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EVIDENCE OF THE TOXICITY EFFECT OF *TETRACLINIS ARTICULATA* ON THE DISEASE VECTOR *CULEX PIPIENS* FROM MOHAMMEDIA

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ABSTRACT

Objective: To determine the larvicidal activities of *Tetraclinis articulata* (Family Cupressaceae) extracts against *Culex pipiens*. **Methods:** Twenty late III instar larvae were exposed to various concentrations (1-3.5 %) of aqueous extract, (1-6 %) of floral water, (50-200 ppm) of essential oil, and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC50 values of the *Tetraclinis articulata* wood extracts was determined following Probit analysis. The susceptibility status of *Culex pipiens* was determined against four insecticides: temephos, chlorpyriphos, DDT and deltamethrin under the laboratory conditions. **Results:** Results showed varying degree of larvicidal activity of the three extracts of *Tetraclinis articulata* against *Culex pipiens* mosquitoes with LC50 values ranging between 1,3(%) to 295(ppm). The extracts had strong toxicity activity against Cx. pipiens as it provided 100% mortality for 48h of aqueous extract (3.5%), followed by floral water (72 h) at 5% and essential oil (24h) at 200ppm providing 50% of mortality. The susceptibility status against chemical insecticides displayed high resistance to temephos and to chlorpyriphos and a resistance to DDT and deltamethrin. **Conclusions:** From the results, it can be concluded the extracts of *Tetraclinis articulata* was an excellent potential for controlling *Culex pipiens* mosquitoes.

KEYWORDS: The susceptibility status of *Culex pipiens* was determined against four insecticides: temephos, chlorpyriphos, DDT and deltamethrin under the laboratory conditions.

INTRODUCTION

Mosquito-borne diseases affect over than two billion people in the world. The genera of *Anopheles, Culex* and *Aedes* are vectors for several pathogens responsible for these diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever, chikungunya and others that threaten over than 1.1 billion of people worldwide (WHO, 2016). All these mosquito genera cause approximately 1 million deaths and over than 700 millions of infections in the world every year (WHO, 2016). In Morocco, the malaria, waterborne parasitic disease, still be a problem in public health (Alaoui Slimani, 1999). However, according to the ministry of health (directory of epidemiology and fight against diseases), the situation is no longer alarming in Morocco. The last active outbreak detected in 2002 was located in the province of Chefchaouen. In 2003, only residual cases were recorded in this same province. The parasitic species present is the benign form: *Plasmodium vivax*. However, the luck of effective vaccine or specific antiviral treatment leads to an intensive use of chemicals to fight the mosquito. The uncontrolled and repeated use of these chemicals in the fight against mosquitoes pushed these insects to develop a phenomenon of resistance. However, certain vegetable plants have shown their bio-effectiveness through their botanical extracts against mosquito larvae and adults. In everyday life, humans resort to using plants that have proven repellent activity to ward off insects, including mosquitoes. In fact, there are few molecules that have been extracted from plants for industrial purposes or for daily use as a biocide against mosquito larvae and adults and include rotenone, nicotine and pyrethrins. Although the pyrethrins from the flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum* are not very stable and very expensive in their extraction, they are the basis of the class of pyrethroids currently used in the fight against adults and in the impregnation of mosquito nets. The traditional use of herbal insecticidal preparations dates back a very long time (Philogene et al., 2005) and herbal products such as pyrethrum, neem and their products such as rotenone, nicotine and pyrethrins and essential oils have a long history of human use to protect stored foodstuffs or to keep pests away from human homes. Substances of plant origin have thus shown their bio-effectiveness through their properties against mosquito larvae and adults, and even if currently few biocidal molecules come directly from plants, extracts and products of plant origin have been used to development of semi-synthetic or synthetic products currently used in the fight against adults and in the impregnation of mosquito nets. In general, the use of plant extracts as a possible alternative that can replace chemical neurotoxic insecticides still in question. Medicinal and aromatic plants known by their secondary metabolites contained in their cells, contributed in healing many diseases that concern the human being. However, in mosquitoes, only the synthetic pyrethroids, are effective recognized chemical insecticides family derived from the flower of *Chrysanthemum cinerariaefolium* that contained the pyrethrin as bioactive molecule. Since its discovery, the pyrethrin was used mainly for its repellent properties, it impregnates the textiles for concentrations less than 4% (Singh et al, 2012).

In a large scale, now and since many studies were conducted on plant extracts against mosquito larvae mainly around the world. They have been evaluated as sources of natural insecticides against several genera of mosquito: *Cx. pipiens, Ae. aegypti, An. Stephensi* etc… showing the effectiveness of the extracts as potential biocide. Nevertheless, no molecule was described as functional and effective bioactive molecule against mosquito larvae. Here, we tested the larvicidal activity of the aqueous extract and floral water of the wood of *Tetraclinis articulata* (Thuya) on *Culex pipiens* mosquitoes from the region of Mohammedia.

MATERIALS AND METHODS

Biological material

The mosquitoes subject to the tests were collected at the larval stages in a breeding site of an open drain which corresponds to wastewater discharges in Ouled Hamimoun from Mohammedia prefecture. The captured mosquitoes were morphologically identified before carrying out the tests.

Figure 1: Breeding site of Ouled Hamimoun of *Culex pipiens* adults and larvae from Mohammedia region.

Plant Material

We tested the extracts of the wood of *Tetraclinis articulata* collected from Essaouira region. We dried and grounded the wood and preserved it in dark plastics in the laboratory until further use.

Insecticides

The sensitivity status of *Cx. pipiens* was determined respectively with 4 insecticides belonging to the most used family: temephos and chlorpyrifos (organophosphates), delthamethrine 0.05% (pyrethroides) and DDT 4% (organochlorines).

Susceptibility tests

The sensitivity tests were carried out according to the method recommended by the WHO (WHO, 2005; 2017). Larval tests were conducted on third and fourth instar larvae collected directly from the field. We prepared many concentrations in 95% ethanol from insecticide stock solutions until obtaining specific concentration range with at least two concentrations providing mortalities between 0 and 50% and two more between 50 and 100%. We placed sets of 20 larvae in plastic cups containing 99 ml of distilled water and 1 ml of insecticide solution at the required concentration. Five replicates of 20 larvae per concentration, and 4 to 5 concentrations of each insecticide were used for each. The LC_{50} and LC_{90} were calculated using log-probit software and compared to that of a sensitive strain to determine their resistance rate.

Adult sensitivity tests were carried out on unfed females obtained from nymphs collected at the same time as the larvae. 20 unfed females were placed in OMS tubes containing the insecticide treated papers with 5 replicates per test. Two controls were used for each test containing insecticide untreated papers and 20 non-fed females each. Tested mosquitoes were then provided with 10% sugar solution and maintained at 28 ± 2 °C and 80 ± 10% RH, and then overall mortality was observed after 24 hours. The percentage of knockdown mosquitoes was noted during the period of insecticide exposure. The evolution of this percentage as a function of time was analyzed using a log-probit model in order to determine the knockdown times, KdT_{50} and KdT_{90}.

Plant extracts

1- Preparation of *Tetraclinis articulata* aqueous extract

The aqueous extract of Thuya wood was prepared following the method of Aouinty et al. (2006). We chose to study the larvicidal effect of the aqueous extract of the plant because the larval stage of mosquitoes takes place in water, demonstrating the effectiveness of the plant extract, then, requires that it is soluble in water. For this, we chose water as the solvent. The aqueous extraction of Thuya wood is made from dry plant matter where the products are more concentrated. It is carried out according to the following steps.

- Wash the plant fragments with tap water then with distilled water.
- Drying of the plant part (wood) in an oven heated to 40°C for 48 hours to 92 hours.
- Grinding of dried plant material until reduced to powder.
- Preparation of an infusion from one liter of water brought to the boil and 100g of vegetable powder, by stirring the mixture for 30 minutes using a magnetic stirrer.
- Filtration of the mixture on Wattman paper (3mm). The filtrate obtained represents a concentration of 100g/l or 10%, considered as initial concentration.

2- Preparation of *Tetraclinis articulata* floral water and essential oil

Floral water was prepared from the extraction of essential oil from Thuya wood by hydrodistillation using a Clevenger type apparatus (Meyer-Warnod, 1984). 100 g of wood powder and distilled water were added using a funnel into the hydrodistiller flask. Then, the oil bath is brought into contact with the flask and all are brought to a boil for 4 hours. We check that the water is circulating well in the refrigerant. The heat leads to the vaporization of the water which, in its vapor state, takes with it all the volatile substances which will condense after cooling, giving a two-phase mixture consisting of floral water and essential oil in the essence part of the hydrodistiller. Finally, the floral water is separately recovered by decantation in an Erlenmeyer flask and the essential oil in a test tube with an amber screw cap at a temperature of 4°C.

Biocide Tests

We tested the toxicity of three botanical extracts from Thuya wood following WHO recommendations with slight modifications (WHO, 2005). From stock solution at 10% of each botanical extract, six concentrations were prepared (1%, 1.5%, 2%, 2.5%, 3%, 3.5%) and (1%, 2%, 3%, 4%, 5 %, 6%) for the aqueous extract and floral water respectively. For essential oil, three concentrations were prepared: (50ppm, 150ppm, 200 ppm). 20 larvae of the 3rd instar larvae were placed in plastic cups filled with distilled water with the designated concentration. For each concentration, five repetitions were carried out and two controls were prepared for each test. Each control included 100 mL of distilled water and received the same larvae number. The mortality was counted after 24 hours, 48 hours and 72 hours. The LC_{50} and LC_{90} values were obtained using the probit method of Finny (1971).

Figure 2: Production of plant extracts from the wood of *Tetraclinis articulata*; 1- extraction of essential oil by hydrodistillation using the clevenger; 2- Essential oil; 3- Floral water; Testing the toxicity effect of the wood of *T. articulata* on *Cx. pipiens* larvae from Mohammedia region.

RESULTS

Table 1: Susceptibility test of *Cx. pipiens* to temephos.

Concentration mg/l	Total exposed	Number of dead	% Mortality
0.00125	20 20 19 20	1 1 0 2	
Total	79	4	5
0.0025	19 19 19 20 20	1 1 3 2 1	
Total	97	8	8
0.005	20 19 18 19 19	9 7 6 5 7	
Total	95	34	36
0.0125	19 19 19 20 19	16 14 12 16 13	
Total	96	71	74
0.025	20 18 20 20 20	19 18 19 20 20	
Total	98	96	98
Control	22 19 20	1 0 1	
Total	61	2	3.2
LC50 (mg/l)	0.0073		
LC90 (mg/l)	0.0179		
RR50	25.5		
Statut	R		

Temperature of water : 23-24°C

Table 2: Suscpetibility test of *Cx. pipiens* to chlorpyriphos.

Concentration mg/l	Total exposed	Number of dead	% Mortality
0.001	17 17 19 18 15	0 0 2 0 2	
Total	86	4	4.6
0.005	18 17 16 20 18	5 3 2 1 1	
Total	89	12	13.5
0.01	19	4	

	19	7	
	16	3	
	19	6	
	18	6	
Total	91	26	28.6
0.025	23	11	
	18	15	
	18	15	
	18	12	
	20	13	
Total	97	66	68
0.05	17	14	
	20	19	
	21	20	
	20	19	
	23	17	
Total	101	89	88.1
Control	22	0	
	22	0	
	21	0	
Total	65	0	0
CL50 (mg/l)	0.019		
CL90 (mg/l)	0.051		
RR90	127.5		
Statut	R		

Temperature of water : 23-24°C.

Table 3: Test of susceptibility of *Cx. pipiens* to DDT (4%).

Exposition time	Total exposed	Number of dead	% Mortality
4 heures	22	5	
	18	2	
	20	5	
	19	3	
Total	79	15	19
Control	20	0	
	20	0	
Total	40	0	0
Statut	R		

Temperature : 25°C. Relative Humidity : 80-90%.

Table 4: Knock down % of *Cx. pipiens* to DDT (4%).

Time mn	Total exposed	Number of Kdr	Kdr %	KdT50 mn	KdT90 mn
30	79	0	0	566.74	2342.95
60	79	0	0		
90	79	7	9		
120	79	7	9		
150	79	10	12.6		
180	79	10	12.6		
210	79	15	19		
240	79	16	20		
Control	40	0	0		

Table 5: Test of Susceptibility of *Cx. pipiens* to delthamethrine 0.05%.

Exposition time	Total exposed	Number of dead	Mortality %
1 hour	20	12	
	20	13	
	20	10	
	18	12	
Total	78	47	60
Control	20	0	
	20	0	
Total	40	0	0
Statut	R		

Temperature : 25°C Relative Humidity : 80-90%

Table 6: Knock down % of *Cx. pipiens* to delthamethrine.

Time mn	Total exposed	Number of Kdr	Kdr %	KdT 50 mn	KdT 90 mn
10	78	0	0	20.20	34.32
20	78	35	45		
30	78	60	77		
40	78	62	79		
50	78	63	81		
60	78	65	83		
Control	40	0	0		

Table 7: Toxicity effect of the wood aqueous extract of *Tetraclinis articulata* against *Cx. pipiens* larvae.

Tetraclinis articulata	Doses %	% of mortality control	Total exposed during 24h/48h/72h	Dead after 24h/48h/72h	% of mortality after 24h/48h/72h	Living after 24h/48h/72h	% of living after 24h/48h/72h	DL_{50} after 24h/48h/72h	DL_{90} after 24h/48h/72h
Aqueous extract	1	0	80/80/80	3/10/17	3.75/12.5/21.2	77/70/63	96.2/87.5/78.7	2.450/1.816/1.328	3.065/2.463/2.11
	1.5		80/79/79	7/20/54	8.75/25.3/68.3	73/59/25	91.2/74.7/31.6		
	2		78/76/76	15/49/61	19.2/64.5/80.3	63/27/15	80.8/35.5/19.7		
	2.5		79/78/78	37/72/75	46.8/92.3/96	42/6/3	53.2/7.69/3.85		
	3		80/80/80	75/79/80	93.7/98.7/100	5/1/0	6.25/1.25/0		
	3.5		80/80/80	78/80/80	97.5/100/100	2/0/0	2.5/0/0		

Table 8: Toxicity effect of the wood floral water of *Tetraclinis articulata* against *Cx. pipiens* larvae.

Tetraclinis articulata	Doses %	% of mortality control	Total exposed during 24h/48h/72h	Dead after 24h/48h/72h	% of mortality after 24h/48h/72h	Living after 24h/48h/72h	% of living after 24h/48h/72h	DL_{50} after 24h/48h/72h	DL_{90} after 24h/48h/72h
Floral water	1	0	80/79/77	10/11/22	12.5/13.9/28.6	70/68/55	87.5/86/71.4	3.727/2.184/1.552	6.30/4.376/3.177
	2		80/75/73	12/19/39	15/25.3/53.4	68/56/34	85/74.7/46.6		
	3		80/80/80	24/60/71	30/75/88.7	56/20/9	70/25/11.2		
	4		80/80/80	51/73/77	63.7/91.2/96.2	29/7/3	36.2/8.75/3.75		
	5		78/77/77	57/72/77	73/93.5/100	21/5/0	26.9/6.49/0		
	6		80/80/80	73/78/80	91.2/97.5/100	7/2/0	8.75/2.5/0		

Table 9: Susceptibility of *Culex pipiens* larvae to essential oil of the wood of Thuya.

Thuya extract	Doses ppm	% of Mortality control	Total exposed during 24h	% of Mortality after 24h	% Mortality corrected by formula of Abbott after 24h	DL_{50} after 24h	DL_{95} after 24h
Essential oil	50	8	70	40	35	295	140 187
	100		71	38	33		
	200		66	53	49		

1- Susceptibility of *Cx. pipiens* larvae

The results of sensitivity test of *Cx. pipiens* to temephos and chlorpyrifos are recorded in tables 1 and 2. The LC_{90} is 0.018 mg/l for temephos and 0.051 mg/l for chlorpyrifos. The LC_{90}s obtained on this strain are significantly higher than the LC_{90}s of sensitive strains. These results confirm the presence of resistance in *Cx. pipiens* to chlorpyrifos and temephos.

2- Susceptibility of *Cx. pipiens* adults

The sensitivity tests of *Cx. pipiens* against 4% DDT showed very strong resistance. 81% of the exposed population survived, after an exposure of 4 hours, at the discriminatory dose (table 3). DDT no longer seems to have a shock effect on this population of *Cx. pipiens* since after 4 hours the Kd did not exceed 20% (table 4).

The exposure of the same population to 0.05% delthamethrin also showed significant resistance. Only 61% mortality was obtained after one hour of exposure (table 5). The KdT_{50} and KdT_{90} noted during exposure were respectively 20.20 and 34.32 minutes (table 6).

3- Toxicity of aqueous extract, floral water and essential oil of *T. articulata* to *Cx. pipiens* larvae

After have counted the numbers of dead larvae after 24, 48 and 72 hours of exposure time, larval mortality percentages of each concentration and those of control are being used to calculate the mortality averages. The larval susceptibility to aqueous extracts, floral water and essential oil are reported in table 7, 8 and 9 respectively. As it can be seen, compared to the control group, all extracts showed considerable larvicidal activity when tested against 3rd instar larvae of *Cx. pipiens*. The mean larval mortality values calculated were tested by Probit winDL version 2.0 to fit a linear regression between the log of the insecticide concentration and the probit of mortality and to estimate the lethal concentrations (LC_{50} and LC_{90}) with their 95% confidence intervals (CI).

The highest mortality percentage (100%) occurred at a 3% concentration of AE after 72h and at 3.5% concentration after 48h and 72h whereas the floral water caused the same mortality percentage of 100% at 5% concentration after 72h and 6% after 72h. The lowest mortality percentage was at the lowest concentrations (1% and 1.5% of AE after 24h; 1% after 24h and 48h and 2% after 24h of FW). Concerning the essential oil, dose of 50 ppm gives 35% of mortality, 100 ppm gives 33% and 200 ppm gives 49%. Theoretically, 408 ppm will give 100% of mortality.

DISCUSSION

In this study we investigated the resistance status of *Cx. pipiens* mosquitoes from Mohammedia against four insecticides that have been historically used for chemical control of the mosquito vector in Mohammedia: temephos, chlorpyriphos, deltamethrin and DDT; and three botanical extracts: aqueous extract, floral water and essential oil from the wood of *Tetraclinis articulata*. Temephos and chlorpyriphos were used in Mohammedia for larval vector control from the 1990s until now (National Institute of Hygiene archives), while DDT has been used from the 1960s until his interdiction, and deltamethrin from the early 2020s until the present day. Bioassays performed on the *Cx. pipiens* population from Mohammedia revealed high resistance to temephos (RR_{90}= 25.5) and to chlorpyriphos (RR_{90}= 27.5) while *Cx. pipiens* adult populations showed a resistance to DDT (% mortality= 19) and to deltamethrin (% mortality= 60). These results agree with those reported by Aboulfadl et al. (2020; 2022) who demonstrated a high resistance of *Cx. pipiens* populations from Mohammedia to temephos (RR_{90}= 40.1) and to chlorpyriphos (RR_{90}= 48.8), a resistance to DDT (% mortality= 74) and a high resistance to deltamethrin (% mortality= 97). *Cx. pipiens* populations from Mohammedia have been already found to be resistant to temephos and chlorpyriphos since the early 2000s (Faraj et al. 2002). Resistance against DDT and deltamethrin was also shown in the population of Mohammedia in the study of Aboulfadl et al. (2022). The occurrence of resistance is related proportionally with the insecticide quantities and the treatment frequencies (Aboulfadl et al. 2020). In mohammedia, the average quantity of temephos reported from 2015-2020 was 120L on 100ha, being a rate of 1.2 of temephos quantities used (Aboulfadl et al. 2020). Finally, the genetic modification gained by the selection pressure be the key of the distribution of resistance within populations.

In the other side, we carried out sensitivity tests to evaluate the toxic effect of botanical extracts of *Tetraclinis articulata* wood on *Culex pipiens* larvae from the Mohammedia region. For this, the biocidal activity of three botanical extracts was evaluated: the aqueous extract, the floral water and the essential oil. The results obtained show that the dose of 3% of the aqueous extract gave 93.7% mortality after 24 hours, unlike floral water where the same dose also gave only 30% mortality after 24 hours. Therefore, the aqueous extract (AEW) is much more effective and has a remarkably high toxicity with LD_{50} = 2.4 (after 24 hours) unlike floral water (FWW) which has a LD_{50} = 3.7 (after 24 hours). The toxicity of *Tetraclinis articulata* on *Culex pipiens* was also reported in the study of Aouinty et al. (2006) with a percentage of aqueous extract of 4% giving 100% of mortality and LC_{50}= 530 mg/l. The same author showed that among 20 other plants, the most effective was *Ricinus communis* with 1% of AE that gives 100% mortality and LC_{50}= 600mg/l followed by *Tetraclinis articulata*.

In this study, we chose to study the effect of Thuya (*Tetraclinis articulata*) on the resistant mosquito *Culex pipiens* from the Mohammedia region. Thuya is a species endemic to Morocco also called *Tetraclinis articulata*. It is located, like majority of Moroccan Tetraclinis, in the temperate and hot semi-arid bioclimatic stage. Thuya also grows in sub-humid areas, preferentially on calcareous or siliceous soils and avoids clayey soils. In order to evaluate the bioeffectiveness of these plant extracts, we carried out the study on the larvicidal effect of extracts prepared from *T. articulata* wood on resistant *Cx. pipiens* larvae to chemical insecticides. We carried out sensitivity tests on the same larval population which presented resistance to the four families of chemical insecticides collected from Ouled Hamimoun deposit in Mohammedia with respectively the aqueous extract, floral water and essential oil of *T. articulata* wood. After reading the mortalities calculated after 24 hours, we obtained the results that show that the dose of 2.5% of the aqueous extract gives 47% mortality, and that of 3% gives 93.7% mortality. The percentages of deaths caused by these doses increase after 48 hours (92% and 99% respectively), and after 72 hours (96% and 100% respectively) (Table 7). The LD_{50} and LD_{90} are respectively 2.45 and 3 after 24 hours of exposure to the aqueous extract. These results show that AEW has high bioefficacy against *Culex pipiens* mosquitoes.

In addition, the larvicidal activity of floral water on the larval population showed for the same dose of 3% after 24 hours a mortality of only 30%, while for a mortality of 91% a dose of 6% was required (Table 8). The percentages of mortality caused by the different doses increase as they are exposed to floral water. In addition, the LD50 of FWW ranging from 3.7, 2.2 to 1.5 after 1, 2 and 3 days respectively show that the effect accentuates with time. On the other hand, the bioeffectiveness of the aqueous extract (AE) is much higher with DL50 = 2.4 (after 24 hours) unlike floral water (FW) which showed a DL50 = 3.7 (after 24 hours) and therefore less bioeffective.

The study of the essential oil (EO) of the wood of *T. articulata*, presents a much higher effect than that of the aqueous extract and floral water. An oil yield of 1.3% was obtained. With doses ranging from 50 to 200 ppm only, EO showed greater bioeffectiveness than the aqueous extract and floral water with LD50 and LD90 of 295 and 140,187 respectively. Thuya essential oil mainly consists of α-pinene (30.22%), delimonene (22.29%), widdrol (5.41%) and bornyl acetate (4.76%) (Bourkhiss et al. 2010). α-pinene and limonene being the majority molecules may be responsible for the toxic activity in *Culex pipiens* larvae. They have an identical chemical formula C10H16. However, limonene (LD50= 4400-5200 mg/kg in white rats) presents a higher toxicity than that of α-pinene (LD50= 3700 mg/kg in rats) in mammals and in humans. Limonene is a renal carcinogen specific to male rats, a foetotoxicant in rats and rabbits and induces bone malformations in mice. With regard to mosquito larvae, α-pinene and limonene can act in the digestive tract by tearing the membrane of intestinal cells or even blocking the transmission of nerve impulses. α-pinene and limonene are part of the compounds in the essential oils of several plants including lemon tree, cistus, danifera, cypress, globose eucalyptus officinal, etc. Generally speaking, essential oils have a repellent, curative and preventive effect to these monoterpenes which have both odorous and toxic characteristics.

In Morocco, several studies have shown the effectiveness of plants against mosquito larvae (Ez zoubi et al., 2016; Dahchar et al., (2016); El Ouali Lalami et al.,(2016); El-Akhal et al., (2016); Govindarajulu et al., (2015); El Joubari et al., (2015); El-Akhal et al., (2015); Sayah et al., (2014); El-Akhal et al., (2014); Shivakumar et al., (2013); Azokou et al., (2013); Sakthivadivel et al., (2012); Jouda Mediouni et al., (2012); Bansal et al., (2012); Sedaghat et al., (2011); Khalafalla Taha et al.,(2011); Belaqziz et al., (2010); Magadula et al., (2009); Aouinty et al., (2006); Redwane et al., (2002); Markouk et al., (2000)).

Generally, botanical extracts have been a topic of research regarding mosquitoes for a long time. They contain several toxic molecules called toxins whose distribution differs from one species to another. There are species that are entirely toxic and others whose toxicity is found in only a few parts of the plant. These toxins are generally secondary metabolites that protect the plant against environmental factors. In some, they are always present and in others only reproduce following environmental stress. The main plant molecules responsible for toxicity are: 1- alkaloids (aromatic nitrogen molecules), 2- glycosides (oses linked by a glycosidic bond to an aglycone molecule), 3- terpenes (class of hydrocarbons, molecules essentially composed of carbon (C) and hydrogen (H) atoms). Pyrethroids are synthetic chemical insecticides originating from the Chrysanthemum plant. The plant contains active substances which are toxic for insects but not target for other organisms with the exception of fish, called pyrethrins. It contains Pyrethrins I and II, Cinerins I and II and Jasmolines I and II. These esters result from the combination of chrysanthemic acid and pyrethric acid in one hand, and in the other hand three alcohols including pyrethrolone, cinerolone and jasmolone. The physicochemical properties of pyrethrins make it possible to attack the nervous system of insects, subsequently leading to an inhibition of CSVD repolarization in sensitive mosquitoes. However, they exhibit characteristics of instability and biodegradability upon exposure to light, heat or air.

Finally, the study of the aqueous extract, floral water and essential oil of *Tetraclinis articulata* wood on the resistant larvae of *Cx. pipiens* studied in the Mohammedia region showed a potential toxicity of these three plant extracts with LD50 of 2.4%, 3.7% and 295ppm respectively. These data provide information on the presence of significant bioefficacy on *Cx. pipiens* larvae and therefore the possibility of having a good larvicide based on the wood of this plant.

CONCLUSION

Cx. pipiens mosquitoes from Mohammedia are multiple resistant to insecticides (Aboulfadl et al. 2020; 2022).

Here we show the studied populations displayed high resistance to temephos and chlorpyriphos and were resistant to deltamethrin and DDT.

The population of Mohammedia is the result of the effects of insecticide selective pressure in *Cx. pipiens* mosquitoes, as this species has historically been prioritized for the insecticide treatments, which had led to the appearance and the selection of multiple resistances and the associated mechanism.

Cx. pipiens mosquitoes are resistant to the only chemical insecticide that is still used in vector control strategies locally, temephos and deltamethrin. Thus, the challenge is to manage this resistance in order to prevent the emergence of *Cx. pipiens* borne diseases in Morocco.

Tetraclinis articulata showed high bioefficiency throw its botanical extracts against *Cx. pipiens* larvae, by the essential oil, aqueous extract and floral water respectively. Our results confirm a possible alternative to fight against mosquitoes via the use of plants.

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