PRELIMINARY STUDY OF THE INSECTICIDAL ACTIVITY OF THE MEDICINAL PLANT ANASTATICA HIEROCHUNTICA AGAINST CULEX PIPIENS MOSQUITO

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Abstract - The insects Culicidae are probably the best known and the most feared for both parasitic diseases they can inoculate during their blood meal and the nuisance their presence constitutes. The distribution area of arthropods involved in the transmission of these diseases has continued to expand, placing new human populations in areas at risk of infection. In order to counter the spread of insects and epidemics arising there, several methods involving the use of chemical insecticides were tested. However, apart from their toxicity, some chemicals used in this fight have become less effective because of the resistance developed by some mosquitoes. Scientists then try to find other products accessible, less toxic (natural products especially who are known as bio-insecticides) to lead this fight. In this context, the use of natural molecules (of ecological and economic interest) with insecticidal or insect repellent properties proves to be alternative to the use of synthetic insecticides. The use of medicinal plants as insecticides has been known for a long time. In this study, we tested the larvicidal potential of Anastatica hierochuntica on the larvae of a species particularly replied, very common in cities Culex pipiens. The toxicological assays were carried out using the plant infusion, with different concentrations (15, 30, 60 and 100g/l), on the fourth instars larvae of Culex pipiens, for 24 hours, to determine the two lethal concentrations LC50 and LC90. Results, under laboratory conditions, indicate a variable sensitivity of the treated larvae; this sensitivity is even higher with increasing concentration of Anastatica hierochuntica. The lethal concentrations estimated are 39.87 g/l for LC50 and 228.58 g/l for LC90.

Keywords - Bio-insecticide, Culex pipiens, medicinal plant , Anastatica hierochuntica.

I. INTRODUCTION

Mosquitoes are one of the most important vectors for arboviruses of medical and veterinary importance. The majority of medically important arboviruses such as dengue, chikungunya, and Zika virus, are transmitted by mosquitoes to more than 700 million people annually (Taubes, 2000; Stanaway et al., 2013; Tyagi, 2016) and circulating in the tropics (Weaver & Reisen, 2010). However, globalisation and climate change have contributed to the emergence of arboviruses in more temperate regions (Kilpatrick & Randolph 2012). These diseases are also responsible for huge economic losses, both in terms of health care costs and lost productivity (Jones et al., 2008; karunamoorthi et al., 2009; Tolle, 2009).
The development of effective and safe insecticides against arthropods is very important, because there are no effective vaccines against arthropod-borne viruses (arboviruses) and parasites. Controls of such serious diseases are becoming increasingly difficult. Synthetic pesticides are at the forefront of mosquito-controlling agents, and have been extensively used for mosquito control by either killing, preventing adult mosquitoes to bite human beings or by killing mosquito larvae at the breeding sites of the vectors (Djeghader et al., 2014a,b; Bouaziz et al., 2017). However its deleterious impact on non-target population, pollution and the development of resistance (Schoeters & Hoogenboom, 2006; Bonde et al., 2008; Lima et al., 2011; Belinato et al., 2012) prompted for the search of alternative, simple and sustainable methods of mosquito control. In recent years, the use of environment biodegradable natural insecticides of plants origin have received renewed attention as agents for vector control because they are rich in bioactive chemicals, active against a limited number of species including specific tagret insects, and biodegradable. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Rahuman et al., 2009). In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the medicinal plant Anastatica hierochuntica against the medically important mosquito vector Culex pipiens.

II. MATERIALS AND METHODS

2.1. Rearing of mosquito larvae of Culex pipiens:

The toxicological essays were conducted in the laboratory following the standard methods for testing larval susceptibility (Rehimi & Soltani, 1999). Different instars larvae of Cx. pipiens were obtained from laboratory colonies of the department. Larvae were reared in storage jars containing 500 ml of stored tap water and maintained at a temperature between 25-27 °C, 85% of relative humidity (RH) and a photoperiod of 14:10 (L:D). Larvae were daily fed with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight), and water was changed every four days. The feeding was maintained till the larvae were developed into the pupa stage. The pupae were collected from the culture trays and were transferred to jars containing 500 ml of water with the help of a dipper. The jars were kept in (50×50×50 cm) size mosquito cage for adult emergence. The adults were fed with 10% sugar solution for a period of three days before they were provided by an animal for blood feeding.

2.2. Plant material:

A. hierochuntica (Family: Brassicaceae) locally called 'Kaff-e-Maryam', is a well known desert zone medicinal plant. All parts of A. hierochuntica are famous and used in folk medicine to provide cure against various disease states.

A decoction of Kaff-e-Maryam (A. hierchuntica) has been in use against stomach upset, as antispasmodic, against fatigue, uterine haemorrage, menstrual cramps, and depression. It is believed to be a cure for arthritis inflammations, infections, diabetes, asthma, viral and autoimmune diseases. It is also used for pain relief after surgery. Its freshly prepared decoction is commonly used as local disinfectant, to ease childbirth, as liver tonic, to stop vomiting, to treat mouth ulcers, and provide cure against stomach cancer. All parts of the plant were reported to possess antimicrobial activity.

Based on its frequent medicinal use and popularity among the people of central Asia, Africa, Arabian Peninsula, and else-where, Kaff-e-Maryam was selected for present study. The areal parts of the plant were washed with distilled water, shade dried and ground. The finely ground plant powder (500 g/l hot water) was extracted with infusion using hot water. The extraction was continued till visibly no further extraction is possible. The crude extracts and stored at 4°C. Standard stock solutions were prepared at 1 % by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassays.

2.3. Larvicidal bioassays:

Larvicidal bioassay was performed by method. Insecticidal activity of this plant was evaluated at 15, 30, 60 and 100g/l to produce a range of mortality from 20% to 90% along with control. Mortality of mosquito larvae was determined at the end of 24h recovery period. The number of mosquitoes died was recorded till the last mosquito and lethal concentrations (LC50 and LC90) values were determined.

2.4. Statistical Analysis:

The mortality percentage observed for each stage and concentration was corrected and subjected to the probit analysis. LC50, LC90, confidential limits and the slope were calculated. Data from insecticidal tests were subjected to analysis of variance after angular transformation of observed mortality percentages.

III. RESULTS

The mortality observed during the treatment of the fourth instar larvae of Culex pipiens with different concentrations of the tested plant Anastatica hierochuntica is shown in the figure 1 with rates of 30.66% after exposure to the lowest concentration 10 g/l and 81.33% with the highest concentration 100 g/l (Figure 1).

The results of the statistical analyzes reveal a highly significant effect (P<0.000). The equation and the regression line were determined after a transformation of corrected mortality to probit and the concentrations tested in decimal logarithms (Figure 2). The coefficient of determination (R^2= 0.92) reveals an equally positive relation between the probit and the decimal logarithms of the tested concentrations. The LC50 and CL90 lethal concentrations estimated from the equation and the regression line are 2.88 g /l, respectively, with a confidence limits of 29.75 to 53.42 (mg/l) and 4.98 g / L with a confidence limits of 170.58-306.29 (mg/l) (Table 1).

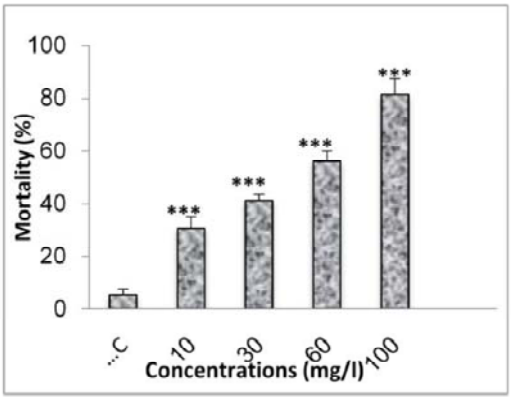

Figure1: Observed mortality of fourth instars larvae of Culex pipiens after treatment with Anastatica hierochuntica for 24h (Means±SD, n=75).

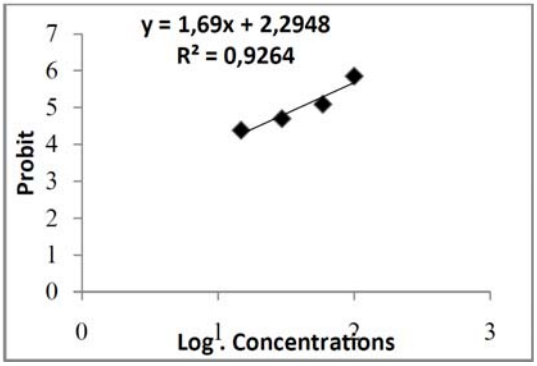

Figure2: Concentrations response relationship for treatment of the Anastatica hierochuntica applied to the newly ecdysed larvae of Culex pipiens (R^2= coefficient of determination).

Table1: Toxicity of Anastatica hierochuntica on the fourth instar larvae of Culex pipiens (LC50, LC90 mg/l and Confidence Limit).

Stage	Slope	LC50	LC90
L4	3.88	39.87 (29.75-53.42)	228.58 (170.58-306.29)

IV. DISCUSSION

Among the widely used methods to control mosquitoes is the use of synthetic insecticides (WHO, 2017). However, the extensive use of these chemicals in urban and semiurban environments has resulted in

the development of resistance among the mosquito populations to these insecticides. Several studies have reported the development of resistance in Cx. pipiens against temephos (Cheikh & Pasteur, 1993), pyrethroids (Scott et al., 2015), Bacillus sphaericus toxins (Nielsen-Leroux et al., 1997; Paul et al., 2005; Darboux et al., 2007), deltamethrin, lambda-cyhalothrin, beta-cyfluthrin, and bifenthrin (Al-Sarar, 2010). Recently, Cx. pipiens has developed resistance against diflubenzuron, one of the most effective mosquito larvicides used in Europe and some other countries (Grigoraki et al., 2017).

In addition to resistance, some mosquitocides may be associated with diverse environmental hazards, such as accumulation of non-biodegradable residue, biomagnification in the food chain, and toxicity to human and animal health (Rawani et al., 2009). Therefore, there is a need for novel insecticides that are effective, cheap, and ecologically friendly such as medicinal plants. The present study is the first to report the insecticidal effect of Anastatica hierochuntica against Culex pipiens larvae. The bioassay with Kaff-e-Maryam showed a high toxicity against the treated fourth instars larvae compared to the control. The efficacy of natural products as insecticides has been developed in Algeria through a multitude of recent work (Amira et al., 2018; Djeghader et al., 2018). Several studies have documented the efficacy of plant as the reservoier pool of bioactive toxic agents against mosquito larvae (Deepa et al., 2015; Afzal et al., 2018; Al-Mekhlafi et al., 2018; Trivedi et al., 2018).

Generally the active toxic ingredients of plant are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components (Rattan, 2010).

This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway) (Rattan, 2010).

CONCLUSION

Today, Environmental safety is considered to be of paramount importance. Plant could be an excellent alternative source for mosquito larvicides due to their ecofriendly nature. This study reveals the excellent larvicidal potentiality of Kaff-e-Maryam (A. hierchuntica). Further investigations are needed to elucidate this activity against a wide range of mosquito species and also to identify active compounds for the mosquitocidal activity.

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