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Environmental Contamination and Toxicology

# Toxicity of *Euphorbia canariensis* Latex to Some Developmental Stages of *Drosophila melanogaster* (Diptera: Drosophilidae)

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*Euphorbia canariensis* is a member of the family of Euphorbiaceae (spurge). *E. canariensis* distributed to all of the world from Canary Islands. The columns of *E. canariensis* could be seen which is popular in cultivation as a grafting stock. It was reported that the several species of this family have some toxic properties. *Euphorbia esula* L. is also a human health hazard. These plants produce a white latex, which was cause severe dermatitis in humans and animals. According to Hoger's Handbuch, human overdoses result in burning mouth, nausea, diarrhea, dilated pupils, collapse with pallor, temporary blindness, arrhythmic pulse, vertigo, cramps etc. (List & Horhammer 1969-1979). The same effects have been shown black rat (Yokel et al. 1981; Gundidza & Kufa 1993 ). *E. tirucalli* L. is well- known as a fish poison and insect repellent for ants and mosquitoes ( Webb et al. 1984). It has been shown to cause keratoconjuctivitis in dogs (Osore 1984 ). Planorbidae snails were killed by very low concentrations of latex of *E. milii* (Oliveira-Filho & Paumgartten 2000). Cattle and horses usually avoid *E. esula* L. and *E. cyparisias* L. but should they eat it, its milky latex may cause sickness and even death (Best et al. 1980; Batra, 1983; Rees & Fay 1989). There was no survival at the highest concentration (10 mg/l) of the latex on *Melanoides tuberculata* (Giovanelli et al. 2001). The latex of this plant has also been reported by Chopra et al. (1958) as having abortifacient and emmenagogic effects.

There are many investigations in the literature about the lethal effects of latex of *Euphorbia* species on different organisms. There is no adequate investigation on the toxic effects of the latex of *E. canariensis*. On the other hand, there is no enough research carried on the effects of latex on *D. melanogaster*. The main reasons for the widely use of *Drosophila* are that it has a short life span (approximately 10 days), and a great variety of offsprings. Besides, different phenotypic characteristics of *D. melanogaster* can easily be distinguished from each other. For these reasons, in this study we aimed to investigate whether the toxic effects of latex of *E. canariensis* on the developmental stages of *D. melanogaster* Meigen (1943) (Diptera: Drosophilidae) Oregon- R, or not.

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## MATERIALS AND METHODS

The latex from *E. canariensis* was obtained from cuts made on the trunk. Oregon R wild type strain *of D melanogaster* was used. Stocks and experimental groups routinely kept constantly at 25 ± °C and 40-60% relative humidity on Standard Drosophila Medium ( SDM). The six different concentrations *of* crude latex were (0.1; 0.5; 1.0; 2.0; 5.0; 10 mg/100 mL) added to 100 ml of SDM and kept at room temperature in dark waiting for 24 hours to diffusion of latex to the medium.

The flies with the same aged were used for experiments and 7 male and 7 female individuals were mated. The culture vials containing only the SDM were used as control. The developmental stages were followed daily. After pupa formation, the parental individuals were removed. Offsprings were counted every day from the first day of eclosion, sexes and phenotypic abnormality were noted. Differences between control and experimental groups were verified using the chi- square test.

20 male and 20 female adult individuals, which were grown in SDM, were transferred to new medium. They were incubated for one day in order to egg laid and then the adult individuals were removed. 72 hr larvae (second instar larvae) grown from these eggs, were transferred to the media which contain the latex at different concentrations (0.1, 0.5, 1.0,2.0,5.0, 10 mg/mL) the offsprings grown in this last media were counted.

## RESULTS AND DISCUSSION

The effect of latex upon the some developmental stages ( metamorphosis, total number of offsprings and liveliness- ratio) of *D. melanogaster* have been investigated. Maternally toxic effects of latex is shown in Table 1.

**Table 1**. Maternally toxic effects of latex on *D. melanogaster*.

| Concentration (mg/100 mL) | Total number of parental individuals | Mortality (%) |
|---|---|---|
| Control | 14 | - |
| 0.1 | 14 | 1 (0.07) |
| 0.5 | 14 | 6 (0.42) |
| 1.0 | 14 | 8 (0.57) |
| 2.0 | 14 | 8 (0.57) |
| 5.0 | 14 | 9 (0.64) |
| 10.0 | 14 | 12 (0.85) |

At the application groups, while the daily investigations were carried out after mating being held, mortality on parents has been observed in accordance with increasing concentration of latex. The number of dead parents at the application groups (0.1-10 mg/mL) has varied between 1-12. The similar maternally toxic effects, we have dealt have also been observed by Giovanelli et al. (2001). As 2.5 mg/mL concentration of latex of *E. splendens* var. *hislopii* starts the mortality on *M. tuberculata* (Thiaridae), at the highest concentration (10mg/mL) there were no survivors. Same species has a molluscicidal action at low concetration (LD90 less than 1.5 ppm) against the vector snails of schistosomiasis (Schall et al., 1991). Maternally toxic effects have been seen on wistar rats (Souza et al. 1997) and planorbidae snails (Oliveira et al. 1999; Oliveira-Filho & Paumgartten 2000). The latex of *E. tirucalli* showed high oxytocić activity in a dose dependent in gravid rat (Osore 1984 ). Again, *E. obovalifolia* were found to have 30-100% killing effects at ticks on cattle (Regassa 2000).

In the experimental groups, the number of offsprings decreased on the basis of the increased concentration of latex (Table 2). While the number of offsprings at the control group was 2100, this number changes between 23-1370 at the application groups. The differences between the number of offsprings obtained from control and experimental groups were statistically important ($P<0.01$). In the application of 10mg/mL latex, neither eggs hatched nor offspring formation was observed.
For us there may be some reasons for the decrease of offsprings according to the increase of concentration. i)The impact of the latex on the egg mases. Using the standardized methodology of the WHO for testing plant- derived molluscicides, a 90% lethal dose (LD90) ranging from 0.13 ppm for *Biomphalaria glabrata* was obtained (Schall et al. 1998; Al-Zanbagi et al. 2001). ii) Embryolethal and maternally toxic effects. The extracts of Euphorbiaceae ( *E. peplus, E. pseudocactus and E. helioscopia* ) are quick acting, and even at low concentrations they only require a short time (6- 12 hr) to cause 100% mortality of *Biomphalaria pfeifferi*. (Shoeb & El Sayed 1984; de Mendonca et al. 1993; Schall et al. 2001). As seen in Table 1 and 2, at the application of 10 mg/ mL latex, no offspring was observed. It is probable that 85% rate of death number at the parental individuals has prevented the new offspring formation. At the egg masses exposed to toxic effects, embryolethal effect occurs and the number of offspring may decrease.

**Table 2.** The effect of latex on the number of offspring of *D. melanogaster*

| Concentration (mg/100mL) | Female % | Male % | Total no of individuals | Total no of abnormal individuals | % |
|---|---|---|---|---|---|
| Control | 1033(0.49) | 1067 (0.51) | 2100 | 3 | 1.428 |
| 0.1 | 756 (0.55) | 614 (0.45) | 1370 a | 6 | 4.379 |
| 0.5 | 51 (0.46) | 59 (0.54) | 110 a | 5 | 45.450 |
| 1.0 | 27 (0.47) | 31 (0.53) | 58 a | 3 | 51.720 |
| 2.0 | 23 (0.58) | 17 (0.42) | 40 a | - | - |
| 5.0 | 10 (0.43) | 13 (0.57) | 23 a | - | - |
| 10.0 | - | - | - | - | - |

*:The differences between sex-ratio is statistically important ($P < 0.05$ ); a: The decreasing of the total number of offsprings according to control group is statistically important ($P < 0.01$ ).

The effects of latex on larval development have been summarized at Table 3. When the adult rate of second instar larvae was investigated, the highest value was seen at the control group. Depending on an increase of latex concentration at the application groups, liveliness- ratio of larvae decreased. At the 10mg/mL concentration too, no larval development occurred. It was found that differences between the liveliness- ratio of control and experimental groups were statistically important (P<0.01), except for the application of 0.1 mg/mL latex (P>0.05).

Similarly, *Chamaesphecia astatiformis* and C. *hungarica* those fed with *E. esula* had a lower larval survival rate (Tosevski et al.,1996). These researchers have also reported that no larval development occurred in the C. *tenthrediniformis*. It is possible that the decreasing of numbers of liveliness larvae is the most effective cause of the decreasing of the number of offsprings (Table 3).

**Table 3.** The effect of latex on the liveliness- ratio of second instar larvae of *D. melanogaster*.

| Concentration (mg/l00mL) | Total number of larvae | Total number of adult individuals (%) |
|---|---|---|
| Control | 100 | 99 (0.99) |
| 0.1 | 100 | 75 a (0.75) |
| 0.5 | 100 | 52 b (0.52) |
| 1.0 | 100 | 48 b (0.48) |
| 2.0 | 100 | 27 b (0.27) |
| 5.0 | 100 | 13 b (0.13) |
| 10.0 | 100 | - |

[a] $\chi^2$ test (P>0.05), [b] $\chi^2$ (P< 0.01).

During the counting of offsprings of $F_1$ generations, wings malformations were observed at the lower doses (0.1,0.5,1.0 mg/ mL) of latex and control group. The numbers of abnormal individuals for control and application groups were 3 (0.142%), 6 (0.437%), 5 (4545%), 3 (5172%) respectively (Table 2). These malformations are both seen on right and left wings ( Fig. 1a-c and Fig. 2a). Besides, thorax abnormalities were also observed on the offsprings ( Fig. 2b).

Similarly, it was reported that latex of different *Euphorbia* species has caused malformations on different animal groups. Embryofeto- toxic effects of *E. milii* latex were observed by Souza et al. (1997) on wistar rats. Fetal growt was retarded at doses > 125 mg latex/ kg body weight and minor skeletal malformations was observed higher at 250 mg/ kg body weight. According to Oliveira et al. (1999) the snail size was seen a minor influence of *E. milii* latex. The extract isolated from *E. bougheii* and *E. leuconeura* exhibited significant skin irritant activity on the mouse ear as well as a tumour promoting activity on the mouse back skin (Gundidza & Kufa, 1993; Vogg et al. 1999). Extract of *E. hirta* caused edema on rat backpaws and on loss of weight, after a chronic treatment during fourteen days at the daily dose of 200 or 400 mg/ kg (Lanhers et al. 1991). This findings are in accordance with our results.

It has been reported that human overdoses result in dilated pupils, nause, arrhythmic pulse, vertigo, cold sweats, etc. and poisoning effects on children (List & Horhammer 1969-1977). Different diterpene esters (phorbol and ingenol ) causes these malformations (Gundidza & Kufa, 1993; Vogg et al. 1999). The presence of ingenane and lathyrane diterpens in *E. canariensis* has also been

**Figure 1.**a- b: Female individuals with malformed right wings; c: a female individual with malformed left wing; X 31.25

**Figure 2.a**: a female individual with unopened left and right wings; b: a female individual with malformed right wings and right thorax. X 31.25.

reported (Marco et al. 1997) and the toxic effect of latex of *E. canariensis* may be caused by these diterpens. According to Wada et al. (1998) these diterpens cause an effect on the inhibition of DNA topoisomerases (topos). We consider that hybrid gene s occurring after single strand or double strand DNA breakage may result in malformations.

The effect of the latex on the metamorphosis of the *D. melanogaster* is shown in Table 4. In control and experimental groups (0.1, 0.5, 1.0, 5.0, 10 mg/mL latex) laid eggs was observed at the second days of mating. Then, in control group, first, second, third instar larvae, prepupa, pupa and adult individuals were formed, respectively. The first adult was observed at the 9th days of mating. In the application of 0.1 mg/ml latex, the metamorphosis was completed in the same period as control. But, at the applications of 0.5-10 mg/mL of latex, metamorphosis was found to be longer during the periods after the egg laid due to the increasing do se of latex. At the highest concentration (10 mg/mL), development after egg laid was found to be stopped.

**Table 4.** The effect of latex on the metamorphosis of *D. melanogaster*.

| Stages of metamorphosis | Days of stages at control and applications | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.1 | 0.5 | 1.0 | 2.0 | 5.0 | 10 |
| Mating | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| egg | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 1th instar larvae | 3 | 3 | 4 | 3 | 5 | 6 | - |
| 2nd instar larvae | 4 | 4 | 5 | 4 | 7 | 9 | - |
| 3rd instar larvae | 5 | 5 | 6 | 7 | 9 | 10 | - |
| Prepupa | 6 | 6 | 7 | 8 | 10 | 11 | - |
| Pupa | 7 | 7 | 8 | 9 | 11 | 12 | - |
| Adult | 9 | 9 | 10 | 11 | 12 | 14 | - |

Findings about the prolongation of metamorphosis at higher concentrations are in accordance with the findings obtained from the assays above mentioned (toxic effect of latex on maternally, egg masses, the number of offspring and liveliness-ratio of second instar larvae ). It is probable that latex effected the different stages of development and so caused the stopping of development.

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