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SYSTEMATIC AND TOXICOLOGICAL STUDY WITH SYNERGY OF THE MOSQUITOES IN EL KANTARA REGION (BISKRA, SOUTHERN ALGERIA)

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Abstract

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Recent years have seen the re-emergence of vector-borne diseases, representing more than 17% of infectious diseases globally. Most of these diseases can be prevented by vector control measures. Series of difficulties which are linked to the fight. Plant insecticides can present interesting alternatives to the chemical insecticides currently used in the fight against these pests. In the first part, we have inventoried the vector species in the region of El Kantara, Biskra, by conducting surveys of three sites. The second part focuses on the biological control of the larvae of the most abundant species in the region, that is, *Culiseta longiareolata*. The faunal inventory of the species of Culicidae in the three sites of El Kantara showed seven species belonging to three genera. The genus *Culiseta* was the best represented, particularly with the species *C. longiareolata* (89.91%). The toxicological activity of the ethanoic extracts of the seeds of *Peganum harmala* and *Citrullus colocynthis* was studied in synergy against the larvae of *Culiseta longiareolata* at different concentrations. The mortality rate increased over time to reach more than 70% after 5 days.

Key words: biodiversity, culicidae, *Culiseta longiareolata*, ethanolic extracts, *Peganum harmala*, *Citrullus colocynthis*.

Introduction

For 170 million years, Diptera (flies and mosquitoes) have formed the most ecologically diverse group of insects, including the family Culicidae. This is the most important family as the mosquitoes belong to this family and form a diversified group in which a large part of the insects are hematophagous (Boudemagh et al., 2013) or vector species capable of transmitting viruses, bacteria, and parasites to both humans and animals, threatening public health (Nabti, 2020).

The fight against mosquitoes has always been a major concern in an effort to protect against the aggression of these hematophagous insects (Guillet et al., 1997). The most effective approach to control is based on sufficient reduction of mosquito larval populations. This control must be adapted to the knowledge of their breeding sites, their behaviors, and their ecologies to ensure the effectiveness of this action (Djogbénou, 2009).

The use of chemical insecticides has been a fundamental tool for insect control, but it has had serious consequences such as the poisoning of people and animals (Regnault-Roger et al., 2004). In addition, insecticide resistance has been reported in all major mosquito vector species (Ranson et al., 2011).

Currently, the fight against insects is entering a new phase, where the botanical world provides means to fight in better harmony with the environment. Many plants synthesize secondary metabolites, which have important biological properties against insect pests (Regnault-Roger et al., 2004).

Peganum harmala is a plant endemic to semi-arid zones, develops in the Saharan zones of the north of the African continent, and extends as far as North India and North China (Abbassi et al., 2003). It occupies the stations of arid hillsides, dry uncultivated fields, rubble, and earthy steppes (Jahandiez, Maire, 1932). In traditional medicine, its seeds have long been used as narcotics, anthelmintics, antispasmodics, and, in some cases, against rheumatism and asthma (Bellakhdar, 1997).

Citrullus colocynthis is a plant native to the sandy desert regions of Africa. It is used in Morocco as a diuretic, anti gonor-

Table 1. Main characteristics of the lodgings chosen in the region of El Kantara**.**

Sites	Cottages	Environment	Altitude	Longitude	Latitude
Site 01 (Oued Boubiadha)	Cottage 1	Natural	775m	5°42‘ 23.55“E	35°13‘ 05.61“N
	Cottage 2	Oasis	762m	5°42‘ 37.55“E	35°13‘ 10.40“N
Site 02 (Oued Elhai)	Cottage 3	Natural	775m	5°42‘ 13.02“E	35°13‘ 00.65“N
	Cottage 4	Natural	775m	5°42‘ 11.93“E	35°12‘ 03.65“N
Site 03 (Basin)	Cottage 5	Urban	762m	5°42‘ 14.53“E	35°12‘ 19.41“N

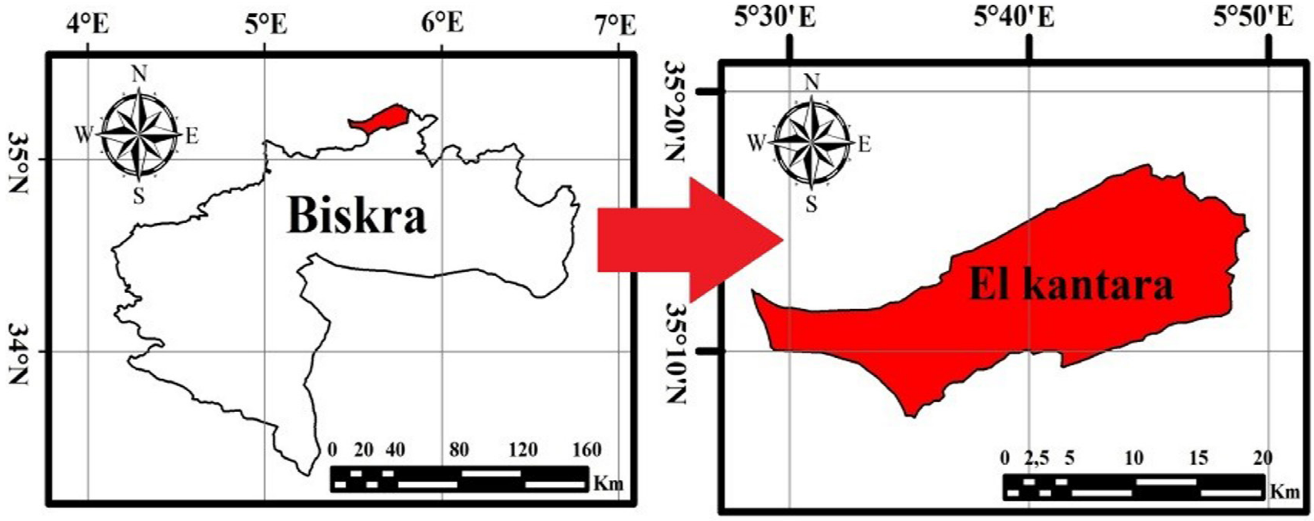

Fig. 1. Geographical location of the El Kantara region (Biskra).

rhagic, and antiepileptic and in India as an antimite for the protection of wool and against the bites of animals and insects with venom. The dried pulp of the fruit harvested before its complete maturity is a violent laxative and is used as an antirheumatic and anthelminthic. The seeds are edible and contain 30–40% of a light yellow oil, which contains an alkaloid, a glucoside, and a saponin. These roasted seeds are rich in lipids and proteins (Fejjal et al., 2011).

Through this study, we have inventoried culicidian species in a broader way by prospecting in some localities in the region of El Kantara (Biskra) to know the faunal composition of the region and to collect information on the habitats of disease-carrying insects. On the other hand, we tested the action of the extracts of two plants, *Peganum harmala* and *Citrullus colocynthis*, in synergy as a larvicide against the larvae (L4) of a locally inventoried mosquito species that is marked as the most frequent in the region of El Kantara – it is *Culiseta longiareolata.*

Material and methods

Study area

The study was carried out in the region of El Kantara (latitude: 35°11´32.51″ N, longitude: 5°40´00.59″ E), which is a commune of the wilaya of Biskra in Algeria in the shape of an oasis located in the southwest of Aures, at 52 km in the north of Biskra and to 62 km to the southwest of Batna. It is located 465 m above sea level (Fig. 1).

Description of study sites

For achieving the aim of the present study, we chose three representative capture sites of the culicidian fauna from an ecological point of view, which are Oued Boubiadha, Oued Elhai, and a basin. The characteristics of each site are detailed in Table 1.

- Oued Boubiadha (S1): It is a pond that is fed by floods of Oued Boubiadha near the palm groves, the water of this soft cottage and the plants surrounding this site are weeds.
- Oued Elhai (S2): The cottages from the overflow of Oued Elhai, the water in these cottages is fresh. Only weeds characterize the vegetation cover.
- Basin (S3): It is a temporary urban lodging in El Kantara. It is an open basin near a house, which is used to store irrigation water. This basin has an area of 3 m^2 and a depth of 1.5 m.

Sampling of larvae

The larvae were collected using a 500-ml capacity ladle (Bendali et al., 2001). Then, they were sorted by larval stage and their breeding was maintained in the laboratory for a taxonomic study.

Breeding

The larvae collected from the study sites were maintained in the laboratory in mass rearing containers containing 250 ml of dechlorinated water and food for the insects. The latter is a mixture of biscuit (75%) and brewer's yeast (25%) (Rehimi, Soltani, 1999). The water was renewed every 2 days, and the containers of breeding were placed in cages (22 × 22 × 22 cm). Breeding was carried out at a temperature of 25 °C and a hygrometry of 70%.

Identification of harvested species

The identification of larvae which reached the fourth stage of development (L4) was done according to the dichotomous keys of Rioux (1958) and using the mosquito identification software (mosquito of Europe) developed by Schaffner et al. (2001), based on a set of very precise morphological criteria and microscopic descriptors.

Animal material

Culiseta longiareolata: It is a pest with complete metamorphosis and is more abundant in warm regions. It belongs to order Diptera and family Culicidae (Villeneuve, Desire, 1965). It preferentially bites vertebrates, especially birds, and very rarely humans; the species is considered a vector of bird *Plasmodium* (Bruhnes et al., 1999).

Plant materials

Peganum harmala: It grows in semi-arid rangelands. All parts of the plant are considered poisonous, and serious poisoning occurs in domestic animals (Mahmoudian et al., 2002).

Citrullus colocynthis: This plant is highly toxic to animals and humans. Signs of poisoning are gastrointestinal pain with diarrhea, vomiting, urinary retention, fatigue, hypothermia, cardiac disorder, and cerebral congestion producing fatal collapse (Charnot, 1945).

These two plants used in the present study were harvested from the region of El Kantara (Biskra).

Preparation of the ethanol extract of synergy

The ethanoic extract of *Peganum harmala* was prepared from 200 g of seeds of the plant ground using a coffee grinder and macerated in 400 ml of ethanol for 24 h at room temperature according to the method of Aouati and Berchi (2015). The mixture was filtered using filter paper, and the filtrate collected was subjected to vacuum evaporation in a rotavapor and then in an oven.

The fruits of the *Citrullus colocynthis* plant were sorted, cut into half, and dried in the open air for a week. The seeds were separated from the pulp, cleaned, and weighed. Then, we ground them into a fine powder using a coffee grinder. Two hundred grams of the seeds was macerated in 100 ml of ethanol for 24 h. Then we filtered the mixture using filter paper. The filtrate collected was subjected to vacuum evaporation in a rotavapor and then in an oven.

The synergy product that we used in our tests consisted of a mixture of 0.5 g of *Peganum harmala* extract and 0.5 g of *Citrullus colocynthis* extract in 1 ml of water.

Preparation of larvae for a control trial

The technique of susceptibility testing standardized by the World Health Organization (WHO, 1963) was used for the treatment of *Culiseta longiareolata* larvae. In a container of 400 ml capacity, we put 15 larvae (L4), 200 ml of spring water, and three concentrations (48, 111, and 149 mg/l) of the aqueous synergy extract prepared previously (aqueous extract of *Peganum harmala* with *Citrullus colocynthis*). Each concentration was applied for 3 repetitions, with a preparation of 20 control larvae.

After 24 h of treatment, the water in the containers was changed and the mortality or malformation that had appeared was compared to the control. This experiment was monitored daily by counting the dead larvae and monitoring the development times of the treated stage and its control.

Exploitation of results

In order to characterize this culicidian population, ecological parameters were calculated to know their relative abundance (F%) (Dajoz, 1971), specific diversity H¢ (Shannon, Weaver, 1963), and equitability (E) (Barboult, 1981).

With regard to the results obtained from the toxicological study, we calculated the lethal times (LT 50% and LT 90%) and the lethal concentrations (LC 50 and LC 90%) for the bioinsecticide used, according to the mathematical procedures of Finney (1971).

Results

Inventory of Culicidae species in the El Kantara region

We identified during the study period 6,898 individuals representing seven species of Culicidae divided into two subfamilies, namely, the Culicinae and the Anophelinae.

The Anophelinae subfamily is represented by two species; on the other hand, the Culicinae subfamily is distinguished by a greater number of species grouped into two genera: the *Culex* genus has four species and the *Culiseta* genus has only one species (Table 2).

We found that the species of *Culiseta longiareolata* was the best represented and the most frequent, with a total of 6,199 individuals and an abundance of 89.91%. It was followed by *Culex pipiens* with 408 individuals and an abundance of 5.92%. These two species were followed by *C. perexiguus* with 164 individuals and by an abundance of 2.38% and *C. laticinctus* with 84 individuals or 1.22% of the population. These species can be considered as the most dominant species in the El Kantara region (Fig. 2).

The abundance analysis showed that the species *Anopheles sergenti* (0.38%), *Culex theileri* (0.19%), and *Anopheles multicolor* (0.06%) were relatively rare in this region (Fig. 2).

The results (Fig. 3) showed that the culicidian population harvested in the region of El Kantara was weakly diversified with a diversity index equal to 0.46. The equitability (E) displayed a value of 0.30, indicating that the El Kantara region is less balanced and is populated by a small number of culicidian species with one dominant species (Fig. 3).

The concentration index for the El Kantara region was indeed 0.81, which means that there is an 81% probability of en-

Table 2. List of Culicidae identified in the region of El Kantara (Biskra).

Subfamilies	Genres	Species identified
Culicinae	*Culex*	*Culex pipiens*
		Culex perexiguus
		Culex theileri
		Culex laticinctus
	Culiseta	*Culiseta longiareolata*
Anophelinae	*Anopheles*	*Anopheles multicolor*
		Anopheles sergenti

Table 3. The toxicological parameters (lethal concentration) of synergy of *Peganum harmala* and *Citrullus colocynthis* with regard to the larvae of *Culiseta longiareolata*.

Exposure time	Regression	LC50 (mg/l)	LC90 (mg/l)
1 day	Y= -0.09 + 2.28X (R^2=0.98)	169.82	616.59
5 days	Y= -1.94 + 4.04 X (R^2=0.99)	52.48	107.15
5 days	Y= -6.06 + 6.63X (R^2=0.93)	46.77	72.44

Table 4. The toxicological parameters (lethal times) of the extract *of Peganum harmala* and *Citrullus colocynthis* in synergy on the larvae of *Culiseta longiareolata.*

Applied concentrations	Regression	LT 50 (Days)	LT 90 (Days)
48mg/l	Y=3.77+1.47X (R^2 =0.99)	6.45	51.29
111mg/l	Y=4.50+2.53X (R^2=0.99)	1.58	5.01
149mg/l	Y=4.76+3.59X (R^2=0.94)	1.15	2.63

countering the same species in this site. This resulted in a very low diversity (D) of the order of 0.19 (Fig. 3).

Effect of the synergy of *Peganum harmala* ***and*** *Citrullus colocynthis* ***on the larvae (L4) of*** *Culiseta longiareolata*

The fourth instar larvae of *C. longiareolata* were susceptible to *Peganum harmala* and *Citrullus colocynthis* used in synergy. This sensitivity was reflected in more or less high mortality rates depending on the concentrations used and especially depending on the time of exposure to the product (Fig. 4).

At the significance level α = 0.05, the statistical analysis showed that there were significant differences between the different mortality rates recorded during the treatment follow-up period at the high dose (149 mg/l) and low dose (48 mg/l) (F: 8.62, *P*: 0.017/*F*= 3.96, *P*: 0.08). The use of a low dose (48 mg/l) of the product studied caused mortality ranging from 18.18% after 1 day of treatment to 53.45% after 10 days. On the other hand, a high concentration (149 mg/l) could kill up to 84.26% of *Culiseta longiareolata* larvae after 10 days of follow-up (Fig. 4). We also noticed that 111 mg/l of the extract studied in synergy caused the death of 73.92% of the population after 5 days of control (Fig. 4).

Concentrations of *Peganum harmala* and *Citrullus colocynthis* in synergy depending on the time of exposure to the product. The fourth instar larvae of *Culiseta longiareolata* exposed for 1.5 and 10 days to *Peganum harmala* and *Citrullus colocynthis* used in synergy showed mortality rates correlating with the concentrations used (Table 3).

The regression line after exposure of the larvae to the treatment for 1 day is of the form: Y = −0.09 + 2.28X (R^2 = 0.98). To ensure a 50% mortality of the larvae after 24 h, the concentration of synergy must be equal to 169.82 mg/l. On the other hand, 616.59 mg/l of this insecticide ensured the mortality of 90% of mosquitoes within 24 h (Table 3).

Fifty percent of mosquitoes could be eliminated after 5 days when a concentration of 52.48 mg/l of synergy was applied, while 90% of the larvae of this species required the use of 107.15 mg/l of synergy (Table 3).

After 10 days of treatment, the LC 50% and the LC 90% were lower, since they did not exceed 46.77 and the 72.44 mg/l, respectively (Table 3).

The results (Table 4) showed that there was a strong positive correlation between the mortality of the larvae and the exposure time (R^2 varied from 0.94 to 0.99).With the concentration of 48 mg/l, the time required to kill 50% of the larvae

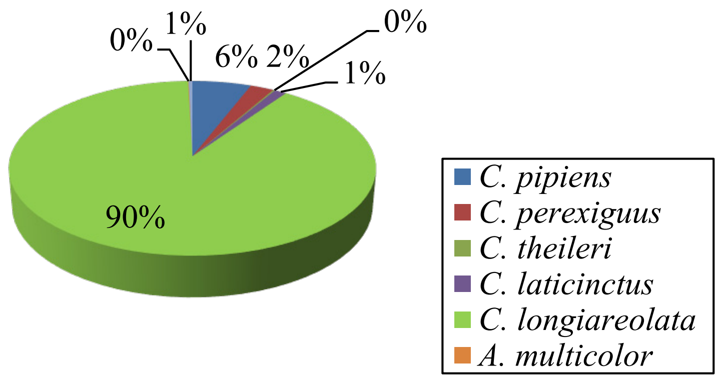

Fig. 2. Relative abundance of the Culicidae species inventoried in the El Kantara region.

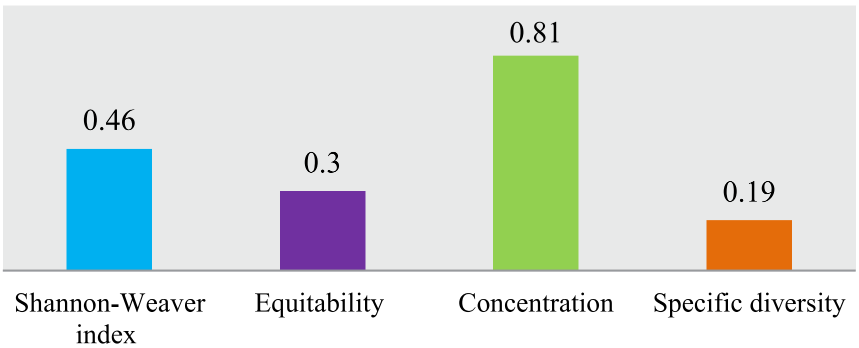

Fig. 3. Ecological indices of structure in the region of El Kantara.

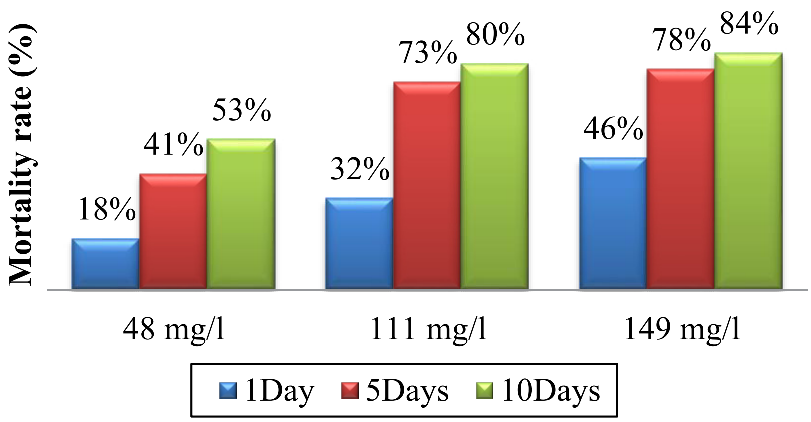

Fig. 4. Corrected mortality rate of *Culiseta longiareolata* larvae treated with three concentrations of *Peganum harmala* and *Citrullus colocynthis* in synergy depending on the time of exposure to the product.

was 6.45 days. This value can go up to 51.29 days to eradicate 90%. On the other hand, the use of 149 mg/l of product ensured the mortality of 50% of the larvae after only 1.15 days of treatment and 90% of the larvae after 2.63 days of treatment (Table 4).

Discussion

The systematic study of mosquitoes, focused mainly on the larval stage, taken from different sites after 5 months of monitoring made it possible to identify seven species of Culicidae, belonging to two subfamilies. Among others, the Anophelinae and the Culicinae, including two species of *Anopheles* have been identified, which are *Anopheles multicolor* and *A. sergenti*, two genera of the subfamily Culicinae have still been found, which are the *Culex* genus *Culex pipiens, C. perexiguus*, *C. laticinctus,* and *C. theileri* and the *Culiseta* genus *Culiseta longieareolata*.

In this study, we concluded that *C. longiareolata* is the most abundant species in the region of El Kantara (89.91%) and is a widely distributed species in the Mediterranean region (Brunhes et al., 2001). Studies indicate its existence in polluted sites and permanent sites of stagnant water with rich or poor vegetation in the regions of Tébessa and Souk Ahras (Hamaidia, 2004). The same results were found by Belkhiri et al. (2021) in the Batna region.

Culex pipiens occupied the second place in terms of abundance in the study area with a value of 5.92% of the culicidian population harvested. It is the most widespread mosquito in the world and able to adapt to different biotopes. It is found in both urban and rural areas, in polluted water, as well as in clean water at a high temperature. Thus, it mainly colonizes freshwater rich in organic matter of plant origin (Messai et al., 2010). Several studies have reported the presence of this species in several localities in Algeria, including the work of Benhissen et al. (2017) in Biskra and Benhissen et al. (2018) in Boussaâda.

The other species had a low frequency, which was generally due to the quality of the water, reduction in the number of females emerging, low quantity of nutrients available, drying up of the breeding sites corresponding to the dry seasons, leaching of breeding sites by precipitation, slowing down of larval development following a drop in temperature, and mortality by invertebrate or vertebrate predators (Berchi, 2000).

In our study, we found that the diversity index H¢ took the value of 0.46 and the fairness index was 0.3, which indicates that the region of El Kantara is less balanced. The specific diversity index (Shannon index) was high when the taxonomic richness was high and the distribution of individuals between taxa was balanced; on the other hand, a low index value reflects a less diversified population with predominant species (Faurie et al., 2003).

Biological control can take many forms, but currently researchers are focusing their attention on control through the use of substances of plant origin. This is what we tried to do in this work.

There has been much concern in recent years about the potential of synergy between the insecticides applied against pests. This work highlighted the existence of a synergistic interaction between the studied plants *Peganum harmala* and *Citrullus colocynthis*. The combined extracts increased the mortality rate when we used low concentrations (53.45%). Mortality was strongly correlated not only with the concentrations used, but also with the treatment time. The mixture caused the death of 50% of the treated insects in just 1.15 days. Studies show that certain products act synergistically in the environment against disease vectors and pests.

The active molecules can vary from one family to another and within the same plant family, the sensitivity of insects can differ depending on the plant used (Guèye, 2011). On the other hand, Aouati and Berchi (2015) indicated that plants are a source of active substances with great potential of wide application range. This application diversity could be due to the diversification of bioactive molecules that make up the herbs. Indeed, several compounds are often cited as responsible for their larvicidal properties.

We have demonstrated the bioactivity of extracts of *Peganum harmala* and *Citrullus colocynthis* in synergy on the mosquito *Culiseta longiareolata*. The insecticidal activity is probably attributed to the toxic substances (alkaloids) of these plants (Habbachi et al., 2014). Several studies have confirmed the toxic effect of *Peganum harmala* against insects. Similar results have been reported by Abbasi et al. (2003) on the locusts *Shistocerca gregaria*, Habbachi et al. (2013) on the larvae of *Drosophila melanogaster*, and Habbachi et al. (2014) on *Culex pipiens* mosquito larvae. On the other hand, Merabti et al. (2015) reported on the toxicity of the aqueous extract of *Citrullus colocynthis* on the larvae of *Culex pipiens* and *Culiseta longiareolata*. Abdul Rahuman and Venkatesan (2008) demonstrated the activity of oleic and linoleic acids extracted from *Citrullus colocynthis* on mosquito larvae (*Aedes aegypti*, *Anopheles stephensi,* and *Culex quinquefasciatus*). This effectiveness lies in the nature of the active ingredient (Bruneton, 1993), which is a molecule of common origin (terpenoids, tannins, and compounds phenolics) (Hopkins, 2003).

A number of studies show that certain products act synergistically in the environment against disease vectors and pests, for example, Chaudhry et al. (2005), Christos et al. (2011), and Habbachi (2013). This can reduce the dose to be applied, and therefore will result in reduced treatment costs (Habbachi et al., 2014).

Conclusion

Our study was devoted to the inventory of the Culicidian fauna in the region of El Kantara, where we could identify the presence of seven different species – two species of genus *Anopheles*, four species of *Culex,* and one species of *Culiseta.* From the density point of view, the species *Culiseta longiareolata* represented a high proportion of the population in El Kantara. This mosquito is reputed to be a vector of diseases in the world such as arboviruses. The rest of the species had a low abundance.

In this work, we highlighted the effect of a treatment based on *Peganum harmala* and *Citrullus colocynthis* when these two compounds were used synergistically on the fourth instar larvae of *Culiseta longiareolata*.

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