

Post Mating Changes in Vaginal Region of Female *Drosophila* Under Normal and Pesticides Treated Conditions

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Abstract

Drosophila punjabensis is a common pest reported from India. Pesticides are known to induce changes in the structure of reproductive organ of *Drosophila* therefore here we examined the effect of Malathion and Chloropyrifos on *Drosophila* at 50% lethal concentration and control condition and results obtained. It was observed that the pesticide treated flies had aberrated vagina affecting their breeding behaviour and their fertility level. This led to the observation that the pesticides induced infertility in the *Drosophila* and therefore this process can be used for similar different common pests it can be replicated to induce effective pest management by using *Drosophila* as model organism.

Keywords: Pesticide; *Drosophila*; Vagina; Mating; Reproductive organ

Introduction

In internally fertilizing organisms, mating consists of a series of coordinated molecular reactions between the sexes that occur in female reproductive tract especially vagina. In many species where females mate and multiply, post mating effects are expected to express rapidly.

Females of insects store sperm and remate several times; therefore, it is likely that ejaculates from several males will compete for the fertilization of the eggs [1]. The insemination reaction is a post mating effect in female *Drosophila* vagina caused by the male ejaculate [2]. The insemination reaction, as postmating effect, which occurs in many species of *Drosophila*, is an enlargement of the vagina caused by the male ejaculate. Therefore, the insemination reaction does not effect the male paternity of offspring. This reaction affects the postmating behaviour of the female. And reported that females will

expel an excess of sperm around 6 to 7 hrs after mating [3]. This suggests that the female performs actively in removing sperm from her vagina. However stated that the insemination reaction disappears gradually from the vagina [4-5]. A gradual clearance of the reaction suggests the strong possibility of an ongoing physiological activity, also discussed the insemination reaction in the context of sperm competition. Insemination reaction in five species was first proposed by [1,6]. *Drosophila melanogaster* female expels a droplet of sperm after mating [6]. *Drosophila mojavensis* has great enlargement of the vagina that disappears gradually [3]. Found that some species discard a large mass of sperm after mating [7].

Because of doubts on appearance and gradual disappearance of vaginal swelling, we decided to compare the changes in vaginal structures of *Drosophila* in different conditions. However still it is not clear, that postmating effect gives any selective benefit to female [8].

Except normal condition, Pesticides induce minor changes in the structure of reproductive organ of *Drosophila*, so this study could be used for similar different common pests and induce effective pest management by using *Drosophila* as model organism. The study emphasizes adverse effect of organophosphate compound, malathion and chlorpyrifos on post mating vaginal swelling in female *Drosophila punjabensis*.

Methods

Drosophila punjabensis were used as the test organism, and to understand the effect of pesticides on *D. punjabensis* two sets of experiments were conducted one test was used as control where no treatment was done whereas the other set (three replicates of each set) had treated flies. This reaction occurs both in control and treated flies. The pesticides were administered by feeding the first instar larvae at LC50 concentration of Malathion and Chlorpyrifos (0.02ppm of malathion and 0.015ppm of chlorpyrifos) in every 30 ml of food medium till they emerged into virgin flies. The healthy virgin flies were selected and pair each of male and female flies was put into each of the replicate vials and left for a day or till mating occurred. Subsequently the size of vagina was observed just after mating in the experimental female flies and then was compared with control virgin flies.

Result and Discussion

In case of control mating the vagina returned to a

normal condition, usually in few hours, but in treated mating it remained swollen for many hours even for several days. Table 1 shows the change in vaginal pouch due to the effect of mating in control and Malathion treated flies. Similar results were observed in the Chlorpyrifos treated flies (Table 2). Insemination reaction showed the effect of mating, swelling and rupturing of vagina of females were observed in all treatments including the control set, however treated females showed higher level of distortion when compared with control flies. The structural changes appears in some species of female vagina and disappears gradually in others suggesting different phenomena, the sperm sac, the mating plug, and vaginal swelling describes a distinct structure associated with a specific female postmating behaviour while explained the enzymatic analysis of the insemination reaction in *Drosophila* [5]. Studies suggest that this could be due to hormonal deficiency due to pesticide could be one of the factors for the swelling and greater distortion in the treated flies. The studies are in progress to know the exact reasons for greater distortion in the treated flies. Insemination in other species of *Drosophila* also revealed that the vaginal region of female become distorted [9-10]. Many of the *Drosophila* species have been recently reported as agricultural pest and causing heavy damage to the agricultural products. These species are more prominent in agricultural field than to fermenting fruit as their natural habitat. *Drosophila punjabensis* is as such common pest reported from India. *Drosophila* has long been a favourite model system for studying the evolutionary changes.

| Crosses | No of females tested | Duration of copulation (in minutes) Mean± S.E. | Duration of insemination reaction(in minutes) | | Enlargement of vaginal pouch (in mm) Mean± S.E. |
|--|----------------------|---|---|---------------------------------|---|
| | | | Begins at Mean± S.E. | Ends at (in minutes) Mean± S.E. | |
| D.punjabensis female X D.punjabensis male | 50 | 4.12 ± 0.23 | 10.39± 0.18 | 1.52 ± 0.16 | 0.25 ± 0.03 |
| Treated (0.2ppm) D.punjabensis female X Treated (0.2ppm) D.punjabensis male | 50 | 3.57± 0.18 | 8.86± 0.42 | 9.07 ± 0.07 | 0.30 ± 0.02 |

Table 1: Duration of copulation and insemination reaction (in minutes) and enlargement of vaginal pouch (in mm) of control and Malathion treated *Drosophila punjabensis* flies

| Crosses | Number of females tested | Duration of copulation (in minutes) Mean. S.E. | Duration of insemination reaction | | Enlargement of vaginal pouch (in mm) Mean± S.E. |
|---|--------------------------|---|--------------------------------------|------------------------------------|---|
| | | | Begins at (in minutes) Mean± S.E. | Ends at (in minutes) Mean± S.E. | |
| D.punjabiensis female* D.punjabiensis male | 50 | 4.14 ± 0.23 | 10.29± 0.17 | 1.53 ± 0.16 | 0.25 ± 0.03 |
| Treated (0.015ppm) D.punjabiensis female* Treated (0.015ppm) D.punjabiensis male | 50 | 3.76± 0.19 | 9.86± 0.45 | 8.27 ± 0.08 | 0.31 ± 0.02 |

Table 2: Duration of copulation and insemination reaction (in minutes) and enlargement of vaginal pouch (in mm) of control and Chloropyrifos treated *Drosophila punjabiensis* flies.

Based on the above study it can be suggested that chloropyrifos & malathion induces developmental and reproductive tract related toxic effects in *Drosophila punjabiensis* and therefore can be used as indicator of non-target organism toxicity. Further if vaginal changes persists for long time duration, they can induce evolutionary changes leading to formation of new species. This study can be used for similar different common pests and induce effective pest management by using *Drosophila* as model organism. But further detailed studies are needed to arrive to this conclusion.

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