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### RESEARCH ARTICLE

#### Response of the mosquito vector, *Culex pipiens* to Manzamine-A as infection barrier against *Wuchereria bancrofti*.

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#### Abstract

*Culex pipiens* mosquitoes thrive in temperate and tropical areas and serve as efficient vectors of *Wuchereria bancrofti* the causative agent of Bancroftian lymphatic filariasis. The antifilarial effect of Manzamine-A, one of unique alkaloid groups isolated from Indo-Pacific sponges was evaluated by exposing the mosquito vectors that were treated with this drug to human individual infected with *W. bancrofti* as well as untreated mosquitoes. After the extrinsic incubation period of the nematode parasites, random samples of the fed mosquitoes were dissected to determine the infection rate. The survival rate of mosquitoes infected with microfilaria (mf) was calculated for both treated and control group. In addition, another group of mosquitoes were examined ultrastructurally to compare between the microfilarial course in both treated and untreated mosquitoes. Analysis of results indicated that Manzamine-A reduced the infection rate and the mf in the infected once didn't penetrate the mosquito midgut to reach the thoracic muscles, the site from which the filarial larval stages develop and move to the head to be transmitted to the next host during a subsequent blood meal.

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#### Introduction:

*Wuchereria bancrofti* is one of three mosquito-borne nematodes that cause lymphatic filariasis. Over 120 million people around the world are infected with *W. bancrofti* and another 1.2 billion are at risk (WHO, 2012). The pathogenesis of lymphatic filariasis is linked to host inflammation invoked the death of the parasite, resulting in altered lymphatic system and the abnormal enlargement of limbs and male genitalia, causing pain and severe disability (Al-Abd *et al.*, 2013). *Culex pipiens* is principle vector of *W. bancrofti* in urban areas of Africa. This species oviposit in stagnant polluted water, and populations are increasing and expanding due to urbanization, irrigation and in the Nile Delta, creation of Aswan High Dam (Michalski *et al.*, 2010). As infectious diseases evolve and develop resistance to existing pharmaceuticals, the marine environment represents a treasure of useful products a waiting discovery for the treatment of fungal, parasitic, bacterial and viral diseases. A small number of marine plants, animals and microbes have already yielded more than 12000 novel chemicals, with hundreds of new compounds still being discovered every year (Donia and Hamann, 2003). One of these chemicals is Manzamine-A, which belongs to a group of unique Beta-carboline alkaloids isolated from Indo-Pacific sponges of genus *Haliclona*. These compounds exhibit a diverse range of bioactivities including cytotoxicity, insecticidal, antibacterial and antimalarial activities.

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## Materials and methods:

### Mosquito rearing:

The colony of *Culex pipiens* was established from larvae and pupae obtained from breeding sites in Abu Rawash, Giza Governorate, Egypt. Twenty-five individuals of 3<sup>rd</sup> instar larvae from the second generation were placed in plastic cups (diameter 12cm x height 7cm) containing 250 ml of Manzamine-A solution. Another group of larvae with the same number were placed in 250 ml dechlorinated tap water as control. The larvae were daily provided with fish food as a diet, which proved to be the most preferable food for their development and a well female fecundity (Kasap and Demirhan, 1992). The emerged adult females were provided with 10% sugar solution on cotton pads.

### The test compound:

The antiparasitic compound used in this work was Manzamine-A, polycyclic marine-derived alkaloids, isolated from the sponge *Halicionasp.* It was from sigma chemical Co. with suggested used concentration: 0.5-1 µg/ml. It was solubilized in dimethyl sulfoxide (DMSO) and kept in a refrigerator under sterilized conditions. The concentration used for treatment was 1 µg/ml.

### Parasites and Parasite exposure:

Source of mf for this study was a volunteer that was acquainted with the details of the study and the nature of her participation. Before exposure to the infected volunteer, the mosquito females emerged from Manzamine-treated and untreated larvae were starved for 24h. The starved females were exposed to volunteer hand and forearm for 20-30 minutes. The infection of the vectors was conducted between 10 PM and midnight (the peak of microfilaraemia activity), Rocha *et al.*, 1991. After exposure the engorged mosquitoes were maintained according to standard procedures in the laboratory at 27°C.

### Calculation of the infection rate:

Ten engorged mosquitoes were anaesthetized and each female was dissected separately after removal of wings and legs for detection of *W. bancrofti* mf. The mosquito's head, thorax and abdomen were teased a part in saline and were examined carefully under binocular microscope and the infection rate was calculated according to Hassan *et al.*, 2013, as following:

$$\text{Infection rate} = \frac{\text{Number of infected mosquitoes}}{\text{Total number of dissected mosquitoes}} \times 100$$

### Calculation of survival rate:

Twenty-five females of both Manzamine-treated and untreated mosquitoes were maintained in plastic cups covered with gauze netting and fed on 10% sugar solution and held for 14 days post blood meal (PBM). Dead mosquitoes were counted and removed daily (Hassan *et al.*, 2013) survival rates were calculated according to the following equation:

$$\text{Survival rate} = \frac{\text{Number of mosquitoes survived for 14 days}}{\text{Number of maintained mosquitoes}} \times 100$$

### Transmission electron microscopy:

Treated and control females were dissected PBM. Grids were examined by JEOL JEM 1010 using image analysis document at Al-Azhar University Regional Center for Mycology and Biotechnology.

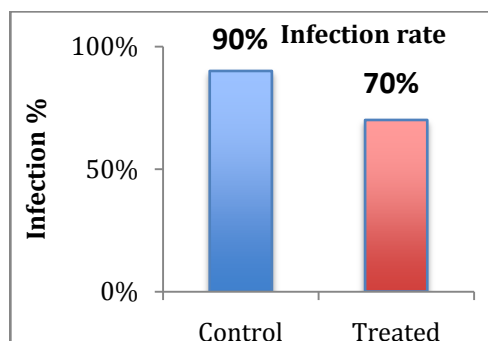
## Results:

The infection rates with *Wuchereria bancrofti* in females of *Culex pipiens* that emerged from 3<sup>rd</sup> larval instar which treated with Manzamine-A as well as the untreated ones were shown in table 1 and figure 1. The treatment with Manzamine-A resulted in reduction of the infection rate, that it was 90% in untreated group while the treated group recorded 70%.

**Table 1:** Infection rates of *C. pipiens* females post blood feeding on *W. bancrofti* microfilaraemic volunteer.

Treatments	No. of dissected mosquitoes	No. of infected mosquitoes	Infection rate*(%)
Control	10	9	90
Manzamine-A	10	7	70

\*Infection rate = No. of infected mosquitoes / Total No. of dissected mosquitoes X100.



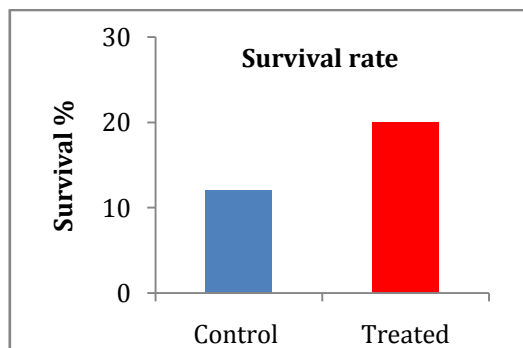
**Fig. (1):** Infection rates of *C. pipiens* females post blood feeding on *W. bancrofti* microfilaraemic volunteer.

Data given in table (2) and Figure (2) illustrated the survival rates of *C. pipiens* females emerged from both treated and untreated larvae. The data revealed elevation in survival rate of the treated group (20% compared with 12% in control group).

**Table 2:** Survival rates of *C. pipiens* females post blood feeding on *W. bancrofti* microfilaraemic volunteer.

Treatments	No. of maintained females	Mosquitoes survived for 14 days	Survival rate*(%)
Control	25	3	12
Manzamine-A	25	5	20

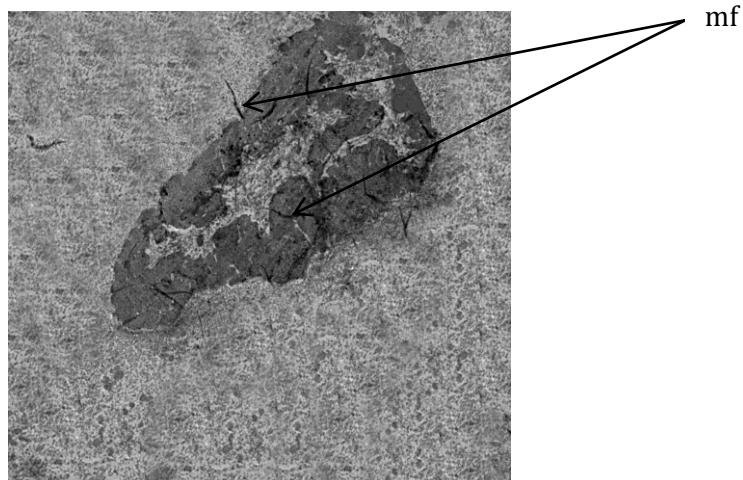
\*Survival rate = No. of mosquitoes survived for 14 days PBM/ No. of maintained mosquitoes X 100.



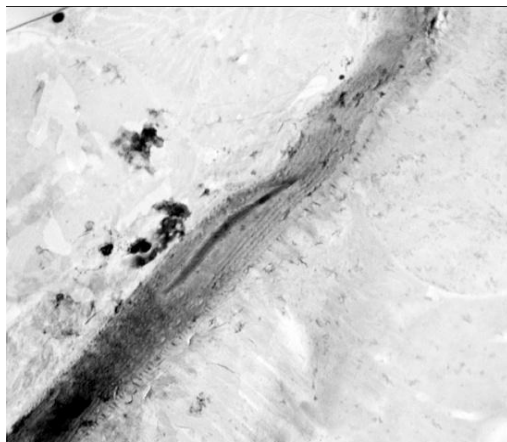
**Fig. (2):** Survival rates of *C. pipiens* females post blood feeding on *W. bancrofti* microfilaraemic volunteer.

The four provided electron micrographs (Figs. 3-6) showed the difference of the filaria presence in the midgut and indirect flight muscles of *C. pipiens* females emerged from both treated and untreated larvae. The micrograph of figure 3 illustrated transversely sectioned midgut of a female emerged from normal larva with microfilariae that succeeded in penetration of the midgut to the hemolymph to migrate and develop in the indirect flight muscles as shown in micrograph 4.

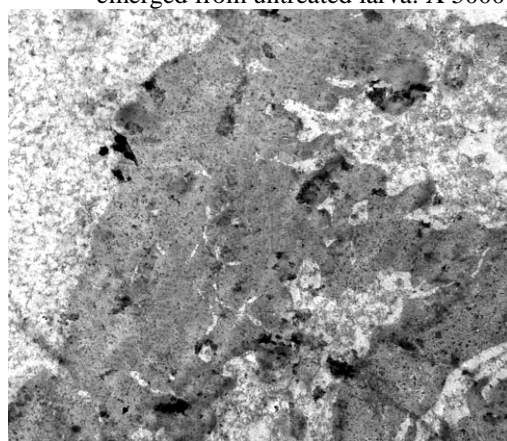
In contrast to the two previous micrographs, figs. 5 and 6 showed that the microfilariae did not penetrate the borders of the midgut and failed to reach the flight muscles. This means that Manzamine-A has an effect on the motility of the parasite and prevents it from reaching the flight muscles. Micrograph 6 showed the tracheoles dispersed between the fibrils of the muscles.



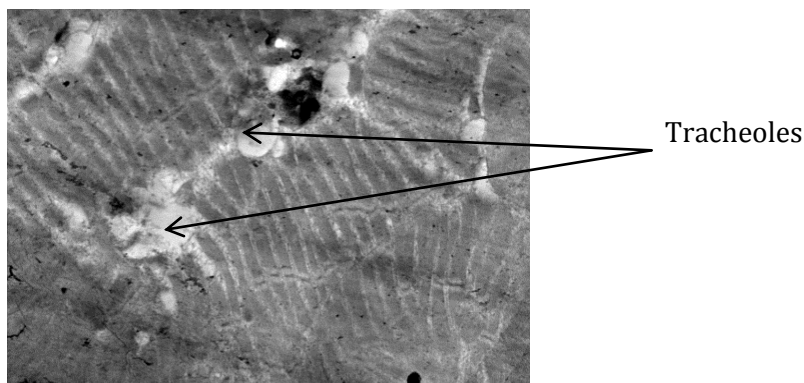
**Fig. 3:** An electron micrograph of midgut in *C. pipiens* female emerged from untreated larva. X 4000.



**Fig. 4:** An electron micrograph of transversely sectioned indirect flight muscles in the thorax of *C. pipiens* female emerged from untreated larva. X 5000



**Fig. 5:** An electron micrograph of midgut in *C. pipiens* female emerged from larva treated with Manzamine-A. X8000



**Fig.6:**An electron micrograph of transversely sectioned midgut flight muscles in the thorax of *C. pipiens* female emerged from larva treated with Manzamine-A. X 8000

### Discussion:

In recent years the marine environment has emerged as a promising source for new lead drugs to combat various infectious diseases (El-Sayed *et al.*, 1996). *Haliclona* sp. are marine demosponge of family Chalinidae in the animal kingdom. The biological activity of these sponges is said to be due to the presence of novel sterols, metabolites including steroids, terpenoids, alkaloids, cyclic peptides, and unsaturated fatty acids. Further analysis on the chromatographic fraction revealed the presence of a mixture of four alkaloids, namely, mimosamycin, xestosponging C, xestosponging D and araguspongin C (Gupta *et al.*, 2012). The fairly common and easily isolated alkaloids from these sponges that are known commercially as the manzamines show dramatic and unexpected improvements in activity against nematodes, malaria, viruses and bacteria (Hamann and El-Sayed, 2004).

The present work provides experimental support for the antifilarial effect of Manzamine-A, that the treatment of *Culex pipiens* mosquitoes with this drug reduced the infection rate with *Wuchereria bancrofti*. This result agreed with Gupta *et al.* (2012) and Lakshmi *et al.* (2009) who reported the antifilarial activity of alkaloids extracted from *Haliclona* sp. against different filarial types and stages.

In a susceptible host, *W. bancrofti* microfilariae migrate from the midgut and develop in the indirect flight muscles. The metamorphosis from the mf to infectious L<sub>3</sub> stage-a process that necessitates a physical transformation from mf to sausage-shaped first stage larvae L<sub>1</sub>, and two subsequent molting events. Under optimal conditions, development takes approximately 10-12 days and the parasites increase in size 4-6 times (Bartholomay and Christensen, 2002). The ultrastructure microscopy of the normal mosquito female in the present work demonstrated this course of the parasite, that the electron micrographs showed the penetration of the mosquito's midgut by mf and the presence of larvae in the indirect flight muscles. On the other hand the electron micrographs of a female emerged from treated mosquito larva showed the failure of mf to reach the muscles. Gupta *et al.* 2012 found that the sponge alkaloids affect the motility of filaria as a mechanism of antifilarial activity that may explain the present work findings.

The parasite development is not benign process; *W. bancrofti* microfilariae inflict debilitating or even lethal damage on the mosquito hosts by disrupting normal cellular and physiological processes in the flight muscles (Bartholomay 2014). These effects supported the present work data, that the survival rate of the normal female mosquitoes was less than that of females emerged from treated larvae. In mosquitoes, midgut infection and escape barriers that have been described include inhospitable chemical environment of midgut lumen that destroys incoming parasites (Higgs 2004). The treatment of mosquito larvae with Manzamine-A might change the chemical environment of midgut resulting in incapability of the mf to escape from the midgut and transform to infectious L<sub>3</sub> stage.

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