Journal of Asian Scientific Research, 2013, 3(10):983-994

Journal of Asian Scientific Research

journal homepage: http://aessweb.com/journal-detail.php?id=5003

EFFECT OF SUBLETHAL DOSES OF CHLORPYRIPHOS-ETHYL ON SOME PARAMETERS OF MATING AND LAYING IN THE LOCUST *SCHISTOCERCA GREGARIA* FORSKAL, 1775 (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT

The objective of this study has been to evaluate the impact of sublethal doses of chlorpiryphos-ethyl on the steps of the mating and oviposition behavior in Schistocerca gregaria and its descendants. The sublethal doses of chlorpiryphos-ethyl were tested on locust imago's five days old (sexually immature). The effects of these treatments on locusts and their descendants were evaluated on the mating (rate of real and false matings) but also on some laying parameters (rate of real and false laying and number of laying / female). The locust imagoes were reared in the insectariums of the Faculty of Biosciences, University Felix Houphouet-Boigny in Côte d'Ivoire between July 2008 and June 2009. Six cages with nesting boxes, each containing 50 locusts (25 males and 25 females) were used. Two cages were used to test the contact effect with 2 ml /cage dose at 0.012 g / l of chlorpiryphos-ethyl sprayed directly on locusts. Two other cages were dedicated to evaluate ingestion effect through providing of leaves of maize (Zea mays L. Poaceae) variety Ferké 79, impregnated with 2 ml of chlorpiryphos-ethyl at 0.15 g / l, to serve as food for locusts. The last two cages were used as controls. It was observed in the offspring of controls population 93% of real *matings against 0% in the offspring of treated individuals. 100% of the offspring of treated locusts do not mate until their death. These treatments significantly reduced the number of layings 3.23 ± 0.2 to 1.32 ± 0.06 (59.13%) and 3.23 ± 0.2 to 0.91 ± 0.08 (71.83%) in the treated locusts respectively on contact effect and ingestion effect. Treatment with sublethal doses of chlorpiryphos-ethyl induced decline in fertility among treated locusts and the sterility in their offspring. These results show that the treatment with sublethal doses of chlorpiryphos-ethyl could be a solution to limit the possibilities for locusts to reach the critical density threshold, and thus reduce their damage.*

Keywords: Desert locust, Chlorpiryphos-ethyl, Sublethal doses, Mating, Laying.

1. INTRODUCTION

In arid and semi-arid regions of Africa, the Middle East and Asia, the desert locust (Forskål, 1775) (Orthoptera: Acrididae) is a constant threat to food security (Lecoq, 2004) and the economical resources (Simpson *et al.*, 1999). In its gregarious, it can cause up to 100% crop loss (FAO, 2012) because it has a huge reproductive potential. A female can lay 4 times per year with an average of 50-70 eggs per laying (De Gregorio, 2000).

Among the methods used for plant protection, chemical control remains the most effective and most accessible (Abdou *et al.*, 2005). Products used against locusts pests; chlorpyriphos-ethyl (organophosphate) is one of the most recommended insecticides (Bayer CropScience, 2006). The dramatic effect of the effectiveness of insecticides often sought, explains the high doses recommended by the (FAO, 2003) with its consequences on the environment, human and animal health (Bell *et al.*, 2001; Story *et al.*, 2011).

Despite these high doses of insecticides, locusts remain a problem for agriculture. It is a problem of resistance (Kikuchi *et al.*, 2012) or methods of spreading that makes locusts are not all affected by the lethal doses. In front of this problem of efficacy, we wondered about the effects and mode of action of chlorpiryphos-ethyl on the reproduction of the locust.

Thus, this work is part of the new opportunities to fight against pests locusts, experimentally determine the effect of sublethal doses of chlorpyriphos-ethyl on skills mating and laying target for treated locusts and their descendants.

The results of this study could contribute to avoid a high level of gregariousness of these locusts and thus reduce their impact on agricultural activities.

2. MATERIAL AND METHODS

2.1. Material

Locusts Schistocerca gregaria used were from the insectariums of Biosciences Department, University Felix Houphouet-Boigny in Abidjan, Ivory Coast. They were fed with young maize plants (*Zea mays* L. Poaceae). The development cycle of *S. gregaria* includes seven stages namely five larval stage followed by the young called winged imago (sexually immature) and adult stage (imago sexually mature). The development cycle of *S. gregaria* has seven stages, five larval stages, one stage of young winged called imago (sexually immature) and an adult stage (sexually mature imago). The average length of imago stage is 19 days (De Gregorio, 2000; Ouali-N'goran *et al.*, 2007). To study the impact of sublethal doses, 5 days old winged imagos (sexually immature) underwent chlorpiryphos-ethyl treatment.

The device consists of locust breeding cages with wood cubic shape of 50 cm side. Three of four side faces are in metal grating of stitches 2 mm. The fourth sides face of plywood 5 cm thick is provided an opening of 20 cm square for later used for manipulation of insects inside cages. The upper and lower faces are of plywood 5 cm thick. Each cage is equipped with a 100-watt bulb lit in order to maintain the high temperature (29-35°C). The relative humidity is 65 to 80%. For the deposition of eggs, Plexiglas tanks, 17.5 × 11.5 × 7 cm, filled with damp sand, are placed inside each cage.

The chemical used is chlorpyriphos-ethyl (organophosphate) and neurotoxic acetyl cholinesterase inhibitor in the body (Bayer CropScience, 2006). It acts by contact, inhalation and ingestion. Its efficiency is 3 to 5 l / ha

2.2. METHODS

2.2.1. Administration of Sublethal Doses of Chlorpyriphos-ethyl on the Locust

Sublethal doses of chlorpyriphos-ethyl were determined by serial dilutions and tested on locust. Among six cages each containing 50 imagos (25 males and 25 females) used to monitor their sexual behavior and that of their offspring, two contained locusts individuals treated by contact effect, two other cages with individual treated by ingestion effect and the last two were control cages (they contained locusts untreated).

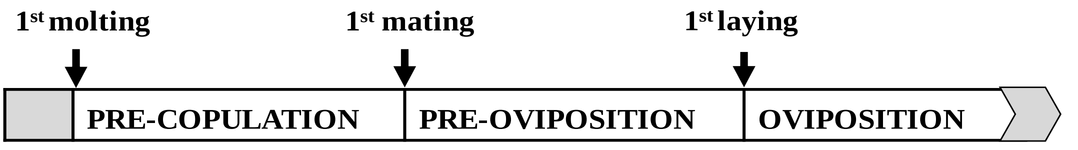
For the contact effect, a concentration of 0.012 g / l of chlorpyriphos-ethyl at a dose of 2 ml /cage was sprayed directly on the locusts during five days while by ingestion, leaves of maize have been impregnated with 2 ml of chlorpyriphos-ethyl in a concentration of 0.15 g / l. After five minutes, the time required for drying drops of insecticide, the leaves were used as a food for locusts.

Finally, these locusts were then fed with untreated leaves. Three repetitions were performed for each treatment. The parameters studied are the mating behavior and the laying. The beginnings and ends of the mating as well as the laying were recorded to assess the duration of mating and the laying. The same parameters were evaluated for control parents, treated invidious and their descendants.

2.2.2. Measuring of Parameters Related to Mating Behavior

These parameters are linked to pre-copulation periods (Figure 1), the rate of locust male that performs sexual displays (approach movement in %), the balance of the male over the female's back (stability of the male over the female) which would show that the nervous system of locusts is not reached, the average length of the mating and the rate of real and false laying. Pre-copulation period is the time between the date of the last molting and the first mating. Duration ranging from the date of molting until the first mating period corresponds to pre-copulation. On the steps of mating, all behaviors of the male and female are observed between pre-copulation periods to the end of the mating. Observations were made during all the experiment and the dates of first clutches were noted. Pre-oviposition period is between mating and first laying.

Figure-1. Different phases from molting to laying



2.2.3. Measuring of Parameters Related to the Laying Behavior

We describe the different stages of the laying and determine the oviposition period, fecundity, number of eggs per laying and fertility. The experiment was continued until the death of all females. The date of the last egg was noted and the laying period is time between the first and the latter laying of eggs.

The beginning and end of matings as well as of layings have been noted for the calculation of the average time of a mating and a laying.

Fecundity (***F***) in *S. gregaria* is considered as the number of layings per female during her life. The number of layings is noted every day until the last clutch.

$$F = \frac{number\ of\ layings}{number\ of\ females}$$

The average number of eggs per laying was determined

$$Average\ number\ of\ eggs\ per\ laying = \frac{\sum Xi}{ni}$$

Xi = number of eggs per laying

ni = number of egg cases considered

As for fertility, it corresponds to the estimate of the rate of emergence. The number of hatchlings emerged was therefore identified every day. The rate of emergence (***TxE***) expresses the percentage of emerged larvae phase 1 (***Ni***) on the average number of eggs laid per female (***No***).

$$TxE = \frac{Ni}{No}\ 100$$

2.2.4. Statistical Analyzes

Data processing was performed using the Epi-Info (EP6). Comparison of means was made by analysis of variance (ANOVA) and the Kruskal Wallis rank. The test was used to assess Barlet homogeneity of variances which determines the choice of the analysis. Thus, quantitative data were subjected to analysis of variance (ANOVA) and qualitative data analyzed Kruskal Wallis test at 5%.

3. RESULTS AND DISCUSSION

3.1. Effect of Treatment with Chlorpiryphos-ethyl on Mating Parameters

We observed that the first mating of *Schistocerca gregaria* occurred between the 16th and 21st days after molting. Before any mating, a wide variety of behaviors have been observed in *S. gregaria*.

Pre-copulation period average measured is 17.6 ± 1.1 days in locust control and 17.5 ± 1.3 days in the treated locusts. There is therefore no significant difference (P = 0.825). Treatment has no effect on the duration of pre-copulation. According to Duraton and Lecoq (1990), this period is 19 days in *S. gregaria* and corresponds to the time of maturation of the reproductive organs. In *Callosobruchus maculatus*, this period does not exist, because once the emergence, the imago is able to mate and lay eggs.

The study of treatment with chlorpiryphos-ethyl on behaviors approach and mating of *S. gregaria* revealed no behavioral disorder in treated locusts.

Whatever treatment (contact effect or ingestion effect), on average, 96.5% of treated locusts have made normal sexual displays. 95.6% of the males were stable on the back of the females during mating. And finally, 90.8% of treated locusts had real mating against 93% of the untreated controls (Table 1). Treatments had statistically no significant effect on the real (P = 0.751) or false mating (P = 0.563) compared to controls locusts. However, we note that the rate of looking of sexual partner is 0% for the descendants of treated locusts, against 97% in the descendants of untreated controls (Table 1).

Outside of normal heterosexual couples, homosexual couples (in males) and two males with abnormal couples superimposed on the back of a female were observed. The rate of these false matings do not vary significantly in locusts treated with contact effect (7.1%) or by ingestion (6.5%) and control locust (5.2%) (Table 1). These false matings can be promoted by an insufficient number of females at the end of a generation. Females have a shorter life span than males (Ouali-N'goran *et al.*, 2007).

The study of these matings parameters in locusts treated by contact or ingestion effect showed that sublethal doses of chlorpiryphos have no significant observable effect on these parameters. This could be explained by the fact that the doses used are low, not only can disrupt the nervous system of treated insects, but also can not affect how serious the vital functions of locusts.

The most impact of effect observed in parameters was noted in the descendants of locusts that have undergone treatments. Indeed, 100% of the locusts do not perform any research nor sexual partner mate. This locust behavior is explained by the fact that they remain sexually immature. A simple observation of non-discoloration of locust integument and the dissection of the insect's abdomen shows atrophy of the gonads. Previous work of Ouali-N'goran *et al.* (2007) on fenitrothion clearly demonstrated it.

Table-1. Effect of treatment with chlorpyriphos-ethyl on some parameters of the mating of S. gregaria.

Treatments	pre-copulation duration (days)	Approach Movements (%)	Stability of ♂ On the ♀ (%)	Average duration of mating (h)	Real mating (%)	False mating (%)
Control (C)	17.56 ± 1.13a	97.6 ± 1.7a	97.1 ± 2.0a	6.7 ± 2.5a	93.0 ± 1.1a	5.2 ± 0.6a
Contact effect (CE)	17.48 ± 1.35a	96.7 ± 2.3a	95.3 ± 2.7a	6.3 ± 2.6a	90.5 ± 1.2a	7.1 ± 0.8a
Ingestion effect (IE)	17.43 ± 1.14a	96.2 ± 2.8a	95.8 ± 2.3a	5.7 ± 2.3a	91.0 ± 1.6a	6.5 ± 0.6a
Probability (P)	*P = 0.825*	*P = 0.773*	*P = 0.727*	*P = 0.686*	*P = 0.751*	*P = 0.563*
Control progeny (Pg/C)	17.53 ± 1.12a	97.0 ± 1.8a	96.8 ± 2.2a	6.8 ± 2.5a	91.4 ± 1.3a	5.6 ± 1.0a
progeny/ Contact Effect (Pg/CE)	0 b	0 b	0 b	0 b	0 b	0 b
progeny / ingestion Effect (Pg/IE)	0 b	0 b	0 b	0 b	0 b	0 b
Probability (P)	*P = 0*	*P = 0*	*P = 0*	*P = 0*	*P = 0*	*P = 0*

C : Control ; **CE** : Treatment by contact effect ; **IE** : Treatment by ingestion effect ; **Pg/C** : progeny of control; **Pg/EC** : progeny of locust treated with contact effect ; **Pg/IE** : progeny locust treated by Ingestion effect. ***P***: Probability; Values with the same letter down the column are not significantly different ($P \geq .05$) from each other according to the Kruskal Wallis test.

3.2. Effect of Treatments of Chlorpiryphos-ethyl on Laying Parameters

Average length of pre-oviposition (P = 0.742) and oviposition (P = 0.894) in treated locusts were not significantly different compared to controls (Table 2). But, in the offspring of treated locusts, these values are zero (P = 0). They have not mated.

Duration recorded during laying in locust is 2 hours to 2 hours 30 minutes against a period of 2 hours 45 minutes to 3 hours (De Gregorio, 2000). This discrepancy is explained by the difference in the number of eggs laid. The eggs are deposited one by one interval of one minute, there will be more eggs to deposit, lay more eggs will be long (Launois-Luong and Latchininsky, 1997).

In nature, looking for a suitable nesting site hatching eggs laid is also a concern for the desert. Two types of oviposition were observed: the real clutches (with egg deposition) and false pundits (drilling holes with recess layings of the abdomen without egg deposition). The study of the effect of treatment on real and false layings could inform about the capacity of treated locusts to control or manage their layings.

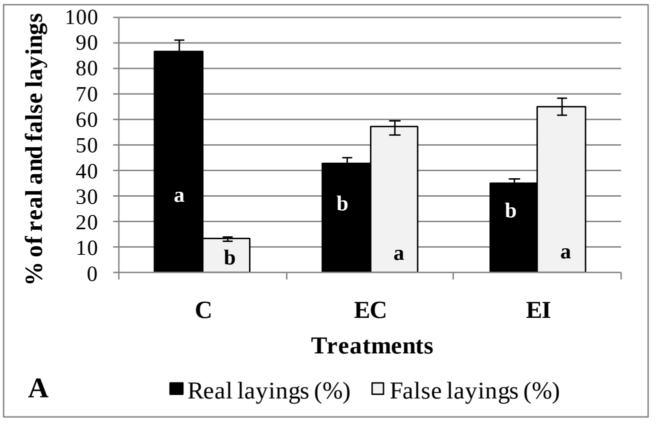
Table-2. Effect of chlorpiryphos treatment on the duration of two parameters on laying

Treatments	**Pre-oviposition duration (days)**	**Oviposition duration (days)**
Control (C)	1.36 ± 0.26 a	13.40 ± 1.06 a
Contact Effect (CE)	1.38 ± 0.32 a	13.14 ± 1.08 a
Ingestion Effect (IE)	1.38 ± 0.20 a	13.08 ± 1.02 a
Probability (P)	*P = 0.742*	*P = 0.894*
Progeny of control (Pg/C)	1.36 ± 0.22 a	13.27 ± 1.11 a
Progeny / Contact Effect (Pg/EC)	0 b	0 b
Progeny / Ingestion Effect (Pg/IE)	0 b	0 b
Probability (P)	*P = 0*	*P = 0*

Pg/C: Progeny of controls; Pg/CE: Progeny of locust treated by Contact effect; Pg/IE: Progeny of locust treated by ingestion effect. Values with the same letter down the column are not significantly different ($P \geq .05$) from each other according to the Kruskal Wallis test.

Treatment with sublethal doses of chlorpyriphos significantly reduced (P = 0.0277) the rate of real layings by 43.7% contact effect, and 51.7% by the effect of ingestion (Figure 2a). This significant reduction in real layings conversely implies a significant increase (P = 0.0206) false layings.

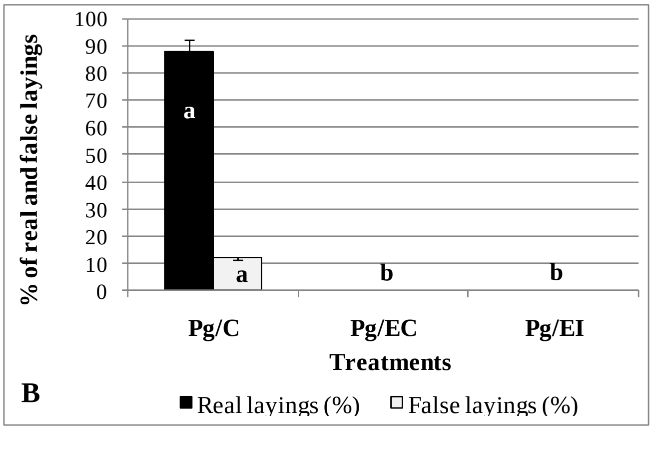
Figure-2a. Treatment effect of chlorpiryphos on real and false laying of locusts treated parents



C : Control ; **CE** : Treated by Contact Effect; **IE** : Treated by Ingestion Effect. Values followed by the same letter are not statistically different at 5% according to the Kruskal Wallis test. Real layings (p = 0.0277) False layings (p = 0.0206).

According to our analysis of these data, treatments provided to locust mitigate their vigilance to lay their eggs in good sites to their hatchings. In offspring of treated locusts, no laying was observed (Figure 2b). This leads toreducethe population ofdescendants.

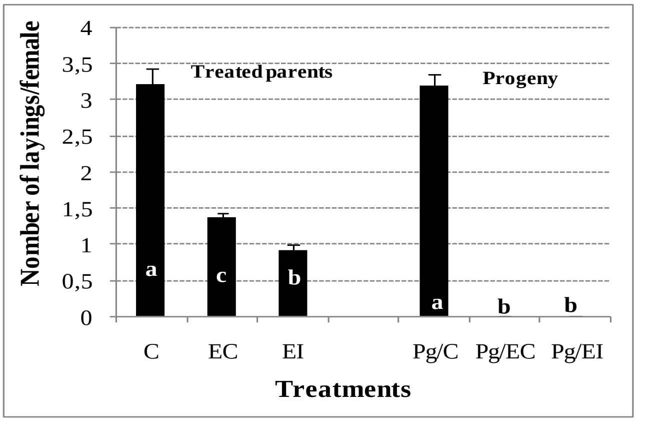
Figure-2b. Effect ofchlorpiryphos-ethyl treatment on real and false laying of des cendants



Pg/C: Progeny of Control; **Pg/EC**: Progeny of locust treated by Contact effect; **Pg/EI**: progeny of locust treated by Ingestion Effect. Values with the same letter are not significantly different (*P* = .05) from each other according to the Kruskal Wallis test. Probability of real and false layings (*P* = 0)

The average number of layings was significantly reduced (P = 0.0265) from 3.23 ± 0.2 to 1.32 ± 0.06 (59.13%) and from 3.23 ± 0.2 to 0.91 ± 0.08 (71.83%) respectively for treated locusts by contact effect and ingestion effect (Figure 3). The number of eggs per clutch also significantly lower (P = 0.033) (Figure 4). It decreases from 51.32 ± 1.06 to 36.13 ± 1.18 (29.6%) for the locusts treated by contact effect, and from 51.32 ± 1.06 to 27.86 ± 01.15 (45.7%) by ingestion effect (Figure 4). Concerning their descendants, the values for these parameters are zero laying (Figures 3and 4). These values are similar to those obtained by De Gregorio (2000) who explains that which female *S. gregaria* lay about 4 times maximum and the number of eggs per laying varies from 50 to 70 in good condition.

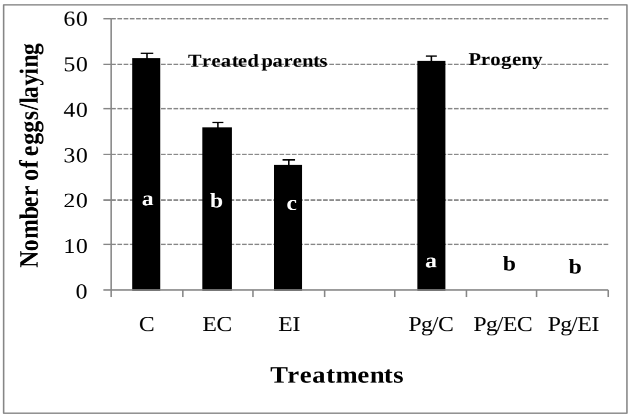
Figure-3. Treatment effect of chlorpiryphos on number of laying by female



Values followed by the same letter are not statistically different at 5% according to the Kruskal Wallis test. Treated parents (P = 0.0265); Progeny (P = 0)

In bad weather, the number of real nesting is 2, maximum numbers of eggs with a 30 to 40 maximum. These poor conditions are similar to treatment with sublethal doses of chlorpiryphos-ethyl inducing 1.32 ± 0.06 and 0.91 ± 0.08 clutches per female and a number of eggs of 36.13 ± 1.18 and 27.86 ± 1.15. Reducing the number of eggs laid by *S. gregaria* after insecticide treatment was reported by Ali *et al.* (2009) under the effect of *Melia azadirachta* extract. Concerning fertility rates, it remains above 90% in controls but never reaches 100% for all individuals treated (Figure 5). This is explained by the fact that either embryos die before maturity, or larval stages 1 die at hatching, or at the time of emergence from the soil.

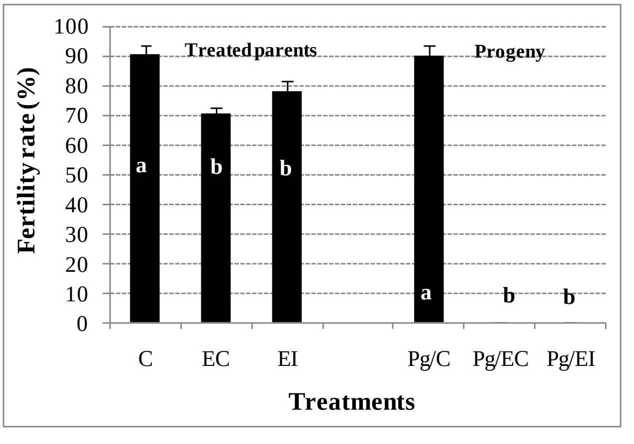
Figure-4. Treatment effect of chlorpiryphos on number of eggs by laying



Values followed by the same letter are not statistically different at 5% according to the Kruskal Wallis test. Treated parents (*P = 0.033*); Progeny (*P = 0*)

After treatment with sublethal doses of chlorpyriphos-ethyl, emergence rate was 71.37% and 78.21% respectively contact effect and ingestion effect compared to 90.45% in controls (Figure 5).

Figure-5. Treatment effect of chlorpyriphos on the rate of fertility



Values followed by the same letter are not statistically different at 5% according to the Kruskal Wallis test. Treated parents (*P = 0.033*); Progeny (*P = 0*)

Following this study, the main results show that sublethal doses of chlorpiryphos-ethyl is acts essentially on the reproduction of the treated locusts and the offspring of these treated locusts.

Concerning the Treated Parents

- The rate of real layings was strongly decreased while the false layings have increased in parallel;
- The number of laying / locust and number of eggs / laying dropped considerably; and finally,
- The fertility rate or the number of larvae that emerged was significantly reduced following treatment.

Among the descendants of locusts treated, the treatment effect was much more noticeable. It was noted that they do not make any research for sexual partner. None of these locusts mates and therefore lays eggs. It is generally that sublethal doses of chlorpyriphos disrupt reproductive function of locusts. Sublethal doses attenuate reproductive function in the parents and completely inhibit it at the descendants which do not reproduce. Indeed, disruption of reproductive function by the action of insecticides was reported by Girardie (1991) and Ramade (1991). The sublethal doses of chlorpyriphos are probably at the origin of chromosomal aberrations that could be transmitted to the descendants of treated locusts. It's known that the development of gametes is dependent on several hormones including juvenile hormone (JH) of allata (CA). Chlorpyriphos is an acetyl cholinesterase inhibitor; there would be no release of acetyl-coA not synthetic juvenile hormone (Gillott and Friedel, 1976). This is what could explain the sexual immaturity or sterility of descendants of locusts that have undergone treatments. The opening of their abdomens shows atrophy of locusts' gonads (Ouali-N'goran *et al.*, 2007).

4. CONCLUSION

The sublethal doses of chlorpyriphos-ethyl applied to imagos of *S. gregaria* 5 days old had no apparent effect on them, with concern pre-copulation periods, pre-oviposition and reproductive period. They reach sexual maturity, mate and lay eggs as untreated controls.

In addition, concerning the fecundity, we note significant decreases in treated locusts by contact and ingestion. Similarly, fertility has declined significantly in the treated locusts by contact and ingestion. Besides, the fact that the descendants of treaties remain sterile reduces the reproductive potential of the desert locust and therefore, its ability to gregarizing and consequently, its pest potential. These results could be taken into account in an integrated pest management program against the locust. In addition, the use of sub-lethal doses of insecticides could reduce environmental pollution by chemicals (sustainable development).

5. ACKNOWLEDGEMENTS

The authors wish to express their profound gratitude to the Head of Department Zoology and Animal-Biology. The University Félix Houphouët-Boigny of Côte d'Ivoire, for permission to publish the study.

Competing Interests

Authors have declared no competing interests.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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