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BIOINSECTICIDAL ACTIVITY EVALUATION OF *PERGULARIA TOMENTOSA* AND *URTICA DIOICA* AGAINST INSECT VECTORS MOSQUITOES AND PHLEBOTOMINE SAND FLIES

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ABSTRACT

Plant extracts are showing to be a good alternative sources, instead of conventional pesticides, for pest and vector insect control agents. Plant extracts and essential oils were used as bioinsecticides products and are exhibited a toxicity to insect pests with a low effects on non target animals and environment. *Pergularia tomentosa* and *Urtica dioica* are plants that have been widely used in traditional medicine since ancient times due to their different medicinal properties. The purpose of the present study was to assess the adulticidal activities of a medicinal plant, *P. tomentosa* and *U. dioica* , against the most abundant insect vectors in Algeria; the domestic mosquito *Culex pipiens* and phlebotomine sand flie *Phlebotomus papatasi*, under laboratory conditions. The toxicological assays were carried out using a ethanolic extracts formulation of *P. tomentosa* and *U. dioica* separately, with different concentrations (150, 200, 300, 400 µl/ml air) on the adults of the mosquito and phlebotomine sand flies. The obtained results showed a highly significant toxic effect of these extract plants for the tested concentrations, with concentration response relation-ship mortality, for the treated adults of both insect species. The lethal concentrations values with confidence limits (LC50 and LC90) against mosquito, were estimated at 179.9, 266 µl/ml *P. tomentosa* and at 216.5 and 383.8 µl/ml for *U. dioica* . Whereas the LC50 and LC90 values of the bioassay against phlebotomine sand flies were higher, with 192.7 and 278 µl/ml for *P. tomentosa* and 184, 315 µl/ml *U. dioica* . It was concluded that *P. tomentosa* and *U. dioica* extracts exhibit an insecticidal effects against adult mosquito and phlebotomine sand flies and could be considered as a potential alternative to the widely used the conventional insecticides.

Keywords: Toxicology, plant extract, vector, insect, mosquito, pphlebotomine

INTRODUCTION

Vector-borne diseases are infectious, caused by parasites, viruses or bacteria that are transmitted by vector insects (Amraoui 2019). Vector insects are playing a crucial role in the spread of various pathogens, that can infect human, animal or plant hosts, thereby contributing to the dissemination of serious diseases. Each year, more than 700,000 deaths worldwide are attributed to vector-borne diseases (WHO 2017). Culicidae and Phlebotominae are among the most distributed insect families found in Algeria (Zeroual et al 2019; Aroussi et al. 2019). Mosquitoes are the most important vectors of vector-borne diseases (Amraoui 2019), such as: dengue, yellow fever, Japanese encephalitis, Chagas disease, onchocerciasis, malaria, schistosomiasis, and trypanosomiasis (Bian 2013). Algeria is affected by Cutaneous Leishmaniasis (CL), which poses a significant public health problem, leading to permanent skin disfigurements, particularly in the southern regions of the country, where Phlebotomine sand flies play a major role in the proliferation of CL (Zeroual et al 2019).

Insects are commonly controlled by chemical insecticides. Although effective, their repeated use has disrupted natural biological control systems and led to resurgence of these insects, resulted in the development of resistance and had undesirable effects on environment, non-target organisms and human health concern (Jones 2008; Adeleke 2010). The increasing concern; over the level of chemical residues in the different environmental sites and especially in food, has encouraged researches to find new alternatives of conventional pesticides (Boudjelida et al. 2005). These synthetic pesticides are expensive and have in many cases only produced moderate results along with major ecological damage (Dhadialla et al. 2005; Aïssaoui and Boudjelida 2014, Aïssaoui et al. 2022). Plants have generated extraordinary interest in recent years as potential alternatives natural insect control agents. Botanical extracts have several advantages over traditional pest control agents; such as specificity, biodegradability and low mammalian toxicity (Bendjedid et al. 2022). The major insect control operations were carried out using synthetic insecticides, such as organochlorine and organophosphate compounds (Aïssaoui et al. 2022). It though these products play a vital role in controlling pests and vector insects, but from their abusive use some negative impacts were emerged on environment generally against the non-target organisms, and a harmful effect specially on human health (Yezli et al. 2024 A).

The application of biodegradable plant compounds is considered one of the safest methods of control of insect pests and vectors (Sivagnaname and Kalyanasundaram 2004; Sun et al. 2006; Alouani 2017; Yezli et al. 2024 A). Several plants used in traditional medicines were tested their mosquito larvicidal activities (Chinnaperumal et al. 2010; Yezli et al. 2024 B). These bioactive chemicals, due to their properties, may act as larvicides, adulticides and ovipositional attractants against different mosquito species (Pratiwi and Nurlaeni 2021: Ragavendran et al. 2024). According to the interest in developing plant insecticides, the present study was carried out in order to evaluate the larvicidal activities and the repellent effects of the medicinal of two plants, *Pergularia tomentosa* L. and *Urtica dioica* L. against a domestic mosquito species, *Culex pipiens* and the phlebotomine sand flies.

MATERIALS AND METHODS

Collection of adult sand flies: The adult phlebotomine sand flies sampling was done out in several localities of the Sidi Okba station (Biskra District) between July and November 2023. In this area 15 locations covering the Biskra region were selected. Sand flies were collected biweekly from indoor habitats (courtyards and stable) and outdoor habitats (house yard, rodent burrow and wall crevices). The adult insect capture was done using sweep net (Figure 1A) and CDC miniature light and (figure 1B). The traps placed in habitat biotopes and the capture was done overnight.

Figure1: Phlebotomine sand flies sampling Materiel: (**A**) sweet net & (**B**) CDC miniature light.

Mosquito rearing: The bioassays, were conducted in the laboratory according to the testing methods for larval susceptibility (WHO 1991). The domestic mosquito, *Culex pipiens* larvae were obtained from laboratory mass colonies. Larvae were reared in storage jars containing dechlorurated tap water and maintained at temperature between 25-27 °C, 85% and a photoperiod of 14:10 (L:D). They were fed with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight), and water is changed every three days. During pupal stage, the pupae were transferred to other jars placed in mosquito cage for adult emergence. The emerged adults were fed with 10% sugar solution (Rehimi and Soltani 1991).

Botanic material: the selected plants, *Pergularia tomentosa* and *Urtica dioica* are harvested from a semi-arid region, Biskra district. *P. tomentosa* is an evergreen plant (Figure 2**A**), perennial shrub belonging to Apocynaceae family and have particular smell (Cherif et al. 2016). The *U. dioica* is known as medicinal plant because of its therapeutic effects to humans (Dhouibi 2022). It is an evergreen plant (Figure 2**B**) is belonging to Urticaceae family, grows in temperate tropical and arid regions throughout the world.

Figure 2: Fresh plant *Pergularia tomentosa* (**A**) & *Urtica dioica* **(B).**

Adulticidal test: The used plant material consists of the *U. dioica and P. tomentosa* leaves was collected during the spring period from the localities, Ain Zâtoot and Biskra regions, respectively. The plant materials were dried in darkness at room temperature for a week. The dried plant leafs were crushed separately using an electric grinder, 100g of the powdered samples was macerated in one liter of methanol (70%) for 24 h. After a preliminary screening of the plants *P. tomentosa* and *U. dioica* was applied by fumigation at different concentrations of 200, 250, 300 and 350 µl/ml of air on a filter paper disk of 2.5 Cm in diameter (Khani and Besayand, 2012). Live adults from mosquitoes *Cx. pipiens* and the phlebotomine sand flies; represented by the most abundant species, *Phlebotomus papatasi* (Zeroual et al., 2019) were introduced into glass bottles of a 250 ml capacity. Three replicates of 10 individuals were made for each concentration. A positive control series were conducted in parallel using ethanol (70%) only. Mortalities of these treated insects, were recorded during 120 min, after treatment and observed mortalities were corrected according to the formula of Abbott (1925). since the mortality is lower than 4%, the observed mortalities were considered and submitted to statistic and probit analyzes and the lethal concentrations (LC50 & LC 90) and their confidence limits (95% LC) were calculated with GRAPH PAD PRISM 6 software.

RESULTS

Adulticidal bioassay against mosquito adults: The results of the toxic effect of *P. tomentosa* and *U. dioica* ethanolic extracts, applied by fumigation for 120 min against adults of *Cx. pipiens* mosquitoes, revealed that the observed mortality values increased throughout the time of observation (Figure 3). The accumulation of the observed mortality under the effect of *P.*

tomentosa varies from 45 % for the 200 µl/ml area concentration, and 95% for the highest concentration of 350 µl/ml area. therefore, the recorded mortality of the mosquito adults treated with the same concentrations of *U. dioica* was 65% for the lowest concentration and reached the 100% before 120 min, the observation time (Figure 3). The results of the statistical analyzes reveal a highly significant effect between concentrations($P<0.001$). Also, the statistical analysis for the comparison of the mortalities of the two used plant *P. tomentosa* and *U. dioica* ethanolic extracts, showed a significant effect between the different and the *P. tomentosa* was more toxic than *U. dioica* extract.

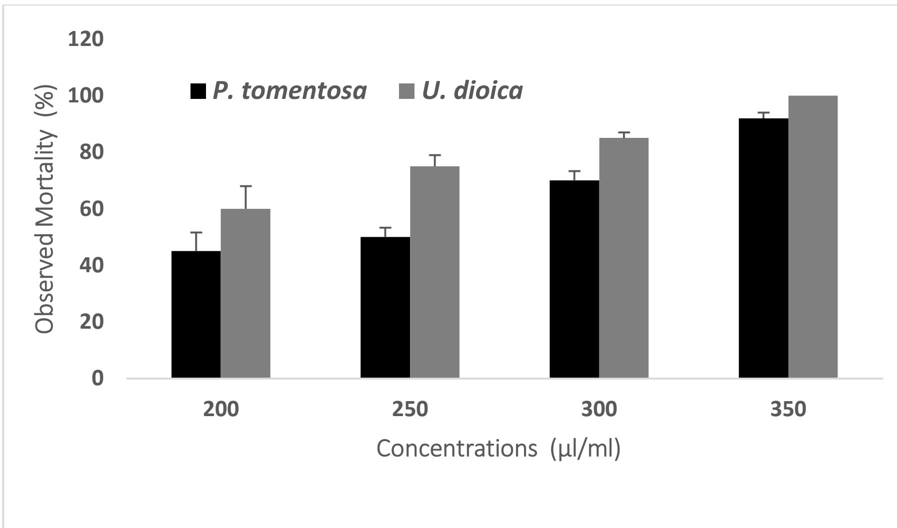

Figure 3. Toxic effect of *P. tomentosa* and *U. dioica* at different concentrations (µl/ml air), applied by fumigation, against adult *Culex pipiens*. Observed Mortality (%): values are significantly different by HSD test at $P < 0.001$.

The equation and the regression line were determined after a transformation of corrected mortality to probit and the used concentrations in decimal logarithms (Figure 4). The curves, for both plants, revealed a concentration response relationship, since the mortality increased with the increase of the concentrations. The coefficient of determination of *P. tomentosa* (R^2= 0.98) and *U. dioica* (R^2= 0.95) revealed an equal positive relation between the probit and the decimal logarithms of the tested concentrations (Figure 4).

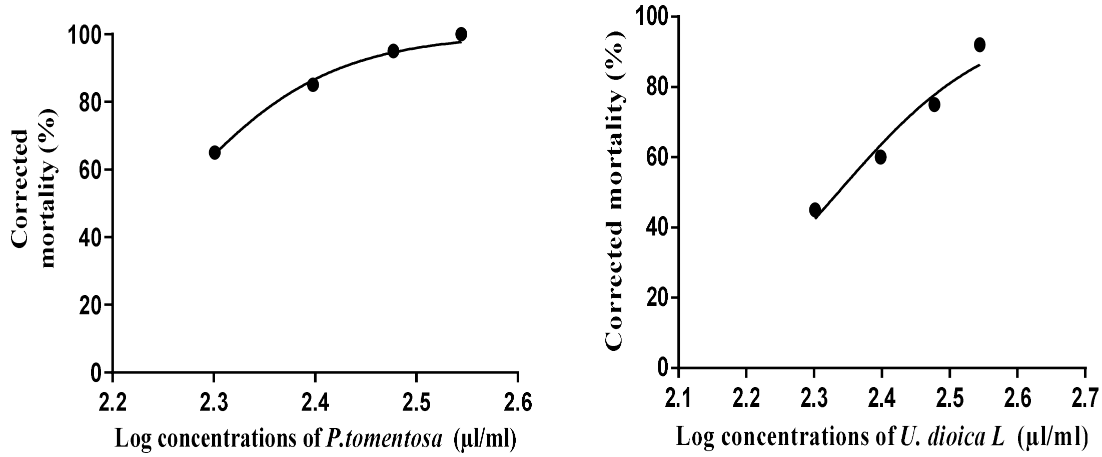

Figure 4: Concentration response relationship for treatment of *P. tomentosa* and *U. dioica* at different concentrations (µl/ml air), applied by fumigation against mosquitoes adults of *Culex pipiens* (R^2= coefficient of determination).

The lethal concentrations (LC50 and LC90) were estimated from the linear regression, at the 95% confidence limits (LLC=Lower LC- UCL=Upper LC) (Table 1). For the concentrations tested, the highest effect activity was observed with the highest one of 350 µg/ml of *the two plant* extracts, used against of *Culex pipiens adult* with the LC50 and LC90 values of 179.9 & 266 µl/ml *P. tomentosa* and 216.5 & 383.8 µg/ml for *U. dioica* respectively (Table 1). The slope values were also calculated and presented in table 1. The plant extract showed dose-response relationship mortality. At higher concentrations, the adult showed restless movement, without mobility, for some times with abnormal wagging and then died.

Table 1. Toxicity of analysis of *P. tomentosa* and *U. dioica* ethanolic extract applied by fumigation for 120 min against adult of *Culex pipiens* mosquitoes. Determination of lethal concentrations (LC 50 & LC90 µl/ml air) and their 95% confidence intervals.

Lethal Concentrations	**LCL >LC> UCL) (µl/ml) Fiducial limits (95%)**	
Plant	***P. tomentosa***	***U. dioica***
LC50	174.5>179.9 >212.7	180.2>216.5 >259.9
LC90	235.6>266>300.2	297>383.8>471.2
R^2	**0.98**	**0.95**
Slope	**0.625** (2.908-8.291)	**3.836** (0.699-6.972)

Adulticidal bioassay against adults of Phlebotomine sand flies: The bioassays results, of the adulticidal activity of the plant *P. tomentosa* and *U. dioica* ethanolic extracts against the adult of the phlebotomine sand flies, are expressed by the recorded observed mortality during 120 min of observation. The mortality percentage for the used concentrations of the two plants are presented in figure 5. The mortality throughout the time for each concentration of *P. tomentosa* is varying between 45% and 92%. Whereas, the toxic effect of *U. dioica* was expressed by a mortality of 65% for the concentration of 200 µg/l and 100 % for the higher concentration of 350 µl/ml. from the present results it was noticed that the insects were more sensitive to *U. dioica* than *P. tomentosa* and this was interpreted with percentage of the mortalities under the effect of the plant extracts. The results of the statistical analyzes reveal a highly significant effect (*P*<0.000) (Figure 5).

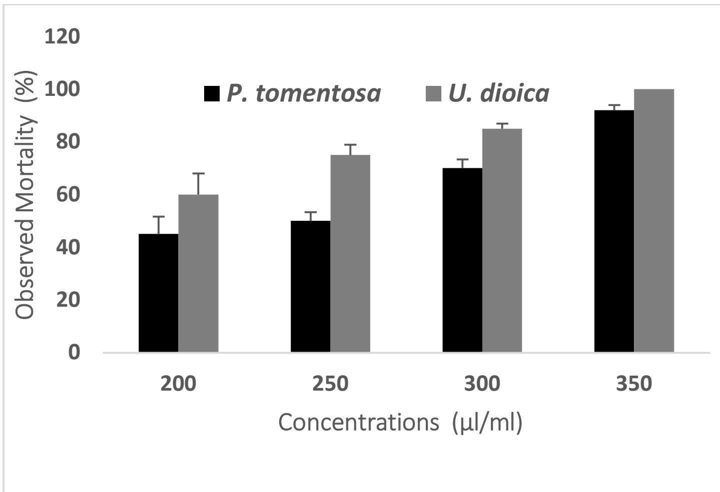

Figure 5. Toxic effect of *P. tomentosa* and *U. dioica* at different concentrations (µl/ml air), applied by fumigation, against adult phlebotomine sand flies. Observed Mortality (%): values are significantly different by HSD test at P < 0.001.

The regression curves were determined, after the transformation of the observed mortality to probit and the tested concentrations in decimal logarithms of both plants *P. tomentosa* and *U. dioica* (Figure 6). The coefficient of determination (R^2 = 0.98, and 0.92 for *P. tomentosa* and *U. dioica* respectively) reveals an equal positive relation between the probit and the decimal logarithms of the tested concentrations (Figure 6). For the tested concentrations, of *P. tomentosa* ethanolic extract the highest toxic activity was observed with the highest one 350µl/ml air against *phlebotomine* sand flies with the LC50 value of 192.7 with a lower lethal concentration of 174.5 and the upper one with 212.7µl/ml. for the LC90 value was 278µl/ml with a lower lethal concentration of 235.6 and the upper limit of the lethal concentration of 327.9µl/ml air (Table 2). Therefore, the results of the used different concentrations of the ethanolic extracts of *U. dioica* applied by fumigation for 120 min against *phlebotomine* sand flies, caused a mortality of larvae with a concentration-response relationship (Figure 6). Table 2 presents the effect of the toxicity analysis. The LC50 and CL90, lethal concentrations were estimated from the regression line which are 184 g /l, and 325 µl/ml, with their confidence limits of 135.8-249.2 (mg/l) and 208.6-475.7 µl/ml, respectively (Table 2).

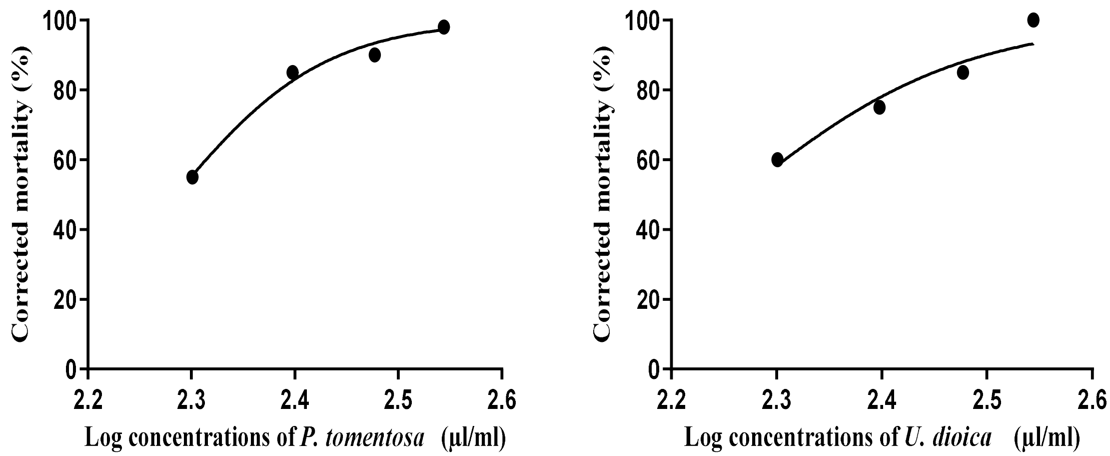

Figure 6: Concentration response relationship for treatment of *P. tomentosa* at different concentrations (µl/ml air), applied by fumigation against mosquitos' adults of phlebotomine sand flies (R^2= coefficient of determination).

Table 2. Toxicity of analysis of *P. tomentosa* and *U. dioica* ethanolic extract applied by fumigation for 120 min against adult of *Culex pipiens* mosquitoes. Determination of lethal concentrations (LC 50 & LC90 µl/ml air) and their 95% confidence intervals.

Lethal Concentrations	**LCL >LC> UCL) (µl/ml) Fiducial limits (95%)**	
Plant	***P. tomentosa***	***U. dioica***
LC50	174.5>192.7>212.7	135.8>184 >249.2
LC90	235.6>278>327.9	208.6>315>475.7
R^2	**0.98**	**0.92**
Slope	**0.625** (2.908-8.291)	**4.085** (0.5263-8.695)

DISCUSSION

The present study indicated that the tested extract plants of *P. tomentosa* and *U. dioica*, exhibit a toxic effects with a concentrations-response relationship mortality; against the insect vectors, the adults of mosquito species *Cx. pipiens* and phlebotomine sand flies, *p. papatasi*. The toxicity of these extracts, expressed by the mortality, increased significantly following the increase in time of exposure and the concentrations. the similar effects were reported, when *Origanum vulg*are (Zahran et al. 2017) and *Myrtus communis* oils were tersted *against* adults of *Culex pipiens*,(Yezli et al 2024B). Several studies have documented the efficacy of plants, as the reservoir pool of bioactive toxic agents against insects (Deepa et al. 2015; Afzal et al. 2018; Al-Mekhlafi et al. 2018; Trivedi et al. 2018). Among the widely used products in control pests are chemical neurotoxic synthetic pesticicides (WHO 2017). However, the extensive use of these chemicals in urban and semi urban environments, has resulted in the development of insect resistance to these insecticides. Several studies have reported the development of mosquito resistance against temephos (Cheikh and Pasteur 1993), pyrethroids (Scott et al. 2015), deltamethrin, lambda-cyhalothrin, beta-cyfluthrin, and bifenthrin (Al-Sarar 2010) and diflubenzuron considered, one of the most effective mosquito larvicides used in Europe and some other countries (Grigoraki et al. 2017). The efficacy of natural products as insecticides has been developed (Amira et al. 2018; Djeghader et al. 2018. Moreover, various authors have reported that adulticidal activity against another mosquito species on essential oils from different plant species, such as *Eucalyptus maculata* (Myrtaceae), *Callistemon linearis* (Myrtaceae), *Cymbopogon citratus* (Poaceae), *Eucalyptus globulus* (Myrtaceae), and *Zanthoxylum limonella* (Sarma et al. 2019; Soonwera and Sittichok 2020). Even, if the compound is of a natural origin, the selectivity is a key issue. The toxicity bioassay results show a adulticidal activity, of plant extracts of *P. tomentosa* and *U. dioica* with a concentrations-response relationship mortality. The insecticidal efficacy of the plant extracts is given not only by their specific chemical profile and total content of major compounds, but also by the mutual ratios of the major compounds, which may lead to both synergistic and antagonistic effect (Pavela 2015). The fumigant toxicity of plants may be attributed to their major monoterpenes, that exist in their secondary metabolites. It was reported some major compounds of the plant oils, such as α-pinene, limonene, α-terpineol, β-pinene, 1,8-cineole, camphor β-citronellol, geraniol, linalool and α-citral had fumigant toxicity against the adults of *Cx. pipiens* (Ma et al. 2014). 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, bio membranes, 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.

The present work represents the first report showing the adulticidal activity of *P. tomentosa* and *U. dioica* against adults of mosquitoes and phlebotomine sand flies. The difference of sensitivity between the vector insects, may be is attributed mainly to the phenotypic resistance (modifications in the target site), metabolic resistance (ability to detoxify insecticides) or behavioral modification. Behavioral changes that minimize contact between insect and the insecticide may cause a severe impact in the insecticide application efficacy, especially if physiological features (Martins et al. 2012) select resistance.

In Algeria, pests and vector insects control programs are based mainly on chemical pesticide. The government interventions are carried out particularly when a reduction of the vector insect's density is needed, especially during the hot periods, to avoid the transmission of a vector-borne infection or to control insect and nuisance mosquitoes from inaccessible breeding areas. From the present study, it was concluded that *P. tomentosa* and *U. dioica* extracts are recommended to be used as an alternative bioinsecticide agents for pest control and better against vector insects, since are showing a significant toxic effect.

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