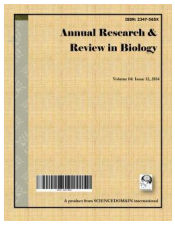

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Antimicrobial Activities of Some Marine Sponges, and Its Biological, Repellent Effects against *Culex pipiens* (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Crude extracts of marine sponges, *Negombata magnifica* and *Callyspongia siphonella* were tested for their antimicrobial activity besides larvicidal, pupicidal, adulticidal and other biological effects against the filarial vector *Culex pipiens*. Three sub-lethal concentrations ranging as 8.4, 28.4 and 47.6 ppm for *N. magnifica* and 44.5, 327.4 and 610.3 ppm *for C. siphonella* were used.

The two marine sponges used in this study were collected from reefs by SCUBA diving. Spicules were prepared by dissolving the soft tissue of small pieces of sponge in sodium hypochlorite and washed with distilled water and ethanol. The extracts were tested for their antimicrobial activity; in addition, the biological activities of the three sub-lethal concentrations used were evaluated against laboratory reared *Cx. pipiens* mosquito.

The results obtained showed interesting antifungal activity against fungal strains tested. *N. magnifica* extract showed quite promising broad-spectrum antibacterial activity due to its capacity to inhibit the growth of almost bacterial strains tested. Based on LC_{50} values, the toxicity of *N. magnifica* extract (47.6 ppm) was higher than that of *C. siphonella* (610.3 ppm). Mean larval and

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pupal durations of mosquitoes treated with *N. magnifica* extract were significantly (P<0.05) prolonged at higher sub-lethal concentrations; meanwhile, *C. siphonella* showed non-significant (P>0.05) prolongation. The *N. magnifica* extract was found to be more effective against the adult emergence than *C. siphonella*. There was a pronounced effect of the tested extracts on the number of eggs laid per female and this effect was concentration dependent, the fecundity of mosquitoes treated with *N. magnifica* extract was significantly (P<0.05) decreased to 86.7±5.8 eggs/♀, compared to 150±8.7 eggs/♀ for the control group, while it was 116.7±5.8 eggs/♀ for *C. siphonella* extract, vs. the control group. Vitellogenin synthesis and ovarian development of *Cx. pipiens* females were highly affected by tested extracts. Six protein bands with high molecular weights (175-90 KDa), which believed to be vitellogenin, were detected in the control group, while this number reduced to two bands in ovaries of *N. magnifica* and *C. siphonella*-treated females. Also, *N. magnifica* extract induced concentration dependent repellent activity against tested females mosquitoes than *C. siphonella* extract. These promising results in relation with antifungal and antibacterial activities open the way for complementary investigation in order to purify and identify active molecules.

The present investigations have helped to focus on some bioactive substances isolated from marine resources; these molecules, which possess antimicrobial and insecticidal activities, could be used as insecticidal agents.

Keywords: N. magnifica; C. siphonella; antifungal; antibacterial; mosquitoes; reproduction; repellency.

1. INTRODUCTION

Mosquitoes top all the insect-vectors in transmission of serious diseases worldwide. *Anopheles stephensi, Aedes aegypti* and *Cx. pipiens* are the mosquitoes vector of malaria, dengue and lymphatic filariasis, respectively. Over two billion people in tropical countries are at risk from mosquito borne diseases and the search for effective vaccines against these diseases is still in progress [1]. The unplanned use of chemical insecticides during the past few decades to control insect pests have resulted in serious consequences such as insect resistance, mammalian toxicity, bioaccumulation through food chains, environmental contamination and risk for human health [2]. This necessitates the search for new sources for insect control agents.

A variety of antimicrobial substances have been isolated from various species of marine sponges [3]. Up to 800 antibiotic compounds have been isolated from marine sponges, a number that corroborates assumptions that sponges appear to defend themselves against infections by producing and/or accumulating secondary metabolites.

Marine sponges are among the richest sources of pharmacologically active chemicals from marine organisms. As infectious microorganisms evolve and develop resistance to existing pharmaceuticals, the marine sponge provides novel leads against bacterial, viral, fungal and parasitic diseases [4].

In the 1970s, natural product chemists embarked on exploitation of bioactive substances from marine invertebrates by using antimicrobial or cytotoxic assays. Later, several pharmaceutical companies joined this effort using more sophisticated assay systems, including enzyme inhibition assays. More than 15,000 marine products have been described [5,6]. So far, several promising candidates for new pharmaceuticals have been discovered from marine sponges [7]. Sponges and their associated microorganisms are responsible for more than 5,300 different products, and every year hundreds of new substances are discovered [8]. These early promises have now been substantiated by an overwhelming number of bioactive substances that have been discovered in marine organisms.

Secondary metabolites of marine organisms differ from that of terrestrial organisms. Bioactive compounds isolated from marine organisms exhibits various biological activities such as anti-cancer, anti-inflammatory, antifungal, antimicrobial and mosquito larvicidal properties [9,10]. The extracts of some marine sponges showed significant insecticidal activity against mosquito larvae and agricultural pests [11].

The aim of the current study was to evaluate the insecticidal properties of active molecules

extracted from natural source (marine sponge), which could be later used as insecticidal agents.

2. MATERIALS AND METHODS

2.1 Sponges Identification

2.1.1 *Negombata (Latrunculia) magnifica* (Keller, 1889)

2.1.1.1 Remarks

Negombata magnifica or toxic finger-sponge, its reddish-brown narrow crooked branches can grow up to 70 centimeters in adult stage. It is extremely toxic because of latrunculin toxin. This sponge forming bright red to orange red branches, medium-sized slightly elevated oscules aligned with the branches. Size 30-70 cm, and preferable occurred depth at 5-25 m.

2.1.1.2 Ecology

This sponge lives on shallow coral reefs in the northern waters of the Red Sea and Indian Ocean. It is a potential source of the cytotoxin (latrunculin B), toxic to its predators.

2.1.2 *Callyspongia (Siphonochalina) siphonella* (Levi, 1965)

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2.1.2.1 Remarks

Callyspongia siphonella is a sponge forming vertical erect tubes clusters from a common base. Its color is usually purple, pink or reddish-brown. It hasn't spicules, so these tubes have smooth consistency. The main skeleton consists of a set of primary horn fibers, exposed from the inside to the outer surface and a second set of fibers that lie at the right angles to the first one. It has a single mouth and adult can grow up to 50-60 cm.

2.1.2.2 Ecology

C. siphonella a species of sponge endemic to the Red Sea, preferring to grow between corals and rocks, or under them it is often found on manmade constructions, from the shallow to deeper water. It feeds on plankton, inhabits reef slopes and has a temperature range of 22-28°C and preferable occurred depth at 2-35 m.

2.2 Mosquitoes Colony

Mosquitoes used in this study were *Culex pipiens* L., they were collected from Abu-Rawash, Giza governorate, then were reared for several generations, in the insectary of medical entomology, zoology department, under controlled conditions of temperature of 27±2°C, relative humidity 70±10% and 12-12 light-dark regime. Adult mosquitoes were daily provided with cotton pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs.

2.3 Collection of Sponge Specimens

Two marine sponges were used in this study, *Negombata magnifica* or toxic finger-sponge and *Callyspongia siphonella* or tube sponge, sponge sampling were done in shallow water at Blue Hole area, northern Red Sea, Egypt. Samples were collected from reefs by SCUBA diving during winter and spring 2016. The samples were cleaned and washed with distilled water and immediately frozen and stored at -20°C. Identification of all specimens has been carefully checked on the basis of morphological characters according to Systema Porifera [12] and the recent update undertaken in the World Porifera Database [13]. All specimens were fixed either for light microscopy in 4% formalin in seawater for 24 h and then preserved in ethanol (70%). Spicules were prepared by dissolving the soft tissue of small pieces of sponge in sodium hypochlorite and washed five times with distilled water and two in ethanol. Cleaned spicules were dried on a slide and described for well identification.

2.4 Preparation of the Crude Extracts

The frozen sponges were cut into ~1 cm3 cubes. The samples of sponge specimen were covered with 200 ml methanol solvent. The macerated specimens were kept at room temperature for a week extraction period and genital shaken. Then, the residue was dried and extracted twice. Samples were filtered through Whatman 542 filter paper, and evaporated using rotary evaporator to obtain soluble extract [14]. Sub-lethal concentrations LC_{10}, LC_{30} and LC_{50} were prepared as stock.

2.5 Experimental Bioassay

2.5.1 Antimicrobial effects

The extracts of, *N. magnifica* and *C. siphonella* were tested for their antifungal activity against

Aspergillus fumigatus and Candida auris; antibacterial activity against different strains of bacteria namely; *Staphylococcus haemolyticus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus sanguis, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis, Corynebacterium diphtheriae* and (Methicillin-resistant *Staph. aureus*; *Staph. Epidermidis*) as G+ve bacteria and *Aeromonas veronii, Klebsiella pneumoniae, Campylobacter fetus, Proteus mirabilis, Acinetobacter baumannii, Serratia plymuthica, Salmonella typhi, Neisseria gonorrhoeae, Enterobacter cloacae* and *Shigella dysenteriae* as G-ve bacteria. These strains were obtained from Regional Center for Mycology and Biotechnology, Nasr City, Egypt. The occurrence of microbial growth inhibition was assessed using a classical diffusion method. In general, this method is based on the visual observation of microbial growth inhibition on agar media and determining the diameter of growth-inhibition zones in mm. 50 µl is the amount used to evaluate the microbial growth inhibition. All tests were done in triplicates and the listed data are the average of the obtained results.

In order to study the biological activity of these sponges, sub-lethal concentrations of each extract was used. The 2nd instar larvae were collected from the established colony and placed in plastic cup containing 250 ml of the extract solution as recommended by (WHO). Control larvae were placed in cups contained 250 ml dechlorinated tap water (25 of 2nd instar larvae/cup). At least three replicates were used in each experiment. *The following biological aspects were tested*:

2.5.1.1 Larvicidal activity

Larval mortality was recorded daily and dead larvae removed until adult emergence. Mortality of the larvae was indicated by a failure to respond to mechanical stimulation. Larval mortality percent (LM%) was estimated by using the following equation: LM% = A–Á/A×100. Where: A= number of tested larvae, Á= number of tested pupae [15].

2.5.1.2 Pupation percent

Pupation percent was estimated using the following equation: pupation% = a/ā×100. Where: a = number of observed pupae, ā = number of tested larvae. The *pupal mortality percentage* (PM%) was estimated by using the following equation: PM% = n–ŋ/n×100. Where: n = number of produced pupae, ŋ = number of observed adults. *Pupal duration* was calculated as the interval between the commencement of pupation and the commencement of adult emergence, it was calculated for each one and then the mean value was taken. The emerged adult males and females were counted and the adult emergence percent (AE%) was calculated by using the following equation: AE%= E/É×100. Where: E = number of emerged adults, É = number of tested pupae [16].

2.5.1.3 Female fecundity

The adult females that succeeded to emerge from larvae treated with sub-lethal concentrations were collected and transferred with normal adult males obtained from the colony by using an electric aspirator recommended by (WHO), and fed with 10% sugar solution for three days. At day five, the starved females were allowed to take a blood meal from a pigeon and allowed to lay egg rafts on clean water. The number of eggs/raft was counted by using binocular microscope and the mean value was taken [16].

2.5.1.4 Electrophoretic protein separation

Forty *Cx. pipiens* adult females resulted from 2nd instar larvae treated with the LC_{50} of each extract were dissected under dissecting microscope 72 hr post blood meal (PBM), ovaries were excised, then put in 0.5 ml sample buffer and kept deep frozen at –20°C to separate the ovarian protein bands of females using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Repellent activities: sub-lethal concentrations from each extract were dissolved in 2 ml water with a drop of Tween 8. The concentration was directly applied onto ventral surface of pigeon after abdominal feathers removal. Ten min later, pigeons were placed for 2 hr in cages containing starved *Cx. pipiens* females. Control tests were carried out using ethanol or water. After treatments, the number of fed and unfed females were counted and calculated as described by [17], where Repellency% =(A%-B%/100-B%)×100. Where A is the percentage of unfed females in treatment and B the percentage of unfed females in control.

2.6 Statistical Analysis

The statistical analysis of the obtained data was done according to [18,19] the analysis was revised and graphics were drawn by Excel for Microsoft office 2010. The obtained data were

assessed by calculation of the mean (M), standard deviation (SD) and student t-test. LC_{50} was calculated using multiple linear regressions [20].

3. RESULTS

3.1 Antimicrobial Activity

The results are presented in (Table 1) and indicated by growth-inhibition zones, it was clear that *N. magnifica* extract had a much more potent antifungal activity than that of *C. siphonella*. Both G+ve and G-ve bacterial strains showed marked increase in the growth inhibition zones when treated with *N. magnifica* extract, except for *P. mirabilis* and *Shigella dysenteriae.* Meanwhile *C. siphonella* extracts showed slight to moderate antibacterial activity.

From the aforementioned results it is appeared that *N. magnifica* extract showed the highest antimicrobial activity against different fungal and bacterial strains tested, where it caused higher growth-inhibitory effects followed by *C. siphonella* extract.

3.2 Biological Effects of Tested Extracts against *Cx. pipiens*

3.2.1 Toxicity

The mortality percentages of *Cx. pipiens* larvae as influenced by different concentrations of *N. magnifica* are illustrated in (Fig. 1). The obtained data indicated that there was a positive correlation between the concentrations and the larval mortality percentages i.e. the increase of *N. magnifica* extract concentration led to increase of LM%. The LM% increased gradually from 10.7% at the concentration of 10 ppm to 94.7% at the concentration of 70 ppm. The LM% among the control group was 1.3%. The calculated LC_{50}, LC_{30} and LC_{10} from the different mortality percentages using linear regression recorded 47.6, 28.4 and 8.4 ppm; respectively.

The larval mortality percentages of *Cx. pipiens* larvae as influenced by different concentrations of *C. siphonella* are illustrated in (Fig. 2). The obtained data indicated that there was a positive correlation between the concentrations and the larval mortality percentages.

Table 1. Antimicrobial activity of *N. magnifica* and *C. siphonella*

Tested microorganisms	*N. magnifica*	*C. siphonella*	Standard
Fungi			**Amphotericin B**
Aspergillus fumigatus	17.2±0.58	15.7±1.2	23.7± 1.2
Candida auris	15.3±1.2	13.7±1.5	19.8± 0.63
Gram positive bacteria			**Ampicillin**
Staphylococcus haemolyticus	24.4±1.7	20.2±0.58	27.4±1.5
Bacillus subtilis	30.3±0.58	17.9±1.2	32.4±2.1
Staphylococcus epidermidis	21.3±0.63	NA	23±1.0
Streptococcus sanguis	19.7±1.5	NA	21.7±1.5
Streptococcus pyogenes	22.4±0.4	20.2±1.5	22.7±1.5
Streptococcus agalactiae	14.3±0.62	NA	22.3±1.5
Enterococcus faecalis	16.3±1.2	15.3±0.5	19.3±0.58
Corynebacterium diphtheriae	20±0.72	NA	20±1.0
Methicillin -resistant microorganisms			**Vancomycin**
Staphylococcus aureus (MRSA)	19.9±1.2	NA	21.6±2.1
Staphylococcus epidermidis (MRSE)	22.1±0.58	NA	22.4±1.5
Gram negative bacteria			**Gentamicin**
Aeromonas veronii	22.1±0.3	NA	23.7±1.3
Klebsiella pneumoniae	21.4±0.62	20.2±0.58	20.2±1.2
Campylobacter fetus	18.1±0.7	NA	21.3±1.7
Proteus mirabilis	NA	17.9±1.2	21.2±1.2
Acinetobacter baumannii	19.3±0.63	23.3±0.63	23.4±1.2
Serratia plymuthica	18.3±1.2	NA	22.3±0.58
Salmonella typhi	15.6±0.6	NA	21.1±0.72
Neisseria gonorrhoeae	15.3±1.2	NA	19.3±0.72
Enterobacter cloacae	17.4±0.5	21.8±0.58	22.4±2.1
Shigella dysenteriae	NA	13.3±1.2	21.3±1.5

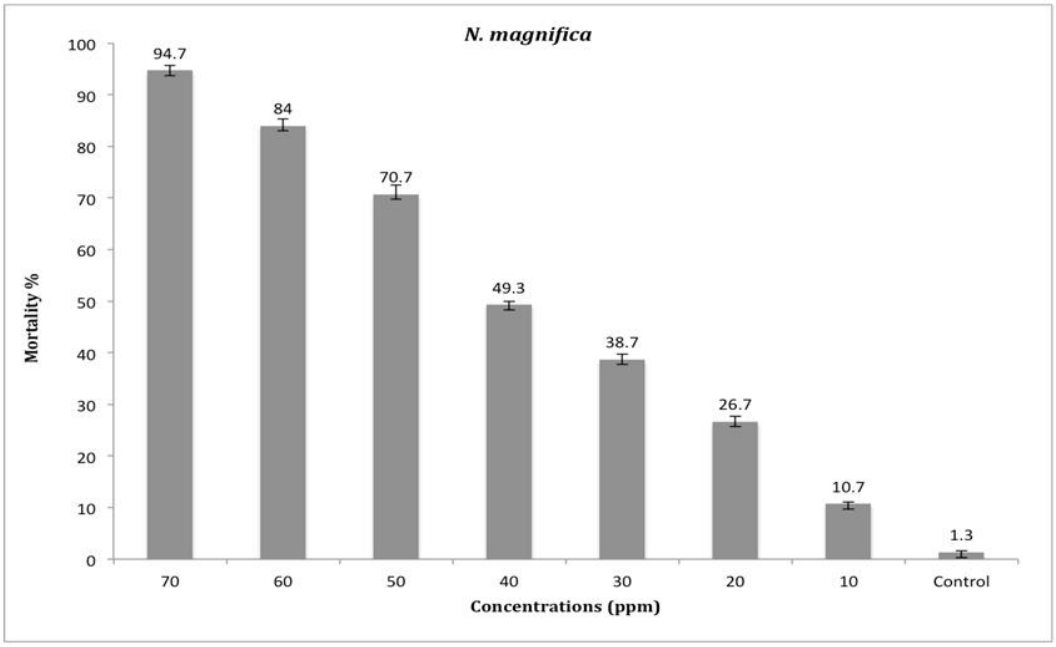

Fig. 1. Effect of different concentrations of *N. magnifica* extract on larval mortality of *Cx. pipiens*

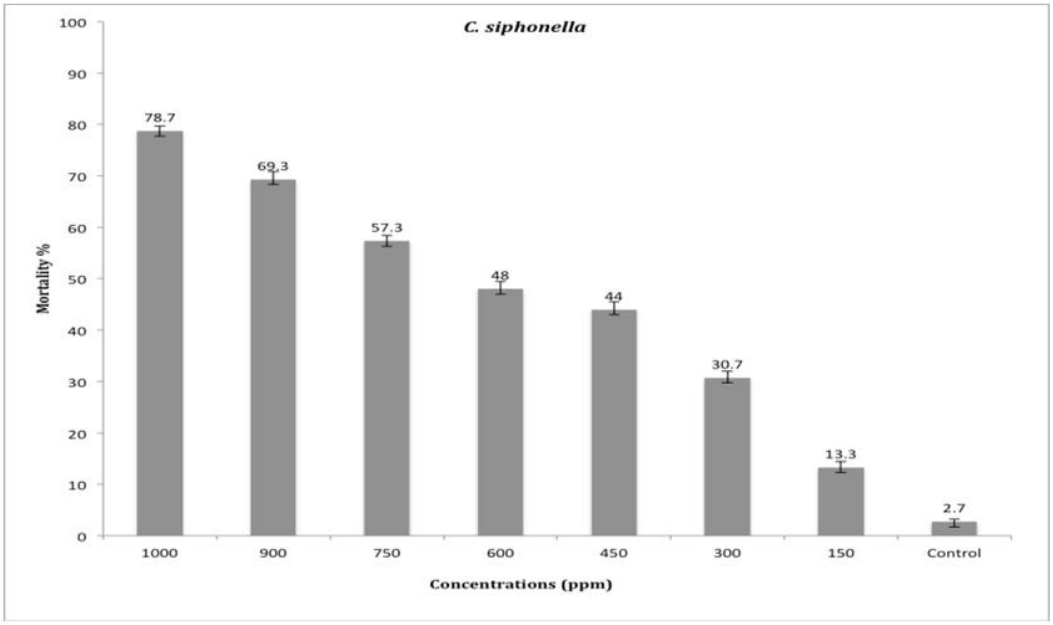

Fig. 2. Effect of different concentrations of *C. siphonella* extract on larval mortality of *Cx. pipiens*

The LM% increased gradually from 13.3% at the concentration of 150 ppm to 78.7% at the concentration of 1000 ppm. The LM% among the control group was 2.7%. The calculated LC_{50}, LC_{30} and LC_{10} from the different mortality percentages using linear regression recorded 610.3, 327.4 and 44.5 ppm; respectively. From the aforementioned results and based on LC_{50} values (Table 2), it is obvious that the toxicity of the *N. magnifica* extract was higher than that of *C. siphonella*.

Table 2. Toxicity of sub-lethal concentrations of *N. magnifica* and *C. siphonella* against larvae of *Cx. pipiens*

Extracts	Sub-lethal Conc. (ppm)		Slope (b)	Correlation coefficient (R^2)
N. magnifica	LC_{50} (ppm)	47.6	1.4238	0.99379
	LC_{30} (ppm)	28.4		
	LC_{10} (ppm)	8.4		
C. siphonella	LC_{50} (ppm)	610.3	0.0707	0.97902
	LC_{30} (ppm)	327.4		
	LC_{10} (ppm)	44.5		

3.2.2 Biological activity

The biological activity of tested extracts against *Cx. pipiens* has been studied. The larval duration, pupal duration, pupal mortality, adult emergence, female fecundity, ovarian proteins electrophoresis and repellency effects were assessed.

Data given in (Table 3) showed that, sub-lethal concentrations of *N. magnifica* extract have significantly (P<0.05) prolonged the MLD to 12.3±0.3 and 13.5±0.9 days, at concentrations of 28.4 and 47.6 ppm; respectively, compared to 10.6±0.6 days for the control group. Meanwhile, there was a non-significant (P>0.05) prolongation of the MLD of the larvae treated with *C. siphonella* extract at sub-lethal concentrations used, vs. the control group.

The mean pupal duration was prolonged as the concentration used increased, the delayed effect was markedly observed in *N. magnifica* extract than the other one, the MPD significantly (P<0.05) recorded 3.5±0.12 and 3.8±0.15 days for LC_{30} and LC_{50} of *N. magnifica* extract; respectively, compared to 2.93±0.12 days for the control group, and it was non-significantly (P>0.05) recorded 2.96±0.05, 3.2±0.2 and 3.2±0.15 days for the sub-lethal concentrations LC_{10}, LC_{30} and LC_{50} of *C. siphonella* extract; respectively, compared to the untreated group.

A positive correlation between the concentrations and PM% was observed. In the case of *N. magnifica*, the highest PM% was 25% at concentration of 47.6 ppm and the lowest PM% was 4.5% at concentration of 8.4 ppm, vs. 0.0% in the control group. On the other hand, concentration of 610.3 ppm gives the highest PM% (15.4%), while the lowest PM% was (4.8%) at concentration of 44.5ppm, compared to the untreated group.

There was a marked reduction in the percentage of the adult emergence at sub-lethal concentrations used, as it recorded 84, 68 and 36% for LC_{10}, LC_{30} and LC_{50} of *N. magnifica* extract; respectively, meanwhile it recorded 80, 64 and 44% for LC_{10}, LC_{30} and LC_{50} of *C. siphonella* extract; respectively, vs. 100% for the control group.

Table 3. The effect of Sub-lethal concentrations of *N. magnifica* and *C. siphonella* against *Cx. pipiens* females

Treatments	Sub-lethal Conc. (ppm)	Tested larvae	MLD (days) ±SD	MPD (days) ±SD	Pupal mortality %	Adult emergence %	Fecundity (Mean ±SD)
N. magnifica	47.6	25	13.5c±0.9	3.8c±0.15	25	36	86.7d±5.8
	28.4	25	12.3b±0.3	3.5b±0.12	5.6	68	111.7c±2.9
	8.4	25	10.3a±0.6	3.1a±0.2	4.5	84	135a±5
C. siphonella	610.3	25	11.3a±0.29	3.2a±0.15	15.4	44	113.3b±5.8
	327.4	25	11a±0.5	3.2a±0.2	5.9	64	116.7a±11.54
	44.5	25	10.3a±0.6	2.96a±0.05	4.8	80	131.7a±5.8
Control	–	25	10.6a±0.6	2.93a±0.12	0.0	100	150a±8.7

Within each column, means with different letter are significantly different (P<0.05)

Results also elucidated a pronounced effect of the tested extracts on the number of eggs laid this effect was concentration dependent. On the basis of LC_{50}, the fecundity of *N. magnifica* extract was significantly ($P<0.05$) decreased to 111.7±2.9 and 86.7±5.8 eggs/♀ for LC_{30} and LC_{50}; respectively, compared to 150±8.7 eggs/♀ for the control group. On the other hand, the fecundity of females resulted from larvae treated with the sub-lethal concentrations of *C. siphonella* extract was non-significantly ($P>0.05$) decreased except for the highest concentration tested (LC_{50}), where it recorded 116.7±11.54 eggs/♀, vs. the control group.

3.2.3 Electrophoretic protein separation

The electrophoretically separated proteins of the ovaries homogenate of *Cx. pipiens* females resulted from larvae treated with the LC_{50} of each extract tested and others of untreated females 72 h, post blood meal (PBM) are shown in (Figs. 3-7). The obtained results displayed that the number of ovarian protein bands was 12 for the females resulted from larvae treated with the LC_{50} of *N. magnifica* and *C. siphonella*, compared to 19 protein bands of the control group.

The results obtained illustrated three major protein bands with area percentages of 12.9 (M.W. 14 KDa), 14.7 (M.W. 39 KDa) and 22.5 (M.W. 170 KDa) among the ovarian protein bands of untreated females. Three major protein bands with area percentages of 16.5 (M.W. 30 KDa), 12.8 (M.W. 42 KDa) and 25.2 (M.W. 161 KDa) were detected in *N. magnifica*-treated females. In addition, three major proteins among the ovarian protein bands of *C. siphonella* treated females, 20.6 (M.W. 14 KDa), 11.9 (M.W. 30 KDa) and 12.3 (M.W. 161 KDa) were detected.

Six protein bands with high molecular weights (175-90 KDa), which believed to be vitellogenin, with area percentages of 22.5 (170 KDa), 0.4 (134 KDa), 1.0 (115 KDa), 3.9 (101 KDa), 0.3 (95 KDa) and 0.1 (90 KDa) were detected in the control group, while this number reduced to 2 protein bands, {25.2 (161 KDa); 8.1 (115 KDa)} and {12.3 (161 KDa); 9.8 (115 KDa)} in ovaries of *N. magnifica* and *C. siphonella*-treated females; respectively.

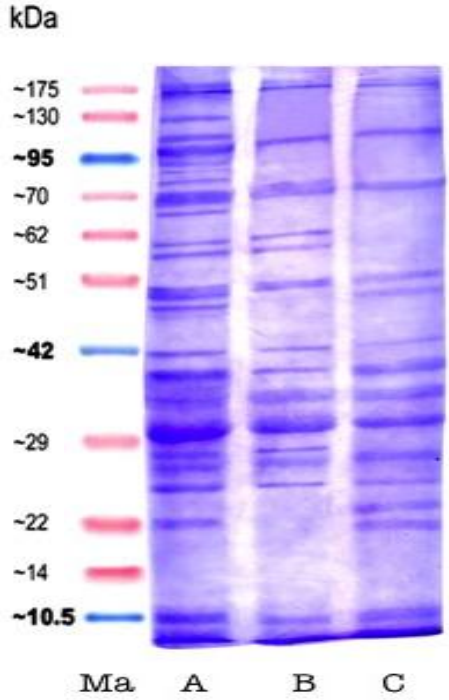

Fig. 3. Zymogram of ovaries homogenate of *Cx. pipiens* females treated with the LC_{50} of tested extracts and others of untreated females. (Ma) marker, (A) Control females, (B) *N. magnifica* treated females and (C) *C. siphonella* treated females

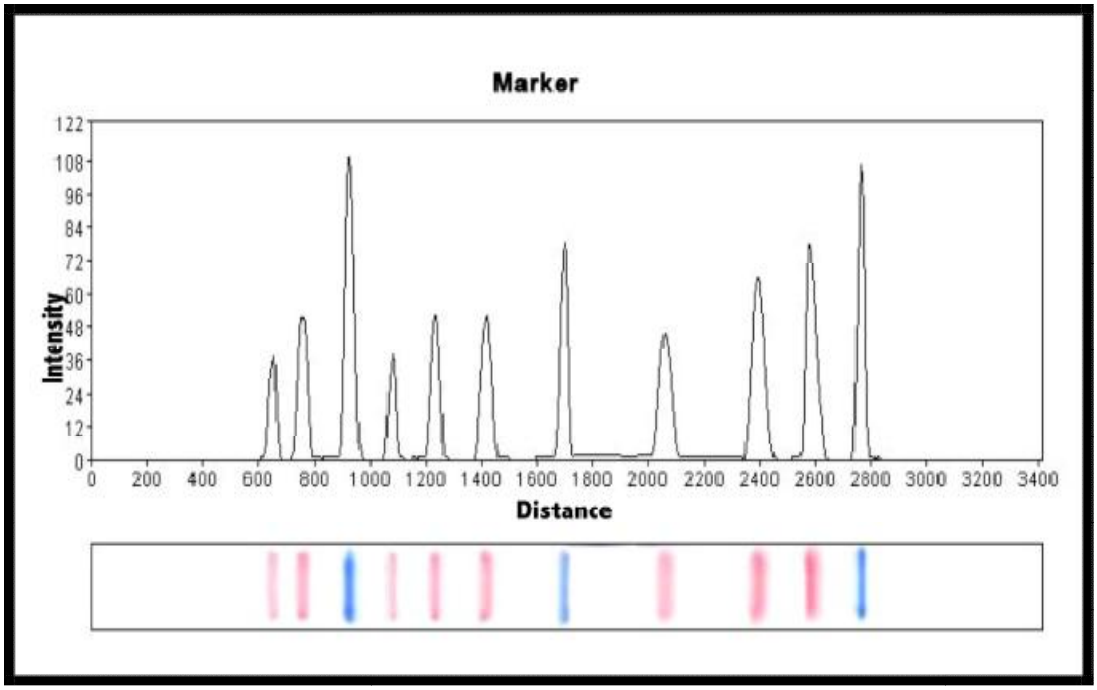

Fig. 4. Densitometric scan of protein electrophoretograms. (Marker)

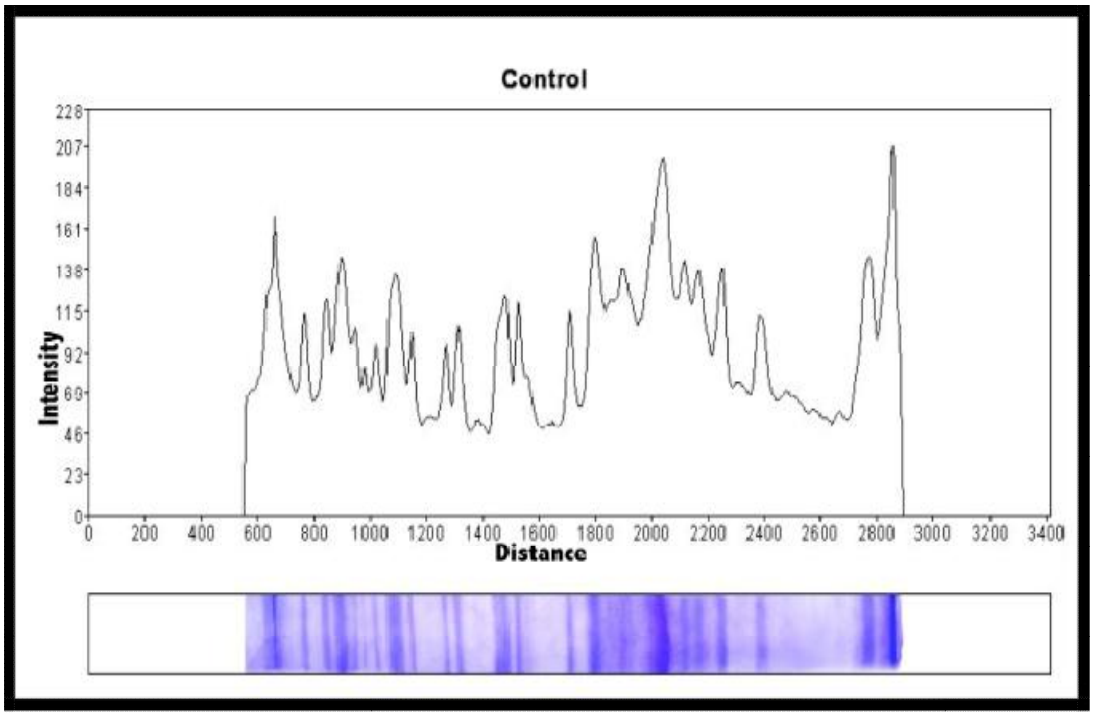

Fig. 5. Densitometric scan of protein electrophoretograms of *Cx. pipiens* ovaries extracts, 72 h post blood meal. Control females

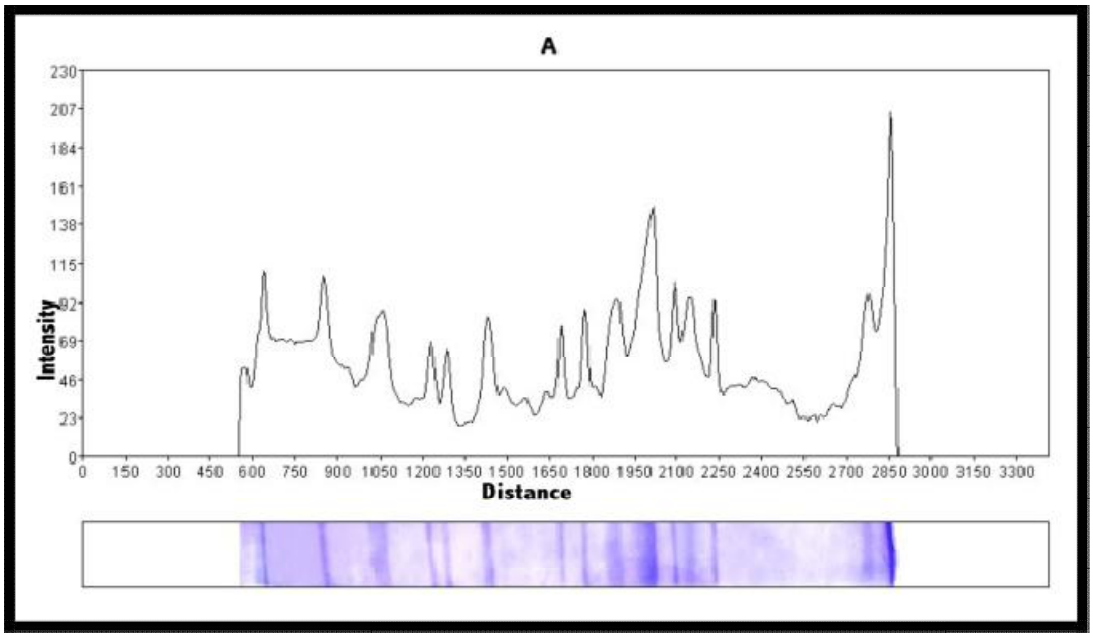

Fig. 6. Densitometric scan of protein electrophoretograms of *Cx. pipiens* ovaries extracts, 72 h post blood meal. (A)- *N. magnifica* treated females

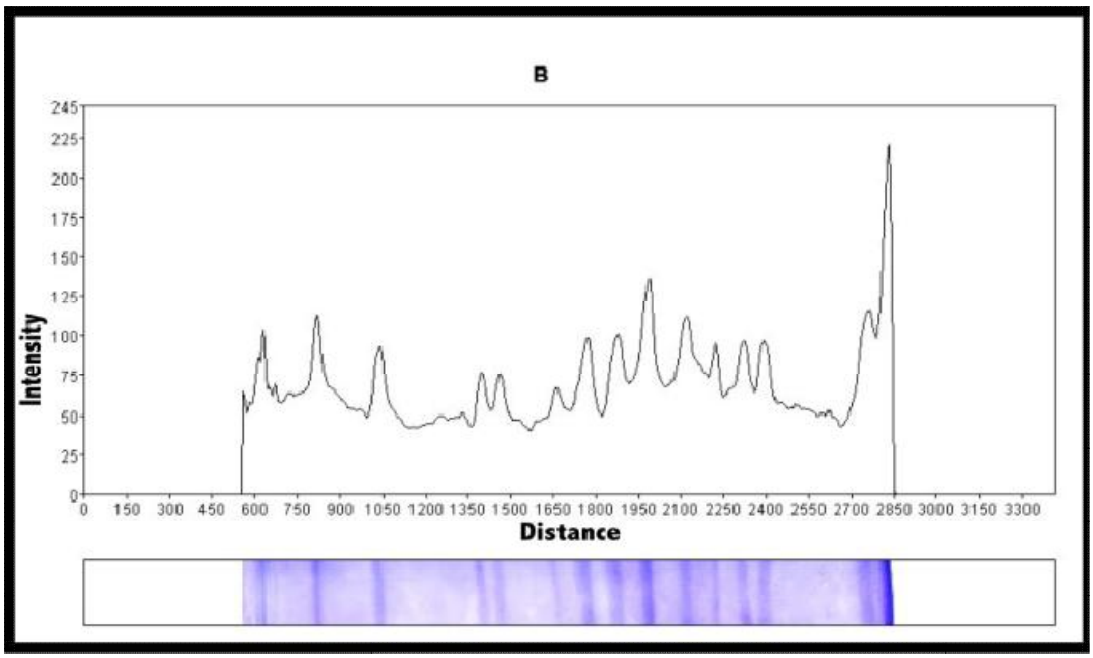

Fig. 7. Densitometric scan of protein electrophoretograms of *Cx. pipiens* ovaries extracts, 72 h post blood meal. (B)- *C. siphonella* treated females

3.3 Repellent Activities

Data given in (Table 4) indicated that, *N. magnifica* extract had a much more repellent activity against *Cx. pipiens* females than *C. siphonella* extract, and this activity was concentration dependent. The repellent activity of *N. magnifica* recorded 19.8, 45.8 and 73.8% for LC_{10}, LC_{30} and LC_{50}; respectively, while it recorded 17.8, 29.8 and 65.8% for LC_{10}, LC_{30} and LC_{50} of *C. siphonella* extract; respectively, vs. 0.0% of control females.

Table 4. Repellency effect of *N. magnifica* and *C. siphonella* extracts against *Cx. pipiens* females

Extracts	Sub-lethal Conc. (ppm)		No. of tested ♀	No. of fed ♀	% of fed ♀	No. of unfed ♀	% of unfed ♀	Repellency %
N. magnifica	LC_{50} (ppm)	47.6	50	13	26	37	74	73.8
	LC_{30} (ppm)	28.4	50	27	54	23	46	45.8
	LC_{10} (ppm)	8.4	50	40	80	10	20	19.8
C. siphonella	LC_{50} (ppm)	610.3	50	17	34	33	66	65.8
	LC_{30} (ppm)	327.4	50	35	70	15	30	29.8
	LC_{10} (ppm)	44.5	50	41	82	9	18	17.8
Control	--	--	50	42	84	8	16	0.0

4. DISCUSSION

The use of marine natural products is an alternative pest control method, which helps to minimize the usage of toxic pesticides and their deleterious effects on insects, livestock, wildlife and on the environment [21]. In recent years, researchers are concentrating on marine organisms to study their biological activities; especially, marine sponges (Porifera) which attracted significant attention from various scientific disciplines [22].

Negombata magnifica a conspicuous bright red branching sponge lives on shallow coral reefs in the northern waters of the Red Sea and Indian Ocean [23-26], it was never seen eaten by fish, when touched it releases a strong smelling reddish juice, this cytotoxic macrolides juice called latrunculins that found to be ichthyotoxic [27]. This toxin causes erratic behavior in fish followed by hemorrhaging, loss of balance and death. Another species used in this study was *Callyspongia siphonella*, this species one of the most widespread sponges in the northern Red Sea [25,28,29]. It is a rich source of triterpenoids [30] and this chemical product may hold promise for cancer treatment [31,32].

Fungal infections remain a major direct cause of death in patients who are treated for a malignant disease, and emerging resistance is also an important problem [33,34]. *Candida* is most often associated with serious invasive fungal infections, but other *Candida* spp. and yeast-like organisms have emerged as etiological agents of severe mycoses. Tested extracts especially *N. magnifica* exhibited interesting antifungal activity against *A. fumigatus* and *C. auris*.

The discovery of the antibiotics in the first half of the 20th century left the society and the scientific community unprepared for the emergence of antibiotic-resistant bacteria. This resistance has rapidly spread, and the infections caused by *Staph. aureus* and other resistant strains of pathogenic bacteria are currently a considerable problem. Even vancomycin, which was the last resource for the treatment of infections by Methicillin-resistant *Staph. aureus*, recently has been rendered ineffective [35]. Clearly, the emergence and clinical significance of drug resistant bacterial infection has created an urgent need for the rapid and continued development of new classes of antibiotics.

The results obtained revealed interesting antibacterial activity against the different bacterial strains tested when treated with the extract of *N. magnifica*, where it caused remarkable growth-inhibitory effects followed by the *C. siphonella*, these findings may be comparable with the previous findings [36], where they reported a high incidence of antibacterial activity against pathogenic bacteria treated with marine sponge crude extracts.

The results obtained showed also that, the used extracts exert some biological effects on the larva, pupa and adult stage of *Cx. pipiens*. The survival potential of the larval stage was highly affected by extracts tested. The LM% increased as the concentrations of extracts increased, *N. magnifica* was much more toxic against the larval stage followed by *C. siphonella* extract. These results may be in harmony with the previous findings [37], where they reported high toxicity of eleven marine sponges against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50} at <50 ppm).

The obtained results revealed that, both tested extracts caused toxic effects on different stages of *Cx. pipiens* mosquito, the MLD, MPD, pupal mortality %, adult emergence and fecundity were significantly affected at higher concentrations,

and this effect was concentration dependent. Concerning the effect of marine sponges on reproduction, reports on its toxic effects on insect reproduction are rare. The data obtained were in harmony with those obtained by [38], where the marine sponge *Cliona celata* methanol extract caused 100% ovicidal activity against *Cx. quinquefasciatus* and 72% ovicidal activity against *A. aegypti*

In many insect species, proteins are selectively incorporated into the yolk of developing oocytes and eventually comprise 70-90% of the total yolk protein [39]. Because these proteins are essential for yolk formation and characteristic of only egg maturing females, they have been termed vitellogenins or female-specific proteins [40].

The effects of tested extracts on vitellogenin synthesis and ovarian development were tested by evaluating intensity and the number of protein bands in *Cx. pipiens* female's ovaries. Six protein bands with high molecular weights, which believed to be vitellogenin, were detected in the control group. While this number reduced to 2 protein bands in ovaries of *N. magnifica* and *C. siphonella*-treated females. The present results may be comparable to the previous findings of [41], where they found that three female-specific protein bands with high molecular weights were detected in the untreated vitellogenic *Oncopeltus fasciatus* females.

Remarkable repellent activity was also presented by tested extracts, where, *N. magnifica* showed a much more repellent activity against *Cx. pipiens* females than *C. siphonella* extract and this activity was concentration dependent. These results maybe comparable with [38], where they tested the repellent activities of marine sponge *Cliona celata* extracts against *Cx. quinquefasciatus* and *Ae. aegypti*.

5. CONCLUSION

The present investigations have helped to focus on some bioactive substances isolated from marine resources; these molecules, which possess antimicrobial and insecticidal activities, could be used as insecticidal agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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