid *et al.*, 2001, Hanafi *et al.*, 2011 and Farajollahi *et al.* 2011).

Mosquito control larvae have been achieved by the use of synthetic organic insecticides such as organophosphorus, chlorinated, carbamate, pyrethroid, and insect growth regulators (WHO, 2006). However, because of many problems of environmental pollution by using these synthetic insecticides, and the development of insecticide resistance in many species of mosquito vectors; considerable efforts were being made to find alternative control methods safer to non-target organisms, and to be selective agents (Barnard, 1997, Abd-El Samie and Abdel Basset 2012). Controlling mosquito needs natural sources that added easily to water and can't cause any side effects on plants, humans, and the environment. Plant extracts have potential as mosquito control agents because many of them are selective, may biodegrade to nontoxic products, and easily applied to mosquito breeding sites (Samidurai *et al.*, 2009).

Several studies mentioned that marine extracts of some seaweeds and algae have been reported for their larvicidal activity against different mosquito vectors (Bianco and Santos, 2013; Abbassy *et al.*, 2014; Valentina *et al.*, 2015 and Mahyoub *et al.*, 2016). Some seaweed extracts or compounds act as general toxicants that cause acute mortality to various life stages of mosquito (Abou-Elnaga *et al.*, 2011). However, some extracts can potentially be used as growth, feeding, and reproduction inhibitors (Elbanna and Hegazi 2011; Asha *et al.* 2012). *Ulva lactuca* is a green macroalga called Sea lettuce, was isolated from the marine environment. The chemical structure of *U. Lactuca* contain many biologically active components such as carbohydrates, proteins, lipids, minerals, fibers, phenolic compounds, chlorophylls, carotenoids, flavonoids, alkaloids, terpenes, and phytosterols, so their biological activities were as antioxidants, antimicrobial, and insecticidal activity (Yu-Qing *et al.*, 2016). Extracts of *U. lactuca* showed an insecticidal effect on mosquito larva and prevent any birth of new mosquito (Abbassy *et al.*, 2014, and Mahyoub, 2018). Thus, the present study aimed to investigate the larvicidal activity and inhibition effect of Sea lettuce *U. lactuca* using various organic solvent extracts (acetone, ethanol, and petroleum ether), against *C. pipiens* mosquito.

## MATERIALS AND METHODS

### Collection and Extraction of algae (*U. lactuca*)

The present study was carried out in the Laboratory of medical entomology, Department of Plant Protection, Faculty of Agriculture, Damanhour University, Egypt from April to September 2019. Marine algae *U. lactuca* Linnaeus were collected during low tides between April to September 2019 from submerged rocks on the coast of Abu Qir and Sidi Gaber beach. The collected seaweed was washed first with seawater, tap water, and then with distilled water to remove salt, sand, and extraneous matter.

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The samples were air-dried at room temperature until it becomes brittle. The dried algae were chopped into small pieces using a domestic blender and dried in an oven at 40 °C. For extraction, 60-80 gm of powdered algal material was extracted by the Soxhlation method using acetone, ethanol, and petroleum ether as solvents. The extracts were concentrated by a rotary evaporator. The resultant extractive collected in airtight glass vials (10 cm) and stored in the refrigerator (at 4 °C) for further use.

**Mosquito culture rearing**

The laboratory strain of *C. pipiens* L. which used in the present study was obtained from wild larvae which collected from Damanhour city, El-Behera Governorate, Egypt, in October 2016. Mosquitoes were held at 27±1 °C, 70±5% RH, and a photo regime of 14:10 (light: dark) hr. Adults were provided with a 10% sucrose solution as a food source. A pigeon was introduced twice a week to the adults for blood-feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food.

**Larvicidal bioassay test**

The larvicidal effect of the extracts was conducted following the WHO standard method (WHO; 2005). Briefly, the stock solution of seaweed extracts was prepared by adding 1ml of it to 99 ml of distilled water containing dimethyl sulfoxide (DMSO) as an emulsifier to ensure complete solubility of the extract in water. Batches of 25 early 4*th* instar larvae of *C. pipiens* were transferred to 250 mL cups containing 100 mL of distilled water and a series of concentrations of the extracts. Each concentration was conducted in four replicates. A control group consisted of 1 mL of DMSO and 99 mL of distilled water only. The larvae were given the usual food during the experiments. Mortalities of larvae and pupae were recorded daily. Live pupae were transferred to untreated water in new cups for further observations (adult emergence or death).

**Delayed effects of seaweed extracts**

The values of $IC_{50}$ (concentration which gave approximately 50% inhibition of adult formation) of the present seaweed extracts were prepared and chosen for treating the 4*th* larval instars. Twenty replicates of 20 larvae each were conducted for each concentration. Mosquito females which survived from larval treatments were isolated in clean cages. Seventy-two hours later, emerged females were fed on a pigeon for a blood meal. Each engorged female was kept with a male in a small glass cup, half-filled with tap water and covered by muslin cloth. These couples were fed on a 10 % sugar solution. The number of eggs laid per female and hatchability of eggs was recorded for the 1*st* gonotrophic cycle.

**Statistical analysis**

The SPSS software package was used for computing all the data including statistical parameters such as $LC_{50}$, $LC_{90}$ $IC_{50}$, $IC_{90}$, 95% fiducial limits of upper confidence limit (UCL), and lower confidence limit (LCL), slope the concentration-mortality regression line and Chi-square. Results with $P < 0.05$ were considered to be statistically significant.

## RESULTS AND DISCUSSION

The larval mortalities were 18-89%, 13-88% and 22-79%, when the fourth instar larvae were treated with effective concentrations of acetone (1-9 mg/ml), ethanol (7-16 mg/ml) and petroleum ether (22-36 mg/ml) extracts of *U. lactuca*, respectively. As shown in Table (1), the highest larvicidal activity was recorded for acetone extract which showed a lethal concentration ($LC_{50}$) of 5.004 mg/ml and $LC_{90}$ equal 9.76 mg/ml. Furthermore, the ethanol extract shows larvicidal activity with $LC_{50}$ of 11.70 mg/ml and $LC_{90}$ equal 17.04 mg/ml. However, the lowest efficiency was observed with petroleum ether extract with $LC_{50}$ of 31.69 mg/ml and $LC_{90}$ of 38.11mg/ml, respectively.

Some studies have focused on the insecticidal properties of *U. lactuca* against mosquito larvae (Thangam and Kathiresan 1991, Manilal *et al*. 2011, Abbassy *et al.* 2014 and Mahyoub 2018). Accordingly, the present study explained the larvicidal activity and inhibition influence of Sea lettuce *U. lactuca* extracts against *C. pipiens* mosquito by using several solvents (acetone, ethanol, and petroleum ether). Our results showed that acetone extract was the most effective extract against the fourth instar larvae of *C. pipiens*. These results were in parallel agreement with the results of the previous study of Abbassy *et al.,* (2014), who reported that acetone extract of *U. lactuca* was the most potent as larvicide ($LC_{50}$=5.46 mg/ml) against *C. pipiens* larvae followed by ethanol ($LC_{50}$=12.82 mg/ml), petroleum ether ($LC_{50}$=27.55 mg/ml), and methanol (27.35 mg/ml), extracts respectively. Likewise, Thangam and Kathiresan, (1991), were demonstrated that acetone extract of the marine seaweed *U. lactuca* was active against fourth instar larvae of *Aedes aegypti* with $LC_{50}$ of 91.20 ppm. Also, Mahyoub, (2018), reported that larval mortalities were 17-88% when the fourth instar larvae of *Anopheles d'thali* were treated with effective concentrations of *U. lactuca* equal 200-600 ppm.

**Table 1. Larvicidal activity of different extracts of *U. lactuca* against *C. pipiens* larvae**

| Extracts | Statistical parameters | | | |
|---|---|---|---|---|
| | $LC_{50}$ (mg/mL) (95% CL) | $LC_{90}$ (mg/mL) (95% CL) | [a]Slope ± SE | [b]$X^2$ |
| Acetone | 5.004 (4.55-5.46) | 9.76 (8.94-10.87) | 2.07 ± 0.024 | 3.376 |
| Ethanol | 11.70 (11.70-12.22) | 17.04 (16.13-18.26) | 5.90 ± 0.021 | 2.41 |
| Petroleum ether | 31.69 (31.69-32.27) | 38.11 (36.89-39.91) | 13.91±0.022 | 0.74 |

**95% CL: Confidence Limits, larval mortality in control 2%, a: Slope of the concentration-mortality regression line ± standard error, b: Chi square value.**

The sublethal effects of *U. lactuca* extract against *C. pipiens* larvae were observed until death or emergence. Therefore, in the present study, cumulative mortalities during larval development to pupae and adults have been taken as a standard for evaluating the *U. lactuca* extracts against *C. pipiens*. The records showed that larval treatments with the effective concentrations of the acetone, ethanol, and petroleum ether extracts caused 24-95%, 15-90%, and 28-90% inhibition of adult emergence, respectively. Tacking $IC_{50}$ values into consideration, the acetone extract proved to be the most effective extract against *C. pipiens* (3.91 mg/ml), followed by ethanol (11.31 mg/ml), and petroleum ether (30.42 mg/ml) extracts respectively (Table 2).

The previous results were in partial agreement with other studies (Yu *et al*., 2014, Ke-Xin *et al*., 2015, Mahyoub, 2018). Where the larvae of *Anopheles d'thali* were affected by using a seaweed extract of *U. lactuca* and the values of $IC_{50}$ and $IC_{90}$ were 335.3 and 588.3 ppm respectively (Mahyoub, 2018). Also, Ke-Xin *et al.,* (2015) reported that methanol extract of *Sargassum binderi* resulted in the strongest inhibition effect in adult emergence (98.67%) of *Ae. aegypti*. Furthermore, the extracts of nonpolar solvents of *U. lactuca* showed higher insecticidal activity than extracts of polar solvents (Hadear, 2019). The larvicidal activity of the seaweed might be due to that seaweed contains effective components (i.e. alkaloids, flavonoids, terpenoids, saponins, and steroids) with larvicidal properties against mosquitoes (Cetin *et al.,* 2010, Kalimuthu *et al.,* 2014 and Yu *et al.,* 2014). Also, the response of mosquito larvae could depend on the type of extract and its effective concentrations.

**Table 2. Effect of different extracts of *U. lactuca* on the inhibition of adult *C. pipiens* emergence**

| Extracts | Statistical parameters | | | |
|---|---|---|---|---|
| | $IC_{50}$ (mg/mL) (95% CL) | $IC_{90}$ (mg/mL) (95% CL) | aSlope ±SE | b$X^2$ |
| Acetone | 3.91 (3.46-4.33) | 8.21 (7.55-9.03) | 1.35±0.025 | 4.74 |
| Ethanol | 11.31 (10.78-11.83) | 16.69 (15.80-17.88) | 5.68±0.021 | 3.06 |
| Petroleum ether | 30.42 (29.82-30.94) | 35.79 (34.96-36.96) | 15.97±0.023 | 0.28 |

**95% CL: Confidence Limits, Inhibition of adult emergence in control 4%, a: Slope of the concentration-mortality regression line ± standard error, b: Chi-square value.**

The possible delayed effects of the *U. lactuca* extracts on the reproductive potential of mosquito adults of *C. pipiens* that emerged from the larval treatment with $IC_{50}$ values of acetone (3.91 mg/ml), ethanol (11.31 mg/ml), and petroleum ether (30.42 mg/ml) extracts are shown in Figures (1, 2). The three extracts of *U. lactuca* caused a reduction in the egg-laying capacity of mosquito females (Fig. 1). Moreover, the acetone and ethanol extracts caused the highest reduction ($P < 0.05$) in the mean number of eggs (42.50±4.11, and 49.25±3.11 eggs, respectively) compared with petroleum ether extract (64.75±2.83 eggs), and control (94.25±3.17 eggs, Fig. 1). Furthermore, the acetone, ethanol, and petroleum ether extracts caused a decrease in eggs produced by mosquito females by about 54.90%, 47.75%, and 31.29 %, respectively ($P < 0.05$) as compared with control.

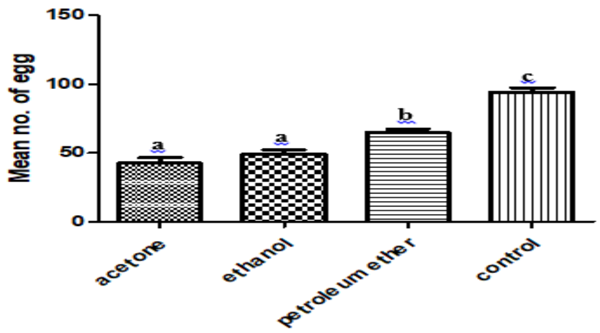


**Fig.1. The mean number of eggs (Mean ± standard error of the mean [SEM]) produced by mosquito females treated with $IC_{50}$ values of acetone, ethanol, and petroleum ether extract of *U. lactuca* or control group.**

**Different letters denote significant differences at P < 0.05.**

With regards to the hatchability of eggs, the same trend of decrease ($P < 0.05$) was recorded for mosquito females that emerged from larval treated with $IC_{50}$ values of acetone, ethanol, and petroleum ether extracts of *U. lactuca* (Fig. 2). The vital activity for acetone extract caused the highest decrease ($P < 0.05$) on the egg hatching percentage compared with other extracts and control group (61.50%, 74.25%, 82.25%, and 94.75% for acetone, ethanol, petroleum ether extracts, and control group, respectively, Fig. 2). Moreover, the acetone, ethanol, and petroleum ether extracts caused a decrease in the hatchability of eggs produced by mosquito females by about 35.09%, 21.64%, and 13.19%, respectively ($P < 0.05$).

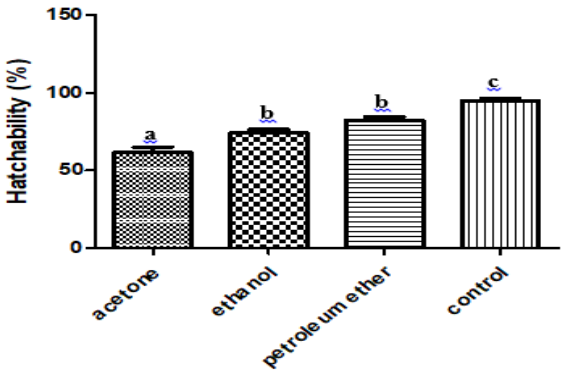


**Fig. 2. The hatchability percentage (Mean % ± standard error of the mean [SEM]) of eggs produced by mosquito females treated with $IC_{50}$ values of acetone, ethanol, and petroleum ether extract of *U. lactuca* or control group.**

**Different letters denote significant differences at P < 0.05.**

To the present knowledge, research on the delayed effects of the *U. lactuca* extracts on the reproductive potential of mosquito is scarce. However, seaweed extracts exhibited effects toward various life stages of other insects. The seaweeds, *U. fasciata and U. lactuca* caused a reduction of body wet weight, adult longevity, female fecundity, and hatchability percentage of red cotton bug *Dysdercu cingulatus* (Asha *et al*., 2012). Also, brown seaweed *Padina pavonica* exhibited nymphicidal and ovicidal activities against red cotton bug *Dysdercus cingulatus* (Sahayaraj and Kalidas 2011). Besides, the benzene extract of *P. pavonica* reduced the egg hatchability and the survival rate of *D. cingulatus* eggs from 53.33% to 0.00%. (Sahayaraj and Kalidas 2011). Too, the present study mentioned that *U. lactuca* extracts have a negative potential effect on the mean number and hatchability of eggs produced by mosquito females. These might be due to that *U. lactuca* extracts contain valuable resources, of precious chemical components that have biological activity against egg production by the mosquito females. However, long term follow-up trials were needed to explain the possible delayed effects of larval treatments with *U. lactuca* extracts on biological and behavioral aspects of mosquito adult.

## CONCLUSION

It is evident from the present study results that treatment with seaweed extracts, Sea lettuce *U. lactuca* showed promising mosquito larvicidal activity against *C. pipiens*. Also, these marine extracts could be suitable alternatives to synthetic chemical insecticides in the future as they are relatively safe, ecological, and are easier to use.

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## النشاط الإبادي اليرقي والتثبيطي لمستخلصات الطحلب البحري *Ulva lactuca* علي بعوضة *Culex pipiens*

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هدفت هذه الدراسة إلى معرفة النشاط الإبادي اليرقي والتثبيطي لمستخلصات الخس البحري *Ulva lactuca* باستخدام مذيبات عضوية مختلفة (الاسيتون والايثانول والأثير البترولي) ضد يرقات بعوضة الكيوليكس*Culex pipiens* . أوضحت النتائج أن جميع المستخلصات أظهرت مستويات متفاوتة من النشاط الإبادي اليرقي والتثبيطي كما لوحظ ان مستخلص الأسيتون كان الاكثر فعالية في تثبيط ظهور الحشرات الكاملة بنسبة 95 ٪ وكذلك كان التركيز الازم منه لقتل 50 ٪ من الحشرات المعاملة ($LC_{50}$) مقداره 5.004 مجم / مل) كما كانت قيمة $LC_{90}$ تساوي 9.76 مجم / مل. علاوة على ذلك نتج عن المعاملة بالمستخلصات الثلاثة من *U. lactuca* حدوث انخفاض في قدرة البعوض البالغ علي وضع البيض. إلى جانب ذلك تسبب مستخلص الأسيتون في حدوث أكبر نسبة انخفاض في متوسط عدد البيض ونسبة الفقس. مما سبق تقترح الدراسة الحالية أن استخدام مستخلصات الأسيتون والإيثانول والأثير البترولي من *U. lactuca* يمكن أن يكون طريقة فعالة وآمنة وأسهل وأقل تكلفة إلى حد ما في مكافحة بعوضة الكيوليكس *Culex pipiens*.