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Study of the Stimulating and Attractive Factors of Oviposition of *Culex pipiens* (Diptera; Culicidae) in Different Environments

Benhissen Saliha[1.2]*, Hedjouli Zakaria[1], Asloum Abdelmadjid Yagoub[3], Masna Fatiha[4.5], Bounadji Siham[2], Habbachi Wafa[1]

[1]*Applied Neuroendocrinology Laboratory. Department of Biology, Faculty of Sciences, University of Badji Mokhtar, Annaba, Algeria.*
[2]*Department of Natural and Life Sciences, Faculty of Sciences, University of Mohamed Boudiaf, M'Sila, Algeria.*
[3]*Ecology of terrestrial and aquatic systems. Department of Biology Faculty of Science. University of Badji Mokhtar, Annaba, Algeria.*
[4]*Department of Natural Sciences, ENS Taleb Ebderrahman, Laghouat, Algeria.*
[5]*Mechanics Laboratory Team, Department of Mechanics, University of Amar Telidji, Laghouat, Algeria.*

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Abstract

The study of the chemical and physical factors that induce egg-laying is important for understanding mosquitoes' ecology. These substances may also help assess and control mosquito populations. With this in mind, we have highlighted the attractiveness of *Culex pipiens* gravid females concerning the containers' color and surface, which has enabled us to show that females of this species are always attracted to large containers of yellow. The ethological tests were made with four biopesticides on the attractiveness of *C. pipiens* females. It has been observed that the highest densities of the eggs are deposited in the container which contains the biopesticides extracts compared to that which includes the spring water with a significant difference between the two environments (*p*: 1.00). This choice is due to the presence of odorous molecules which attract the mosquito.

Keywords: Attractiveness, bioinsecticide, color, *Culex pipiens*, oviposition, surface.

1. Introduction

Oviposition is in the life of insects, a fundamental act that allows them to secure their offspring. It provides the entomologist with valuable information about the relationship between the animal and the environment [1].

Depending on their genus and species, mosquitoes use various breeding sites to develop their larvae. Different parameters such as color, humidity, and the presence of certain volatile chemicals play a role in females' choice of oviposition site [2]. For most species, females lay eggs in temporary roosts, where the water depth is shallow, in particular, to limit predation [2, 3].

The presence or absence of attractive and stimulating factors such as chemical and physical factors play a significant role in the choice of egg-laying sites, especially in complex species of the *C. pipiens*. Most of the work devoted to this subject has been carried out in the laboratory, the chemical and physical factors have attracted the attention of many authors.

*Email: hedjouli.zakaria@hotmail.com

Such as Haddow and Gillett [4], who devoted their first work on this subject to *Aedes aegypti*, using farmed mosquitoes, exposed to normal fluctuations in daylight, were able to observe an egg-laying cycle with a peak in the afternoon. Fay and Perry [5] also attended those gravid females of *Aedes aegypti* were influenced in their choice by the nature of the substrate lining the spawning vessels. They also showed that various solutions (of sodium benzoate in particular) had great attracting power.

To this end, our objectives in this work are to study the influence of physical and chemical criteria; four bio-insecticides: *Peganum harmala, Daphne gnidium, Bacillus thuringiensis krustaki* and Spinosad ($C_{41}H_{65}NO_{10}$, It comes from the fermentation of a bacterium *Saccharopolyspora spinosa*) on the choice of spawning sites in the pregnant female of *C. pipiens*.

2. Material and methods

2.1. Biological material

The study was carried out on *C. pipiens* (Linnee, 1758) (Diptera: Culicidae). It is widespread in all world regions, especially in temperate and Mediterranean regions [6].

2.2. Breeding method

The fourth instar larvae of *C. pipiens* were collected from different places in Biskra (Algeria). The larvae are fed daily with a mixture of biscuit and yeast (25%; 75%). At the same time, the adults were fed a 5% sugar solution and given a blood meal from a chicken on the third night after emergence.

Breeding was carried out under laboratory conditions of 25 ° C, relative humidity of 70%, and a photoperiod of 14 light hours per day [7]. They were fed from a sweet solution of grapes.

2.3. Adult oviposition behavior test

In the laboratory, we studied the attractiveness of the pregnant female of *C. pipiens* via the physical criteria (The size: small or large, and the color of the containers: white or yellow) of the spawning environment. After mating, twenty-nine females of *C. pipiens* were isolated in a cage (20 x 20 x 20 cm). The cage contains two containers filled with different volumes of water (240ml and 310ml) or of a different color (yellow or white container). Once deposited by the females, the eggs were counted using a stereomicroscope (Gr: x4).

We have also studied the attractiveness of the pregnant female of *C. pipiens* via the chemical criteria (the water used in the container either natural source water, or water contains one of the bioinsecticides) of the spawning environment and this by giving the females a choice between two containers. The first contains 200ml spring water, and the other has 190ml spring water mixed with 10ml of bioinsecticide (aqueous extract of *D. gnidium* (300g / l); aqueous extract of *P. harmala* (300g / l); *Btk* (1g / l); spinosad (1 g / l)).

2.4. Statistical analysis

The results of the oviposition choice tests are compared using simulation of Monte-Carlo, based on a Chi2 test at a threshold of $p = 0.05$ [8].

3. Results

3.1. Oviposition choice test C. pipiens according to physical criteria

The attractiveness of gravid females (without a blood meal) and pregnant females (with a blood meal) results from *C. pipiens* to different egg-laying vessel surfaces and colors are listed in Table 1.

Depending on the colors of the vessels, the gravid females of *C. pipiens* were undoubtedly attracted to the color yellow for both pregnant females who have had a blood meal and those without a blood meal (62% to 76% respectively) (Table 1).
In contrast, large container surfaces only attract gravid females of *C. pipiens* without a blood meal, with an attractive rate of 83%. The attractive rate for pregnant females with a blood meal was 45% for large vessels (Table 1).
Statistical analysis shows significant differences between the choices of pregnant females without a blood meal (Table 1). The number of eggs of pregnant females with a blood meal varies between 474 and 834 eggs. Regarding the eggs laid by pregnant females without a blood meal, there are 1065 eggs in the yellow containers and 993 eggs in the large containers (Table 1).

Table 1: Choice of oviposition of pregnant *C. pipiens* females according to the physical criteria of the spawning grounds.

	With blood meal				**Without blood meal**			
	YC	**WC**	**LC**	**SC**	**YC**	**WC**	**LC**	**SC**
Attractive	18	10	13	16	22	6	24	5
Number of eggs	834	474	596	695	1065	213	993	162
P	0,832 NS		0,904 NS		$0,998^{(*)}$ S		$1,000^{(*)}$ S	

[*YC: Yellow container; WC: White container; LC: Large container; SC: Small container; *Significant*]

3.2. Oviposition choice test C. pipiens According to chemical criteria

The oviposition choice tests made it possible to highlight the role of different insecticide molecules in determining the laying sites by the pregnant females of *C. pipiens*.

Table 2 shows that containers containing *D. ginidium*, *P. harmala*, spinosad, and *Btk* attract *C. pipiens* pregnant females, with significant attractiveness rates ranging from 86% to 93%. The spring water containers attract only 7 to 14% of the females tested (Tab. 2). Statistical analysis by the Monte Carlo test shows significant differences between the spawning choices (Table 2).

The number of eggs laid in these treated environments (*D. ginidium*, *P. harmala*, spinosad, and *Btk*) varies between 572 eggs in the environment containing *Btk.* and 1153 eggs, including the environment the aqueous extract of *P. harmala*. The control environment only included 89 to 164 eggs. The hatching rate is around 80% in most environments tested (control and treated) (Table 2).

Table 2: Choice of oviposition of pregnant *C. pipiens* females according to the physical criteria of the spawning grounds.

	Aqueous extracts of plants				Synthetic products			
	D. ginidium	**Control**	***P. harmala***	**Control**	**Spinosad**	**Control**	***Btk***	**Control**
Attraction	27	2	26	3	25	4	25	4
Number of eggs	895	89	1153	137	883	160	572	164
Hatching rate	81,78%	49,43 %	82,82 %	89,05 %	81,65 %	81,25 %	79,02%	79,87 %
P	1,00* S		1,00* S		1,00* S		1,00* S	

[*Control: spring water (* Significant)*]

4. Discussion

In the wild, the females of *C. pipiens* mostly lay eggs at the beginning and the end of the night. As various authors have shown for other species of mosquitoes, this rhythm is mainly conditioned by several variations [9].

Our results concerning the influence of water volume on the attractiveness of *C. pipiens* pregnant female, reveals that the most numerous spawns would be deposited on the surface of the enormous water masses (16 spawns) on the other hand in small roosts (13 spawns) for females with blood meal and females without blood meal are deposited (24 eggs) in the large container and five eggs in the small container. Subra [1] shows that it would then be necessary to specify an existing relationship between the structure of the roost and the fluctuation of the number of eggs. De Meillon *et al.* [10] showed that tanks containing water from breeding sites did not attract *C. fatigans* females equally if placed at different heights above the ground. The number of clutches collected was all the less, the farther away from the soil surface.

The color of the container also plays a role in the attractiveness of the pregnant female, great stimulation was found reflected in the high number of spawns in the colored roosts for the tests studied. Based on our results, gravid females were not attracted equally to all types of container, and the color of roosts was a significant factor in choosing spawning grounds. So, color is one of the factors determining the choice of spawning grounds. The pregnant female of *C. pipiens* with or without a blood meal, however, prefers yellow vessels rather than white vessels; we noted a significant difference between the two types of containers (*p*: 1,000), which is means, that the female of *C. pipiens* where more attractive to yellow color than the white color, that maybe because the female of *C. pipiens* can detect the yellow color easyer than the white color, as observed by Field and Matsui [11], for *C. tritaeniorhynchus* in the laboratory. Various colored waters are more attractive to gravid females of *C. tritaeniorhynchus* than crystal clear waters.

Plant selection by insects consists of a behavioral sequence in response to one or more stimuli associated with the plant [12]. Allelochemicals (attractants or repellants) in plants play a significant role in accepting the plant by the insect. Witzgall *et al.* [13] have shown that odour signals and visual signals are essential for species survival. These scent signals are the chemical signals that guide insects to their preferred food and on which a phytophagous insect

is based in choosing a nesting site [14, 15]. Our results showed that females prefer containers containing plants water to lay their eggs with a rate of 80%.

In addition, the choice of the laying site depends on several factors, including the amount of organic matter in the roosts [16], the presence of algae and bacteria in the environments [17, 18], nitrogen, phosphorus, and potassium (NPK) levels in water [19].

In this context, we studied the attractiveness of two bacteria, *Btk* and spinosad. The latter affect the sensitivity of the female of *C. pipiens* is the number of attractions in an environment containing these bacteria, unlike in a spring water environment. This sensitivity causes a very significant difference (*p*: 0.001) between the two samples. Gjullin [20] and Hirakoso [21] show that various varieties of larval foods and bacteria obtained many clutches of *C. pipiens*, in fresh manure, emitting a strong and characteristic odour, while no female laid eggs in pure water.

Chitinases and chitobiose are synthesized by a large number of fungi and bacteria, but insects can also produce these two enzymes. During larval molts, the production of these enzymes is under the hormonal control of the insect and, more particularly, of ecdysone or molting hormone [22]. The synthesis of these enzymes by the larval stages of insects would suggest that the waters are home to large populations of mosquito larvae.
Other molecules such as lauric acid and palmitoleic acid are described as spawning stimulants for female *A. aegypti* and are present in their eggs [23]. These fatty acids are described as spawning inhibitors for pregnant females of *C. quinquefasciatus*, maybe because these acids contain the same composition as the pheromones produced by the mosquitoes, this effect being dose-dependent [24].

5. Conclusion

The oviposition behavior of *C. pipiens* was determined based on color and space. The results show that females prefer yellow lots than the white lots with ample space to lay eggs. We also determined the attractiveness behavior of pregnant females of *C. pipiens* to biopesticides, the presence of these products in the water forcing pregnant females to lay eggs on the surface of treated water rather than on spring water. These are important observations that could help in making attractive traps for pregnant females.

Statements on compliance with ethical standards and standards of research involving animals

“This article does not contain any studies involving animals performed by any of the authors.”

Disclosure and conflict of interest

“Conflict of Interest: The authors declare that they have no conflicts of interest.”

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