



## The ameliorative potential of the ethanol extract of the plant *Ocimum basilicum* on *Biomphalaria alexandrina* snails exposed to the insecticide Bestacid

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### ABSTRACT

Bestacid E.C. is a commercial insecticide may contaminate the snail habitats. *Biomphalaria alexandrina* snails act as bioindicators of environmental pollution. *Ocimum basilicum* plant showed many pharmacological effects. The present investigation studied the potential protective effect of *O. basilicum* ethanol extract on Bestacid- induced toxicity on *B. alexandrina* snails. The results indicated that Bestacid E.C. had a molluscicidal effect on snails, where, LC<sub>50</sub> was 25 mg/l. Continuous exposure of snails to the sublethal concentration (LC<sub>25</sub>) of Bestacid for two weeks followed by two weeks of recovery decreased their survival, reproductive rates (R<sub>0</sub>) and total blood count than the control ones and caused several histopathological alterations in the hermaphrodite gland. Treating *B. alexandrina* snails with Bestacid and the same equivalent or double concentration of the ethanolic extract of *O. basilicum* plant for two weeks led to an improvement of the biological, histological and hematological alterations induced by Bestacid E.C., where, it caused amelioration in the tissues of the hermaphrodite gland histology and this mirrored on survival and reproductive rates and increased the mean total number of hemocytes. It was concluded that using eco-friendly material like *O. basilicum* may help in the amelioration of the toxic effects of the chemical pollution.

### INTRODUCTION

Pesticides are widely used all over the world to eradicate pests (Tataji and Kumar, 2016). They were considered as potent pollutants of the water environment with undesirable effects on non-target organisms such as fish and water animals (Guner, 2016). Invertebrates played as bioindicators to monitor environmental pollution in the ecosystem (Ibrahim *et al.*, 2018). Among invertebrate animals, freshwater snails are commonly used in acute and chronic toxicity studies of chemicals (Wilson-Sanders, 2011). *B. alexandrina* snails are aquatic gastropods of the family Planorbidae and they were distributed in Africa and the Middle East (Ibrahim and Abdalla, 2017). These snails served as food for many vertebrate and invertebrate predators (Dillon, 2000) and intermediate host of *S. mansoni* that caused one of the neglected tropical diseases called schistosomiasis (Abd El-Ghany and Abd El-Ghany, 2017).

In Egypt, pesticides are intensively used in agriculture (Abdel-Ghaffar *et al.*, 2016) and reach water ecosystems through run-off resulting in the pollution of aquatic habitat (Hassanein *et al.*, 1999). Bestacid E.C. is a broad spectrum insecticide, that can be sprayed to control flies, mosquitoes, cockroaches, dust mites, ants and fleas on pets. It is composed of 40% Chlorpyrifos-methyl, 20% Acetamiprid and 10% Fenprothrin. Chlorpyrifos-methyl is an organophosphorous insecticide effective against a wide range of insects in crops of commercial importance; 20% Acetamiprid pesticide has been classified as an “unlikely” human carcinogen. In mammals, acetamiprid has relatively low acute and chronic toxicity and there was no evidence of carcinogenicity, neurotoxicity, mutagenicity or endocrine disruption. Raj and Joseph (2015) stated that chronic exposure of *Oreochromis mossambicus* fish to the sub-lethal concentration of acetamiprid 5.99 ppm (LC<sub>50</sub>) increased activity of lactate dehydrogenase in their liver, brain and gill tissues during all the exposure periods when compared with the control. 10% Fenprothrin is one of the widely used pyrethroid insecticides that had neurotoxic effects in rodent models (Xiong *et al.*, 2016). It was used to control many species of mites (except rust mites) and insects (e.g. whitefly, lepidopterous larvae, leaf miners, leaf worms and bollworms). These pesticides caused numerous physiological and histological alterations of the fresh water fauna (Regoli and Principato, 1995).

Using insecticides in aquatic media was a worldwide problem that caused serious environmental consequences (Merian, 1991). The toxicity of insecticides resulted in the release of reactive oxygen species (ROS) and the Free radicals that might cause degenerative effects to proteins, nucleic acids and lipids, finally lead to cell death (Moradas-Ferreira *et al.*, 1996; Bai *et al.*, 2003).

To ameliorate the side effects of these insecticides, the use of medicinal plants that have antioxidant properties could be used. *Ocimum basilicum* known as sweet basil, is a perennial herb of the Lamiaceae family has an antioxidant, anti-aging, anticancer, antiviral and antimicrobial capacities (Sakr and Al-Amoudi, 2012) and its essential oil was used in the treatment of liver fibrosis (Ogaly *et al.*, 2015). It is native to Asia, Africa, South America, and the Mediterranean (Grayer *et al.*, 1996). It is widely used in folk medicine to relieve anxiety, diabetes, digestive disorder and a variety of neurodegenerative disorders (Zeggwagh *et al.*, 2007).

In Egypt, Bestacid E.C. is a commercial mixture formulation of 3 insecticides used for agricultural purposes and then became a part of the snail's habitat. Therefore, the present study aims to use ethanol extract of *O. basilicum* as antioxidant plant to minimize the chemical toxicity resulted from pollution with Bestacid E.C. insecticide to the freshwater snail *B. alexandrina*.

## MATERIALS AND METHODS

### Snails:

*B. alexandrina* snails (8 - 10 mm) from Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt were kept in plastic aquaria (16 x 23 x 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/ L) and covered with glass plates. Oven dried lettuce leaves and blue green algae (*Nostoc muscorum*) were used for feeding and water in the aquaria was changed weekly.

### Synthetic insecticide:

Bestacid E.C. insecticide is one of the commercial mixture formulations that composed of 40% chlorpyrifos-methyl, 20% Acetamiprid and 10% Fenprothrin. It

was manufactured by limited Royal Chemical Company (Japan), Egypt, local registration number (56420).

#### **Plant material**

*Ocimum basilicum* L. (Family Lamiaceae) which is known as sweet basil, is a common vegetable in Africa. Identified and collected by the Medicinal Chemistry Department in Theodor Bilharz Research Institute.

#### **Ethanol extract of *Ocimum basilicum*:**

Dry powder of the whole plant was totally extracted by soaking it in ethyl alcohol (0.5 kg/liter) for seven days. Then the solvent was filtered and distilled under vacuum and the crude extract residues were used in preparing a series of concentrations in terms of weight/volume.

**Investigation of antioxidant activity of Ethanol extract of *Ocimum basilicum* (EEB) on *B. alexandrina* snails:** *B. alexandrina* snails as groups each group 10 snails exposed for each concentration of EEB (10, 20, 30, 40, 50 and 100 ppm) for 24 hours then recovery for 24 hours then observe number of dead snails there is no dead in exposed snails so EEB have powerful antioxidant activity against various antioxidant systems in vitro, moreover, these extracts can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications. It can also be used in stabilizing food against oxidative deterioration (Gulcin *et al.*, 2007) and it was reported that extracts obtained from spices had antioxidant activities (Gülçin, 2006b).

#### **Investigation of molluscicidal activity of Bestacid EC:**

A stock solution of 1000 ppm was prepared from the insecticide on the basis of V/V using dechlorinated tap water. Series of concentrations were prepared and ten snails were incubated for each concentration (WHO, 1983). Another snail group of the same size was dished in dechlorinated water only as control. Three replicates were prepared for each group and control. Exposure and recovery periods were 24 h each at  $25 \pm 2$  C°, and pH 7.4. Mortality rate and lethal doses were recorded for each group. The toxicity of Bestacid E.C. has been expressed as LC<sub>50</sub> and LC<sub>90</sub> (Litchfield and Wilcoxon, 1949).

#### **Bioassay tests:**

*B. alexandrina* snails (8–10 mm) were divided into five groups as follows:

- 1- control group.
- 2- Snails were continuously subjected to the sublethal concentration LC<sub>25</sub> of the insecticide alone (21.64 mg/l) 24 h/week for two weeks followed by two weeks of recovery.
- 3- Snails were continuously subjected to plant extract alone at the same concentration (LC<sub>25</sub>) of the insecticide (21.64 mg/l) for two weeks followed by two weeks of recovery.
- 4- Snails were continuously subjected to LC<sub>25</sub> of the insecticide and with the same concentration of plant (21.64 mg/l) for two weeks followed by two weeks of recovery.
- 5- Snails were continuously subjected to the sublethal concentration LC<sub>25</sub> of the insecticide and with the double concentration of plant (2 x 21.64 mg/l).

The exposure for two weeks, then, the snails were removed from the experimental test solution, and washed thoroughly with dechlorinated tap water, and transferred to containers with fresh dechlorinated tap water for another two weeks. Three replicates, each of 10 snails/L, were prepared for each concentration, the following parameters were weekly recorded:

**Survival rate:**

It was calculated according to Frank (1963) by the following equation:

$$\text{Survival rate} = \frac{\text{Number of survived snails}}{\text{Total number of exposed snails}} \times 100$$

**Reproductive rate (R<sub>0</sub>):**

It is the summation of LxMx during the experimental period, where, Lx (the survival rate as a proportion of the correct one), Mx (the number of eggs/snail/week) (El-Gindy *et al.*, 1965).

**Total hemocytes count:**

A small portion of the shell which situated directly above the heart was removed and a capillary tube was inserted into the heart to collect the hemolymph as described by Nduku and Harrison, (1980). Whole hemolymph sample from 8 snails of the same group was collected at the same time, pooled in Eppendorf vial (1.5 ml) and kept in ice-bath. For total hemocytes count, the number of cells was counted using a Bürker- Turk hemocytometer (Der Knaap *et al.*, 1981) by using 10 µl of hemolymph of each group.

**Histopathological examinations**

Snails (8-10 mm) exposed to the experimental materials were dissected. The digestive and hermaphrodite glands of each snail were separated gently from the soft parts, fixed, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. Sections were microscopically examined and photographed by a Zeiss Videocamera, Germany.

**Statistical analysis**

Probit analysis is used to define the lethal concentration values (Finney, 1971). Values were expressed as mean ± S.E., and the obtained data were analyzed using SPSS v. 17.0 for Windows (SPSS Inc2008).

**RESULTS**

The lethal concentrations (LC<sub>90</sub> and LC<sub>50</sub>) of *B. alexandrina* snails after exposure to Bestacid E.C for 24 h were 31.7 and 25 mg/l, respectively (Table 1).

Table 1: Molluscicidal activity of Bestacid E.C. against *B. alexandrina* snails.

Insecticide	LC <sub>50</sub> (mg/l)	Confidence limits of LC <sub>50</sub> (mg/l)	LC <sub>90</sub> (mg/l)	Slope	LC <sub>25</sub> (mg/l)
Bestacid EC	25	23.5- 26.5	31.7	1.26	21.64

The present results showed that exposing *B. alexandrina* snails to LC<sub>25</sub> insecticide gradually decreased their survival rate, while, snails exposed to the same concentration of the ethanolic extract of *O. basilicum* plant (21.64 mg/l) survived well lived through out the 4 weeks of the experiment. Exposure of snails to LC<sub>25</sub> of Bestacid E.C insecticide, then to the same or double concentration of the ethanolic extract of *O. basilicum* plant led to significant increase in their survival rate compared to those exposed to the insecticide alone (Table 2).

The reproductive rate (R<sub>0</sub>) of the exposed *B. alexandrina* snails to LC<sub>25</sub> of Bestacide was significantly decreased than the control ones by 91.1% (P<0.001), while, snails exposed to the same concentration of the ethanolic extract of *O. basilicum* in significantly affected. Exposure of snails to LC<sub>25</sub> of Bestacid E.C. insecticide, then to the same concentration or double concentration of the ethanolic

extract of *O. basilicum* plant caused pronounced reduction rates of 84.45 and 82.8%, respectively (Table 2).

Table 2: Survival rate (Lx), fecundity (Mx) and reproductive rate (Ro) of *B. alexandrina* snails exposed to sub-lethal concentration (LC<sub>25</sub>) of Bestacid E.C insecticide and the same or double concentration of ethanolic extract of *O. basilicum* plant for two weeks followed by 2 weeks of recovery.

Weeks	Control			Bestacid E.C.			<i>Ocimum basilicum</i>			Bestacid E.C.+ <i>O. basilicum</i>			Bestacid E.C.+ 2 <i>O. basilicum</i>		
	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx	Lx	Mx	LxMx
<b>0</b>	1.00	10.2	10.2	1.00	10.2	10.2	1.00	10.2	10.2	1.00	10.2	10.2	1.00	10.2	10.2
<b>1</b>	1.00	10.2	10.2	0.8	2.8	2.24	1.00	9.2	9.2	0.8	4.5	3.6	0.8	4.2	3.36
<b>2</b>	0.95	12.5	11.87	0.65	1.9	1.23	1.00	4.8	4.8	0.65	3.6	2.34	0.6	3.9	2.34
<b>3</b>	0.90	9.3	8.37	0.2	0	0	0.89	8.3	7.38	0.42	0.3	0.126	0.5	1.8	0.9
<b>4</b>	0.90	9.5	8.55	0	0	0	0.85	7.7	6.54	0.05	0	0	0.2	0.5	0.1
<b>Ro =</b>															
<b>Σ Lx</b>	38.99			3.47***			27.92			6.066***			6.7***		
<b>Mx</b>															
<b>Reduction %</b>				91.1			28.39			84.45			82.81		

\*\*\* = Very highly significant compared to control at p< 0.001

Regarding the total hemocyte count ( Fig.1) shows that the exposure of *B. alexandrina* snails for two weeks to LC<sub>25</sub> of Bestacid, significantly decreased the mean total number of hemocytes (p<0.001) compared to the control ones, while, snails exposed to the same concentration of the ethanolic extract of *O.basilicum* increased the total number insignificantly. Snails exposed to LC<sub>25</sub> of Bestacid E.C insecticide with either the same concentration or double concentration of the ethanolic extract of *O.basilicum* plant caused a highly Fig.1: Total number of hemocytes/ mm<sup>3</sup> of *B. alexandrina* snails exposed to the sub-lethal concentration (LC<sub>25</sub>) of Bestacid E.C insecticide and the same or double concentration of ethanolic extract of *Ocimum basilicum* plant for two weeks followed by 2 weeks of recovery.

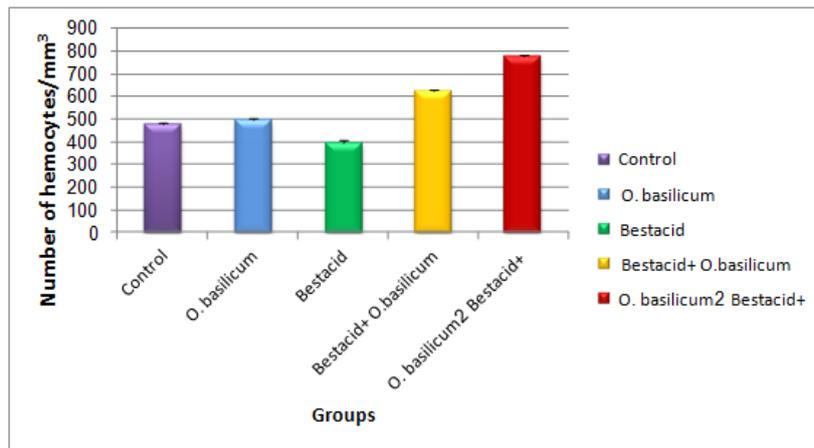


Fig.1: Total number of hemocytes/ mm<sup>3</sup> of *B. alexandrina* snails exposed to the sub-lethal concentration (LC<sub>25</sub>) of Bestacid E.C insecticide and the same or double concentration of ethanolic extract of *Ocimum basilicum* plant for two weeks followed by 2 weeks of recovery.

The hermaphrodite gland of the normal *B. alexandrina* snails is composed of a number of cub-shaped acini connected together by a connective tissue (plate A, Fig.1). Each acinus is enveloped by a thin epithelial sheet which comprises the various stages of both male and female gametogenic cells. The male reproductive

cells (spermatogonia) are differentiated into clusters forming primary and secondary spermatocytes. The spermatids are small, spindle-shaped with small heads and very long tails. On the other hand, the female oogenic cells filled the acinar lumen as primary, secondary oocytes and mature ova. The mature ova contain large accumulations of yolk material and are surrounded by a follicular membrane.

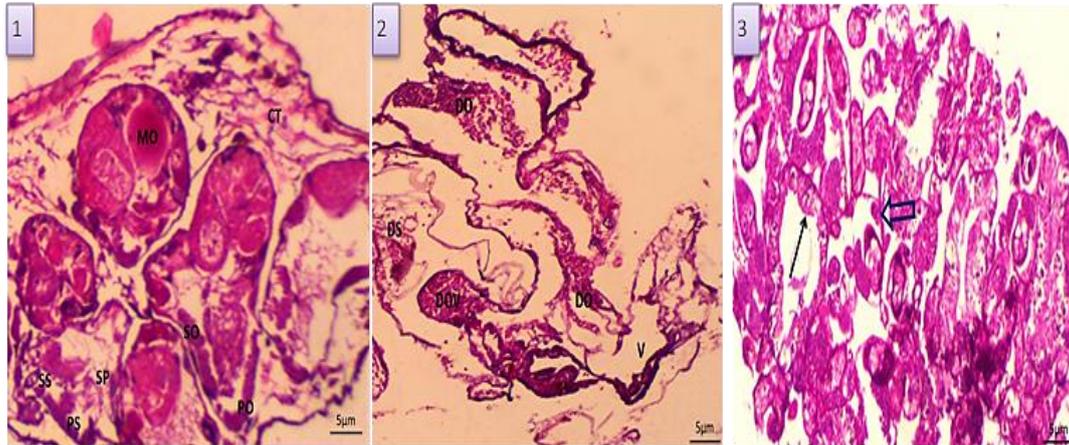


Plate A: Light micrographs showing sections in hermaphrodite gland of *B. alexandrina* snails (H& E) (x40): Fig.1: normal *B. alexandrina* snails with normal CT: Connective tissue; PO: Primary oocyte; SO: Secondary oocyte; PS: Primary spermatocyte; SS: Secondary spermatocyte; SP: Sperms; MO: Mature ovum. Fig.2: *B. alexandrina* snails exposed to the sub-lethal concentration LC<sub>25</sub> of Bestacid.E.C. insecticide. DO: Degenerated oocyte; DS: Degenerated spermatogonia; V: vacuole; DOV: Degenerated ovum. Fig.3: snails exposed to ethanol extract of *Ocimum basilicum* showing healthy, normal morphology shape of acini represented as complete oogenesis (arrow) and complete spermatogonia (curved arrow).

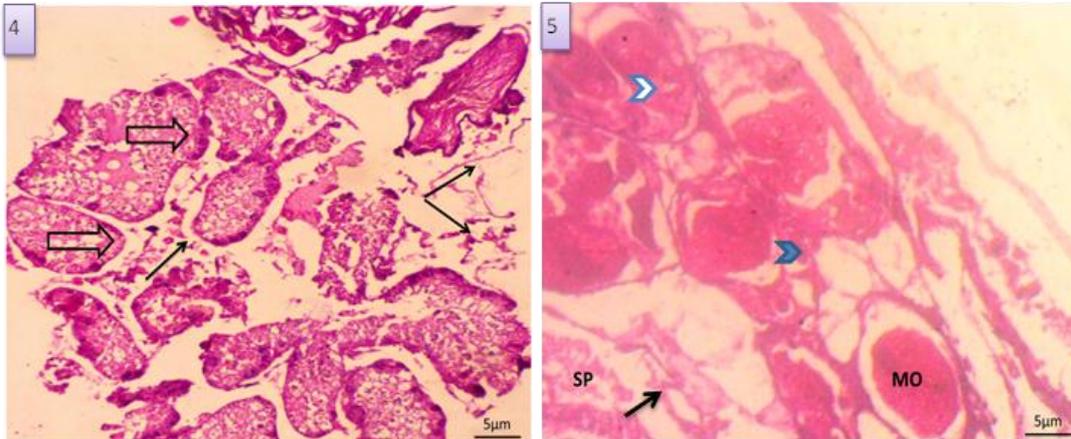


Plate B: Light micrographs showing sections in hermaphrodite gland of *B. alexandrina* snails (H& E) (x40): Fig.4: snails exposed to sub-lethal concentration LC<sub>25</sub> of Bestacid.E.C. insecticide and the same concentration of ethanol extract of *O. basilicum*, showing reduction in toxicity features of Bestacid, as less degenerated oocytes (curved arrow) and less amount of broken connective tissue (small arrow). Fig.5: snails exposed to sub-lethal concentration LC<sub>25</sub> of Bestacid.E.C. insecticide and double concentration of ethanol extract of *O. basilicum*, showing reduction in toxicity features of Bestacid and less degenerated cells (head of arrow) and few degenerated spermatogonia (small arrow).

Snails exposed to the insecticide had degenerated oogonia and spermatogonia with loose connective tissues (plate A, Fig.2). When snails exposed to the ethanol extract of *O. basilicum* only there was no significant reduction in their fecundity compared with the control group and that extract kept the snail's capacity better than control as shown in the morphological shape of the hermaphrodite gland (plate A,

Fig.3). The group that subjected to LC<sub>25</sub> insecticide and the same concentration of the ethanol extract of *O. basilicum*, there is an improvement in their histological sections, where there is less degenerated oocytes (plate B, Fig.4). The effect of insecticide and the double concentration of ethanol extract of *O. basilicum* was slight compared to that of Bestacid alone as less degenerated cells and mature ovum were observed (Plate B, Fig. 5).

## DISCUSSION

Freshwater snails can act as bioindicators of environmental pollution (Ibrahim *et al.*, 2018). The present results showed that the lethal concentrations (LC<sub>90</sub> and LC<sub>50</sub>) of Bestacid E.C insecticide on *B. alexandrina* snails after exposure for 24 h were 31.7 and 25 mg/l, respectively. This insecticide can be considered as a molluscicidal material because on the basis of the standardized method of World Health Organization, the median lethal concentration (LC<sub>50</sub>) for any molluscicidal material must not surpass 100 ppm (WHO, 1993).

The present investigation showed that exposure of *B. alexandrina* snails to LC<sub>25</sub> of Bestacid E. C. insecticide for two weeks, gradually decreased their survival rate and highly decreased their reproductive rate (R<sub>0</sub>) than the control ones and by studying the histopathological changes, it caused a complete destruction of gametogenic cells and severe damage in hermaphrodite gland tissues. These findings agree with that recorded by Abdel-Ghaffar *et al.* (2016) who reported that, survival rate of *B. alexandrina* snails, the reproductive (R<sub>0</sub>) and fecundity rates were reduced after their exposure to the sub lethal concentrations of Butralin 48% EC, glyphosate isopropylammonium 48% SL and Pendimethalin 50% EC herbicides. They reasoned this reduction by histological examinations, which showed severe damage in the digestive and hermaphrodite gland cells of the treated snails. Also, Tripathi and Singh (2004) showed that cypermethrin and alphamethrin insecticides inhibit the reproduction and induce oxidative stress for the freshwater snail *Lymnaea acuminata*.

To modulate the toxic effects of the insecticides, medicinal plants with antioxidant properties can be used to reduce their harmful effects (Mossalem, 2018). *Ocimum basilicum* is a widely distributed plant, used as a spice in the Mediterranean diet. Due to the polyphenolic and flavonoid contents, it can be used as chemopreventive agents or adjunctive therapy in the treatment of different clinical conditions (Sestili *et al.*, 2018). Moreover, *O. basilicum* ethanolic extract has antioxidant activity and phenolic content and can be used for curative purposes (Aydemir and Becerik, 2011).

The present study showed that when *B. alexandrina* snails exposed to *O. basilicum* ethanolic extract only, there was no significant changes in their survival, fecundity and reproductive rates compared with control group and that was confirmed by histological sections in the hermaphrodite gland where, it has the same morphological shape of control groups.

Exposure of snails to LC<sub>25</sub> of Bestacid E.C insecticide, then to the same concentration or double concentration of the ethanolic extract of *O. basilicum* plant significantly reduced their survival and reproductive rates than control group. While, histopathological changes in hermaphrodite gland showed that the snail group subjected to the insecticide then to the ethanol extract of *O. basilicum* appeared as slightly affected with toxic effects of Bestacid E.C insecticide, where, it showing less degenerated spermatogonia. These observations could be attributed to the ameliorative effects of *O. basilicum* ethanol extract. Many researches confirmed the

antioxidant property of water soluble ethanol extract of *O. basilicum* as it is used as a kitchen herb and an ornamental plant in house gardens (Bonanni *et al.*, 2007; Shan *et al.*, 2007; Generalić *et al.*, 2012). The radical scavenging and antioxidant activity of the water and ethanol extracts of basil are related to their biofunctionalities such as the decrease of chronic diseases and reserve of pathogenic bacteria growth (Covacci *et al.*, 2001). Ethanol extract of basil has been widely used to assess the free radical scavenging efficiency of a variety of antioxidant substance in food systems (Ozcelik *et al.*, 2003; Elmastaş *et al.*, 2006).

The internal defense system of molluscs is comprised of cellular and humoral components (Le Clec'h *et al.*, 2016). In *B. alexandrina*, there are three main hemocytes categories: hyalinocytes, small undifferentiated hemocytes and granulocytes (Cavalcanti *et al.*, 2012).

The present study revealed that there was significant decrease in total number of hemocytes after exposure to the sublethal concentration (LC<sub>25</sub>) of Bestacid .E. C. insecticide. These results were in accordance with that of Ibrahim *et al.* (2018) who recorded a significant decrease in total number of hemocytes after exposing *B. alexandrina* snails to the insecticide lufenuron 5% EC. Also, these findings were supported by the study of (Mossalem *et al.*, 2013) who stated that the number of hemocytes of *B. alexandrina* snails decreased after exposure to an antimalarial agent called Artemether. The decrease in hemocytes count in treated groups with sublethal concentrations may be resulted from tissues damage in host (Esmaeil, 2009).

While, snails exposed to LC<sub>25</sub> of the ethanolic extract of *O. basilicum* increased the total number insignificantly. Snails exposed to LC<sub>25</sub> of Bestacid E.C insecticide then to either the same concentration or double concentration of the ethanolic extract of *O. basilicum* plant caused a highly significant increase in the mean total number of hemocytes ( $p < 0.001$ ) than that for control group. These results coincide with Helal *et al.* (2003) who observed significant increase in *B. alexandrina* hemocytes after exposure to the water extract of the plant *Euphorbia peplus*. Higher numbers of circulating hemocytes could be due to release of hemocytes from tissue depots as they participate in tissue repair (Nelson *et al.*, 2016).

Moreover, Bakry *et al.* (2012) reported increased number of hemocytes after exposure of *B. alexandrina* snails to the methanol extract of *Azadirachta indica* plant and they stated that these hemocytes involved in the death of some encapsulated parasites or in the production of soluble factors, which could be cytotoxic.

## CONCLUSION

In summary, The insecticide Bestacid E.C. has a molluscicidal activity and can affect the biological system of *B. alexandrina* snails. Ethanol extract of the plant *O. basilicum* has a powerful antioxidant activity and can be used as a food supplement. It can also help in the recovery of tissue damage after exposure to a stressor and in reducing the toxic effects of the chemical pollution. The antioxidant activities of such plant extracts should be evaluated in a variety of model systems using several different indices to ensure the effectiveness of such antioxidant materials.

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**Compliance with Ethical Standards:**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

**Conflict of interest:**

The authors declare that no conflict of interest.

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### ARABIC SUMMARY

القدرة المحسنة لنبات *Ocimum basilicum* (الريحان) على قواقع *Biomphalaria alexandrina* المعرضة للتلوث الكيميائي بمبيد الحشرات بستاسيد

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يستخدم مبيد بستاسيد كمبيد حشري تجاري في الاغراض الزراعية وقد يحدث تلوث في بيئة القواقع. تستخدم قواقع *Biomphalaria alexandrina* كمؤشرات بيولوجية للتلوث البيئي بما يسببه لها من اضرار. ولمحاولة معالجة هذه الاضرار يتم استخدام النباتات الطبية . ولذا الهدف الاساسي من هذا البحث هو دراسة التأثير العلاجي للمستخلص الايثانولي لنبات *Ocimum basilicum* (الريحان) على الاضرار الناتجة في أنسجة القواقع المعرض للتأثير السام لمبيد بستاسيد (مستحلب). أشارت النتائج إلى أن مبيد بستاسيد كان له تأثير سام على القواقع حيث كان التركيز الذي يؤدي الى موت 50% من القواقع 25 مجم / ل. أدى تعرض القواقع للتركيز تحت المميت (LC25) من مبيد بستاسيد لمدة أسبوعين متبوعاً بأسبوعين للتعافي إلى انخفاض معدلات بقائها ومعدلات تكاثرها وقلة في عدد خلايا الدم وتسبب في عدة تغيرات نسيجية في الغدة الخنثية . معالجة قواقع بيومفلاريا الكسندرينا بنفس تركيز المبيد من المستخلص الايثانولي من نبات الريحان أو التركيز المضاعف من المستخلص لمدة أسبوعين أدى الى تحسن في التغيرات البيولوجية والنسجية والدموية، حيث تسبب في تحسين الأنسجة الغدة الخنثية وهذا يعكس على معدلات البقاء والتكاثر وزيادة متوسط مجموع عدد خلايا الدم. وقد استنتج من هذا أن استخدام مواد صديقة للبيئة ولها خصائص مضادة للاكسدة مثل الريحان قد يساعد في تحسين الآثار السامة للتلوث الكيميائي.