Toxicology International, *Vol 28(3), 245-253, 2021, DOI: 10.18311/ti/2021/v28i3/27416*

Original Research
ISSN (Print): 0971-6580
ISSN (Online): 0976-5131

Utilization of Courtship Behavioral Elements to Understand the Toxicity of Methyl methanesulfonate in *Drosophila melanogaster*

Baladare Udayakumar Divyashree* and Venkateshaiah Vasudev

Department of Bioscience, University of Mysore, P.G. Centre, Hemagangothri, Hassan – 573220, Karnataka, India; divyacrp6@gmail.com

History: Received 2021-03-24; Accepted 2021-05-27; Published 2021-08-23

Abstract

A standard, monofunctional alkylating agent, Methyl methanesulfonate, was tested for its effects on courtship behavioral elements in *Drosophila melanogaster*. Different concentrations ranging from 0.1 to 3 mM were analyzed using the larval feeding technique. When the different combinations of crosses were performed to analyze the effects, the results have revealed that male courtship elements such as orientation, tapping, wing vibration and licking were significantly increased compared to controls. Similar data were observed in case of female behavior. Nonetheless, the courtship behavior interms of fitness such as courtship latency and copulation latency were significantly high and copulation duration was significantly reduced, thus emphasizing the effect on fitness and inturn viability. On par with earlier data on toxicity, the present results also demonstrate the drastic effects of Methyl Methanesulfonate (MMS) on the courtship behavior of *D. melanogaster*. Thus, it is opined that courtship elements as a parameter can be utilized to understand toxicity and also it can be analyzed within a short period of time.

Keywords: Courtship Elements, *Drosophila melanogaster*, Methyl methanesulfonate, Toxicity

1. Introduction

It is fully recognized that all the organisms exposed to alkylating agents induce clastogenic, mutagenic, carcinogenic etc., effects[1–6] on one hand and on the other, these agents are also useful in cancer treatments[7–11] which may play an important role in human health and welfare. During 39 years following the discovery of viability parameter for assessing toxicity in *Drosophila melanogaster* by Muller[12], several researchers have failed to add to this inspite of many investigations on toxic effects of radiation and chemical agents. But, as a consequence of the experiments of Luning[13], it was possible to add the rate of development as another parameter to study toxicity and further experimental data brought to light the importance of the said parameters to study the toxicity of environmental agents in *D. melanogaster*. This test system has been proved beyond doubt as the best sub-mammalian test system to screen genotoxic effects of environmental pollutants[14]. However, the use of rate of development and viability to screen toxicity in *D. melanogaster* require a long duration. Hence attempts are being made time and again to get inexpensive, short duration and effective parameters. Eventhough Sturtevant[15] observed the courtship behavior of numerous species of *Drosophila* in 1915 itself, he has used this parameter to understand sexual isolation and sexual selection in evolutionary biology. Nonetheless, to say the least, this has not attracted toxicologists. In this direction, Vasudev *et al.*[16] have made an attempt to understand the utility of mating behavior of *D. melanogaster* to analyze toxicity. They have used pesticides namely Dithane M-45 and Baygon respectively to prove the hypothesis. To prove

*Author for correspondence

beyond doubt the utility of mating behavior as a parameter to analyze toxicity, we proposed to use a standard mutagen Methyl methanesulfonate (MMS), which is having a lot of literature on routine toxicity and the results are presented here.

2. Material and Methods

2.1 Test System

In all experiments, the OK strain of *Drosophila melanogaster* has been used as the material and the same as been obtained from *Drosophila* Stock Centre, Department of Zoology, University of Mysore, Mysore. Flies were maintained in the BOD incubator with 24 ± 1^{0}C.

2.2 Chemicals

The well-known monofunctional alkylating agent, Methyl methanesulfonate (MMS:CAS No. 66-27-3), was obtained from Sigma Chemical Company, USA and was used as the genotoxic agent to study the effects on courtship behavior. Other routine chemicals were from BDH chemical Ltd., Mumbai.

2.3 Preparation of Media

- **Control Media:** Normal standard wheat cream agar food medium was used as control.
- **Chemical Media:** Various concentrations of MMS (0.1, 0.5, 1, 2 and 3 mM) in food medium were prepared according to the procedure of Vasudev and Krishnamurthy[17]. This was distributed to food vials in equal quantities and was used as chemical media for all experiments.
- **Delcour Media:** This media was prepared according to the method of Delcour[18].
- **Collection of Eggs:** The modified technique of Delcour was used to collect the same age of eggs[17].

2.4 Assessment of Rate of Development and Viability

Eggs of the same age (± 6 h) in equal numbers (100 eggs per bottle) were placed into normal and each bottle containing media of different concentrations of MMS (0.1, 0.5, 1, 2 and 3 mM) and allowed to develop. The newly-hatched larvae were continuously fed on the above food media, i.e. larval feeding method[17] was used. The rate of development and viability were analyzed by using the method of Vasudev and Krishnamurthy[19] after the emergence of adult flies.

2.5 Isolation of Virgin Flies for Mating

As said in item 2.4, the same procedure was followed here until the emergence of adult flies. When the adult flies emerged, the virgin females and bachelor males were isolated within three to four hours of eclosion and maintained separately in a normal medium. They were aged for five days for observation of the courtship behavior.

2.6 Courtship Behavioral Assay

Courtship assay was conducted during the morning hours (7-10 A.M.) every day in a room with a temperature of 24 ± 1^{0}C under normal laboratory light conditions. A single virgin female was introduced into Elens-Wattiaux mating chamber without etherizing them, using an aspirator. A bachelor male was added to it and allowed to acclimatize to the chamber for 30 sec. The details of courtship behavior were directly observed through hand lens of 10x magnification. Depending upon the experimental protocols mentioned below, flies were introduced into the observation chamber. Courtship activities such as orientation, tapping, wing vibration and licking which are male-specific activities and ignoring, extruding and decamping which are female-specific activities and copulation duration were recorded separately in the control and treated groups. From these data, courtship latency and copulation latency were analyzed and tabulated. Continuous observations were made for 30 minutes, then if there had been no copulation, that was considered as unmated and that pair was replaced by fresh pair. The mating chamber was washed every time after each observation to remove sex pheromones of previous couples if any.

2.7 Experimental Protocols

- **Control:** Untreated male and female flies were released into mating chambers to observe for their mating behavior.
- **Female Treated:** Female treated with 0.1, 0.5, 1, 2 or 3 mM of MMS was released with control male into the mating chamber.
- **Male Treated:** Male treated with 0.1, 0.5, 1, 2 or 3 mM of MMS was released with control female into the mating chamber.
- **Both Treated:** Both male and female flies treated with 0.1, 0.5, 1, 2 or 3 mM of MMS were released into the mating chamber.

Twenty-five pairs were observed for each experiment and we repeated the experiments thrice. As a whole, we recorded 75 observations each for treated and control groups.

2.8 Statistical Analysis

The data obtained from the individual experiments were subjected to statistical analysis to determine the significance level between the control and treatment groups. The data were expressed as Mean ± SE and compared using a one-way Analysis of Variance (ANOVA). Comparisons among groups were analyzed according to Tukey's post hoc test using the SPSS statistical package version 23.0.

3. Results

3.1 Rate of Development and Viability

The results have revealed that there is dose-dependent increase in rate of development and the viability is significantly reduced to 51.55% in the highest dose (3 mM) tested (data not given) compared to controls.

3.2 Courtship Behavioral Assay

Both in treated and control groups, it has been observed that male engages in a series of actions, which include following the female (orientation), tapping her with his forelegs, vibrating his wings, licking her genitalia with his mouthparts, bending his abdomen to copulate and lastly mounting the female by holding with first forelegs. Thus, in the present study, qualitative data are similar in all experimental regimes analyzed. When we take into consideration the quantitative data, the results have revealed that there are significant differences between treated groups and control (Tables 1, 2 and 3, $p<0.05$). Nonetheless, it is clear from each table that male courtship behaviors like orientation, tapping, wing vibration and licking show a significant increase in a dose-dependent manner compared to controls. The highest effect of MMS is noticed in the highest dose tested of all courtship elements. Tables also reveal the concordant results obtained from different types of experimental schedules. On par with this, even in female rejection elements of *D. melanogaster*, there is an increase in dose-dependent manner compared to controls (Figures 1, 2 and 3). The courtship latency and copulation latency have been shown to be significantly extended in all the experimental treated groups compared to controls (Tables 1, 2, 3, $p<0.05$). On the other hand, the copulation duration is significantly decreased in different treated groups than that of controls (Tables 1, 2 and 3, $p<0.05$). The highest reduction of courtship duration is noticed when both males and females are treated. For example, the courtship duration in this group is 10.44 ± 0.25 min compared to 19.35 ± 0.05 min in control (Table 3). These findings are suggestive of overall ailment in performance of courtship by both male and female under treatment with MMS compared to control.

Table 1. Effect of different concentrations of MMS on courtship elements of *D. melanogaster* when male is treated and paired with normal female

Concentrations (in mM)	Orientation (in numbers)	Tapping (in numbers)	Wing vibration (in numbers)	Licking(in numbers)	Courtship latency (in seconds)	Copulation latency (in minutes)	Copulation duration (in minutes)
Control	4.58 ± 0.07^{a}	3.62 ± 0.07^{a}	4.64 ± 0.05^{a}	2.43 ± 0.04^{a}	10.80 ± 0.16^{a}	1.51 ± 0.02^{a}	19.35 ± 0.05^{a}
0.1	7.07 ± 0.53^{b}	5.12 ± 0.12^{b}	6.60 ± 0.54^{b}	3.47 ± 0.29^{a}	17.34 ± 0.56^{b}	2.71 ± 0.17^{b}	17.88 ± 0.10^{b}
0.5	10.05 ± 0.36^{c}	7.40 ± 0.29^{c}	9.54 ± 0.48^{c}	5.41 ± 0.44^{b}	22.63 ± 0.53^{c}	3.74 ± 0.17^{c}	16.73 ± 0.10^{c}
1	16.34 ± 0.26^{d}	12.54 ± 0.24^{d}	14.25 ± 0.19^{d}	9.44 ± 0.27^{c}	27.12 ± 0.70^{d}	6.24 ± 0.16^{d}	14.79 ± 0.17^{d}
2	21.17 ± 0.18^{e}	19.31 ± 0.24^{e}	21.24 ± 0.36^{e}	13.51 ± 0.35^{d}	33.39 ± 0.43^{e}	9.28 ± 0.10^{e}	12.69 ± 0.19^{e}
3	29.15 ± 0.27^{f}	23.54 ± 0.25^{f}	26.61 ± 0.28^{f}	18.71 ± 0.56^{e}	37.12 ± 0.69^{f}	12.40 ± 0.42^{f}	11.44 ± 0.25^{f}

Note: Data of 3 independent experiments; 25 pairs per experiment were used; Mean with S.E are given. Values with same superscripts are not significantly different ($p>0.05$) whereas values with different superscripts are significantly different ($p<0.05$) from one another.

Table 2. Effect of different concentrations of MMS on courtship elements of *D. melanogaster* when female is treated and paired with normal male

Concentrations (in mM)	Orientation (in numbers)	Tapping (in numbers)	Wing vibration (in numbers)	Licking(in numbers)	Courtship latency (in seconds)	Copulation latency (in minutes)	Copulation duration (in minutes)
Control	4.58 ± 0.07^{a}	3.62 ± 0.07^{a}	4.64 ± 0.05^{a}	2.43 ± 0.04^{a}	10.80 ± 0.16^{a}	1.51 ± 0.02^{a}	19.35 ± 0.05^{a}
0.1	8.25 ± 0.20^{b}	6.19 ± 0.17^{b}	7.40 ± 0.33^{b}	4.42 ± 0.25^{b}	16.47 ± 0.36^{b}	3.12 ± 0.14^{b}	18.57 ± 0.15^{b}
0.5	13.30 ± 0.37^{c}	8.10 ± 0.09^{c}	11.31 ± 0.29^{c}	6.44 ± 0.36^{c}	19.15 ± 0.21^{c}	4.80 ± 0.13^{c}	17.15 ± 0.46^{c}
1	18.68 ± 0.21^{d}	14.52 ± 0.25^{d}	17.37 ± 0.43^{d}	11.26 ± 0.42^{d}	25.39 ± 0.32^{d}	7.38 ± 0.11^{d}	15.68 ± 0.16^{d}
2	24.72 ± 0.19^{e}	21.63 ± 0.21^{e}	23.57 ± 0.26^{e}	15.29 ± 0.71^{e}	31.41 ± 0.40^{e}	10.37 ± 0.31^{e}	13.23 ± 0.17^{e}
3	31.57 ± 0.24^{f}	26.89 ± 0.21^{f}	28.79 ± 0.11^{f}	20.76 ± 0.22^{f}	36.52 ± 0.47^{f}	13.25 ± 0.19^{f}	12.32 ± 0.25^{f}

Note: Data of 3 independent experiments; 25 pairs per experiment were used; Mean with S.E are given. Values with same superscripts are not significantly different ($p>0.05$) whereas values with different superscripts are significantly different ($p<0.05$) from one another.

Table 3. Effect of different concentrations of MMS on courtship elements of *D. melanogaster* when treated male and female is paired

Concentrations (in mM)	Orientation (in numbers)	Tapping (in numbers)	Wing vibration (in numbers)	Licking(in numbers)	Courtship latency (in seconds)	Copulation latency (in minutes)	Copulation duration (in minutes)
Control	4.58 ± 0.07^{a}	3.62 ± 0.07^{a}	4.64 ± 0.05^{a}	2.43 ± 0.04^{a}	10.80 ± 0.16^{a}	1.51 ± 0.02^{a}	19.35 ± 0.05^{a}
0.1	10.66 ± 0.24^{b}	7.38 ± 0.43^{b}	8.87 ± 0.15^{b}	5.11 ± 0.19^{b}	18.45 ± 0.47^{b}	3.96 ± 0.07^{b}	16.69 ± 0.24^{b}
0.5	16.91 ± 0.10^{c}	10.59 ± 0.24^{c}	13.58 ± 0.43^{c}	8.16 ± 0.71^{c}	24.49 ± 0.41^{c}	5.24 ± 0.22^{c}	15.68 ± 0.22^{c}
1	21.71 ± 0.33^{d}	17.72 ± 0.22^{d}	20.71 ± 0.24^{d}	13.64 ± 0.19^{d}	30.53 ± 0.38^{d}	9.25 ± 0.15^{d}	13.51 ± 0.28^{d}
2	28.98 ± 0.25^{e}	23.87 ± 0.16^{e}	27.78 ± 0.24^{e}	18.43 ± 0.37^{e}	35.40 ± 0.39^{e}	12.36 ± 0.34^{e}	11.64 ± 0.36^{e}
3	34.62 ± 0.42^{f}	28.86 ± 0.35^{f}	31.76 ± 0.30^{f}	24.17 ± 0.19^{f}	39.70 ± 0.70^{f}	14.40 ± 0.42^{f}	10.44 ± 0.25^{f}

Note: Data of 3 independent experiments; 25 pairs per experiment were used; Mean with S.E are given. Values with same superscripts are not significantly different ($p>0.05$) whereas values with different superscripts are significantly different ($p<0.05$) from one another.

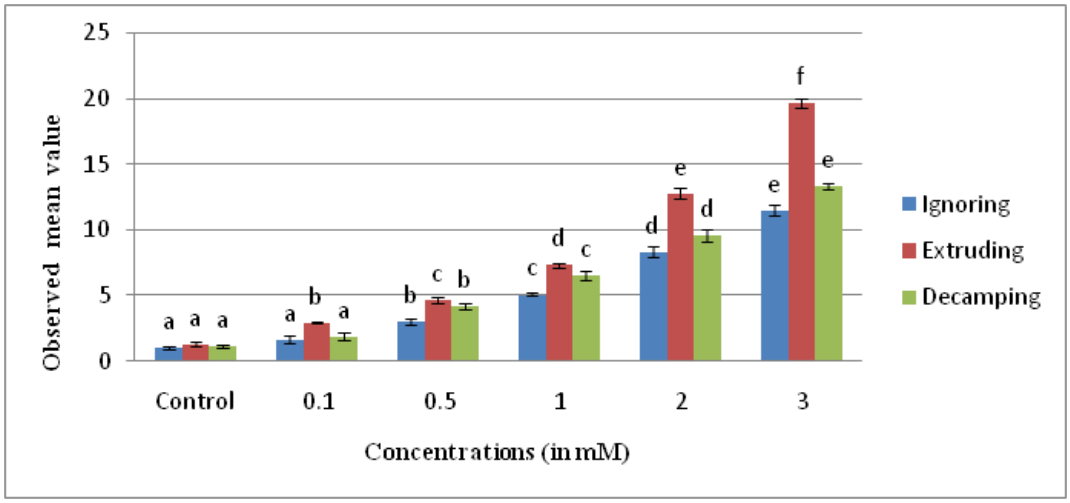


Figure 1. Female rejection courtship elements of *D. melanogaster* after MMS treated male is paired with a normal female.

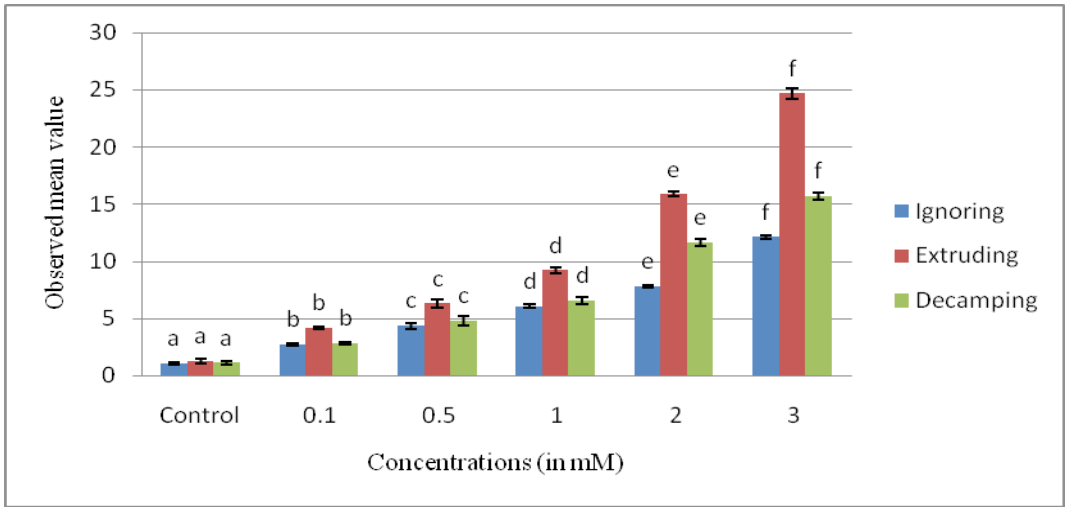


Figure 2. Female rejection courtship elements of *D. melanogaster* after MMS treated female is paired with a normal male.

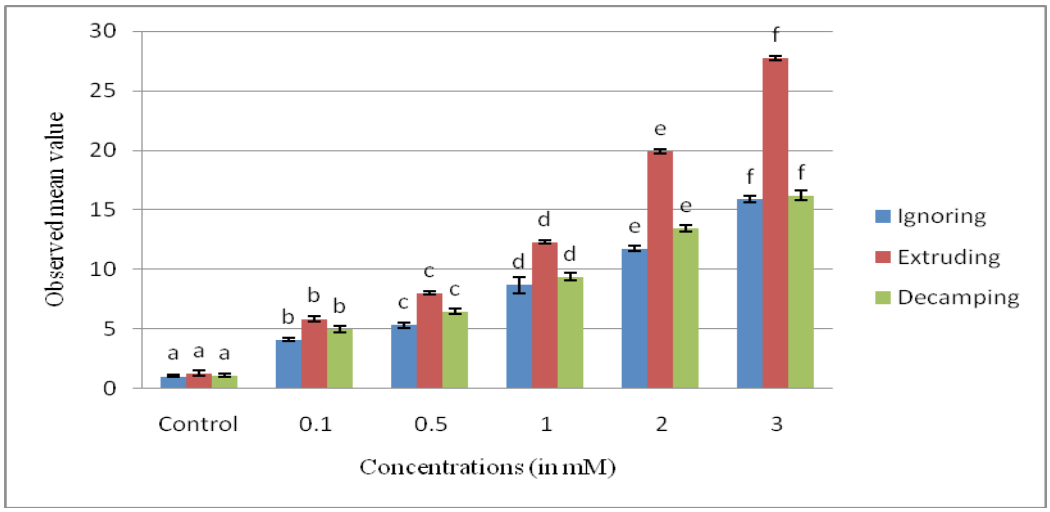


Figure 3. Female rejection courtship elements of *D. melanogaster* after MMS treated male and female is paired.

4. Discussion

The goal of our present study is to establish courtship behavior as a marker to understand toxicity in *D. melanogaster* because of its many advantages, which will be discussed in later part, in concordant with that of well established parameters of toxicity namely rate of development and viability. Further, the rate of development and viability are considered as the best characters to know the effect of a chemical on somatic cells[13]. Outcome of the present data on the effect of MMS reveals that there is significant increase of rate of development and reduced viability in *D. melanogaster* at all doses tested (data not given) and thus justifying the toxic effects of MMS. These results are also on par with the investigations of Luning[13] who showed the same effect in *D. melanogaster* using sodium salicylate, Sorsa and Pfeifer[20] using Organo-mercurials, Vasudev and Krishnamurthy[17,21,22] using Dithane M-45, Cadmium chloride and Baygon.

D. melanogaster to be used as a model organism to understand courtship behavior in toxicology, it must have many advantages. They are: 1. *D. melanogaster* are small but not minute (2.7 mm); 2. Short adult life span, 3. Sexual dimorphism, 4. Single pair mating can be easily achieved, 5. Elaborate courtship behavior, 6. Courtship behavior can be analyzed qualitatively and quantitatively, 7. Many behavior mutants are available, 8. Time taken to analyze courtship behavior is very short compared to rate of development and viability, 9. and limited space to work out. Hence, *D. melanogaster* has been used to analyze the effects of MMS, a standard monofunctional alkylating agent on courtship behavior inturn to get the picture of toxicity. Qualitative data of courtship behavior of present study such as orientation, tapping, wing vibration, licking, curling and mounting are similar to that described by Bostock and Manning[23] and Spieth[24]. There is no variation between treated groups and controls with regard to courtship behavior and thus it is opined that MMS has not affected the courtship elements qualitatively.

Quantitatively, the behavioral attributes during courtship like orientation, tapping, wing vibration, licking and copulation attempts have significantly increased in different experimental regime, thus exhibiting the sign of MMS toxicity in *D. melanogaster* (Tables 1, 2 and 3, $p<0.05$). Similarly, different chemical agents have been tested and showed that they alter the courtship behavior of *D. melanogaster*[16,25-27],. While correlating results of established toxicity parameters and courtship behavior, Vasudev and Krishnamurthy[17,19] and Vasudev *et al.*,[16] have demonstrated that Dithane M-45 significantly increased rate of development and reduced viability, reduced the morphological characters such as body length, wing length and size of pupae on one hand and on the other, the same agent has increased the male and female courtship elements except copulation duration. Furthermore, reduced body size and wing size of *D. melanogaster* due to Dithane M-45 resulted in altered courtship behavior, which is a disadvantage for the flies. Thus, they have opined that *D. melanogaster* can be used as a model to test the effects of sub-lethal concentrations of environmental pollutants using courtship behavior. Present results also prove and strengthen that hypothesis by using standard mutagen, MMS.

Male activity and female receptivity are important for faster successful mating in Drosophila. Vigor of the male *D. melanogaster* is indicated by a parameter namely courtship latency[28] which represents the time between introduction of male and female flies into observation chamber for acclimatization and then initiation of courtship activities[29,30] or it is the time lag before performance of the first courtship behavior (orientation) after pairing[31]. A male with high vigor reacts quickly in the presence of female while a male with less vigor, reacts slowly[32]. On the other hand, it also reflects the receptivity of females. In the present study, significantly longer courtship latency was noticed in experimental treated groups (Tables 1, 2 and 3, $p<0.05$) indicating that males require longer duration to attract females. It also means that increased courtship latency, decreased male vigor.

Copulation latency is measured as the time taken for the male after orientation towards female until initiation of copulation or it is the time lag before successful mounting after pairing[31]. It actually indicates the vigor of male and receptivity of female[16,25,33]. It also shows the time required for males and females to initiate copulation. Higher the vigor of males and receptivity of females, shorter is the copulation latency. That is, a male with high vigor reacts quickly in the presence of a female, where as a male with less vigor reacts slowly. On the other hand, it also reflects the receptivity of females. During copulation latency, courtship acts are performed mostly by males, to increase receptivity of females and to make her sexually excited[34]. In the present study, significant longer copulation latency is noticed in experimental treated groups compared to controls (Tables 1, 2 and 3, $p<0.05$). It is obvious to say that longer copulation latency indicates less vigor of males and also non receptivity of females. It is also known that *Drosophila* males with high vigor has to perform the same courtship act many times to a non-receptive female than to a receptive female and vice versa. It is clear from the results

that the treated males could not maintain high vigor and treated females are less receptive. This result agrees with the finding of Nazari and Hegde[25], Vasudev *et al.*[16] and Roy and Ghosh[27] while studying the effects of Fluoxetine, Dithane M-45 and Alar recepectively on *D. melanogaster*.

According to Spiess[35], the courtship activity of male and female culminates in copulation in *D. melanogaster* and the duration of which depends on the species i.e. copulation duration is variable from species to species. This copulation duration is the time between initiation of copulation to termination of copulation of each pair. During copulation, sperms from the male are transferred to the female reproductive tract and therefore, the duration of copulation has a lot of significance in an animal's life. Guruprasad et al.,[36] suggested that longer duration of copulation permits the transfer of more sperms by males, thus enhancing the fitness of males. It also enhances the fitness of females, because the sperm received by female can fertilize more eggs. In the present study, in the treated groups, the copulation duration is significantly less compare to control (Tables 1, 2 and 3, $p<0.05$). This is more pronounced when both male and female are treated (Table 3) and thus proving that these flies are less fit compared to non-treated ones. These results are concordant to that of previous studies of Vasudev *et al.*,[16], Roy *et al.*,[37] and Roy and Ghosh[27] and On par with this, treated flies are less fecund than control[19]. It is also demonstrated that when flies are treated, the mean daily egg production is reduced with reduced size of imago[19].

Treatment of male or female and subsequent outcome related to individual act of courtship behavior reveals toxicity of MMS in *D. melanogaster* is sex-independent as the score for individual act in alternate treated groups are variable (Tables 1 and 2). Further, from these tables it is also clear that treated females are much more affected interms of individual acts compared to treated males.

The non-receptive female escapes from the courtship activities of male by ignoring and qualitatively not producing any signal from wings, legs, genital and movements of abdomen[38] but quantitatively, this element is significantly increased in different treated groups (Figures 1, 2 and 3, $p<0.05$). Nonetheless, ignoring is more pronounced when female is treated (Figure 2) compared to treated male (Figure 1). However, it is significantly expressed when both male and female treated (Figure 3, $p<0.05$). Extruding is a process in which the non-receptive female contracts and relaxes certain muscles in abdominal region and vaginal plate and is allowed to form a slender tube to deny copulatory position to male[39]. In the present experiments, when females are treated (Figure 2), extruding is more than treated males and control (Figure 1). It is also interesting to note that the number of extruding is highest in both female and male treated group (Figure 3). Similar results were obtained by Nazari[26] using Fluoxetine in *D. melanogaster*. Decamping is the behavior of non-receptive female during which, she attempts to escape male overturns by running, jumping or flying away from the vicinity of male[40]. The frequency of decamping depends on the degree of receptivity of female[41]. Data of the present study reveal that treatments of MMS have resulted in significant increase in the number of decamping compared to their controls (Figures 1, 2 and 3, $p<0.05$). Thus, it is evident from these results that the treatments of MMS make the female non-receptive and non-fit. In *Drosophila*, mate selection is made by the female[42,43]. Because of the expression of non-receptivity of females, the males have to perform their courtship elements such as orientation, tapping, wing vibration and licking more times than the controls (Tables 1, 2 and 3). These results prove beyond doubt, that MMS is toxic even in female courtship elements.

The data of previous studies and the results of present one, when pooled together it is interesting to note that toxicity is expressed in terms of increased rate of development, less viability, less fecundity, reduced size of imago on one hand and on the other, toxicity is noticed in courtship elements in the form of increased orientation, increased tapping, increased wing vibration, increased licking, increased courtship and copulation latency, increased ignoring, increased extruding, increased decamping and decreased copulation duration. Thus, it is strongly opined that courtship act can undoubtedly be used as a parameter of toxicity in *D. melanogaster*, which can be analyzed within a short period of time compared to routinely used parameters.

5. Conclusion

In conclusion, it can be said that MMS is harmful to *D. melanogaster* with a spectrum of adverse effects on different courtship activities, thus characterizing toxic effects of MMS. Furthermore, especially increased courtship and copulation latencies and reduced copulation duration are the indicators of reduced fitness due to toxicity of MMS, similar to that of reduced viability. This may lead to the new direction of research in understanding the toxicity of MMS in particular and other agents in general, on other model organisms and humans.

6. Acknowledgement

The authors would like to thank the Director of Post Graduate Centre, University of Mysore, Hemagangothri,

Hassan, for providing facilities and also grateful to Professor M. S. Krishna, Department of Studies in Zoology, Manasagangothri, Mysore, for assistance during the experiments of this work.

7. References

1. Brewen JG, Payne HS, Jones KP, Preston RJ. Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic male germ cells. Mutat Res. 1975; 33(2-3):239–49. https://doi.org/10.1016/0027-5107(75)90200-6
2. Vogel E, Natarajan AT. The relation between reaction kinetics and mutagenic action of mono-functional alkylating agents in higher eukaryotic systems: I. Recessive lethal mutations and translocations in *Drosophila*. Mutat Res Fund Mol Mech Mutagen. 1979; 62(1):51–100. https://doi.org/10.1016/0027-5107(79)90223-9
3. Vasudev V, Mahmood R, Harish SK, Guruprasad KP. Comparative analysis of error-free DNA repair (adaptive response) induced by EMS and MMS in *Poecilocerus pictus* and mouse. Environmental Pollution and Genetic Risk. M. Hemaprasad and PP Reddy, Ed. M/s. Murthy Graphics, Hyderabad. 1997; 59–68.
4. Kaya B. Anti-genotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents. Turk J Biol. 2004; 27(4):241–6.
5. Pottenger LH, Schisler MR, Zhang F, Bartels MJ, Fontaine DD, McFadden LG, Gollapudi BB. Dose-response and operational thresholds/NOAELs for *in vitro* mutagenic effects from DNA-reactive mutagens, MMS and MNU. Mutat Res. 2009; 678(2):138–47. PMid: 19616119. https://doi.org/10.1016/j.mrgentox.2009.07.002
6. Hartwig A, Arand M, Epe B, Guth S, Jahnke G, Lampen A, Martus HJ, Monien B, Rietjens IM, Schmitz-Spanke S, Schriever-Schwemmer G. Mode of action-based risk assessment of genotoxic carcinogens. Arch Toxicol. 2020; 94(6):1787–877. PMid: 32542409 PMCid: PMC7303094. https://doi.org/10.1007/s00204-020-02733-2
7. Hurley LH. DNA and its associated processes as targets for cancer therapy. Nat Rev Cancer. 2002; 2(3):188–200. PMid: 11990855. https://doi.org/10.1038/nrc749
8. Kaina B, Christmann M, Naumann S, Roos WP. MGMT: Key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair. 2007; 6(8):1079–99. PMid: 17485253. https://doi.org/10.1016/j.dnarep.2007.03.008
9. Ralhan R, Kaur J. Alkylating agents and cancer therapy. Expert OpinTher Pat. 2007; 17(9):1061–75. https://doi.org/10.1517/13543776.17.9.1061
10. Kondo N, Takahashi A, Ono K, Ohnishi T. DNA damage induced by alkylating agents and repair pathways. Journal of Nucleic Acids. 2010; 543531. PMid: 21113301 PMCid: PMC2989456. https://doi.org/10.4061/2010/543531
11. Lossos C, Liu Y, Kolb KE, Christie AL, Van Scoyk A, Prakadan SM, Shigemori K, Stevenson KE, Morrow S, Plana OD, Fraser C. Mechanisms of lymphoma clearance induced by high-dose alkylating agents. Cancer Discovery. 2019; 9(7):944–61. PMid: 31040105 PMCid: PMC6606344. https://doi.org/10.1158/2159-8290.CD-18-1393
12. Muller HJ. Artificial transmutation of the Gene. Science. 1927; 66:84–7. PMid: 17802387. https://doi.org/10.1126/science.66.1699.84
13. Lüning KG. *Drosophila*-tests in pharmacology. Nature. 1966; 209(5018):84–6. PMid: 5956339. https://doi.org/10.1038/210104a0
14. Vogel E, Sobels FH. The function of *Drosophila* in genetic toxicology testing. Chemical mutagens. Boston, MA: Springer; 1976; 93–142. https://doi.org/10.1007/978-1-4684-0892-8_4
15. Sturtevant AH. Experiments on sex recognition and the problem of sexual selection in *Drosophila*. Anim Behav. 1915; 5(5):351. https://doi.org/10.1037/h0074109
16. Vasudev V, Gurushankara HP, Vishwaprakash Mahadimane P, Khalandar D, Shamprasad BR. Effects of fungicide Dithane M 45 in *Drosophila melanogaster* on courtship behavior. Dros Inf Serv. 2013; 96:94–8.
17. Vasudev V, Krishnamurthy NB. Toxicity of Dithane M-45 on *Drosophila melanogaster*. Experientia. 1979; 35(4):528–9. https://doi.org/10.1007/BF01922750
18. Delcour J. A rapid and efficient method of egg collecting. Dros Inf Serv. 1969; 44:133–4.
19. Vasudev V, Krishnamurthy NB. Effect of Dithane M-45 on the rate of development, viability, morphology and fecundity in *Drosophila melanogaster*. J Mysore Univ. 1982; 29:79–86.
20. Sorsa M, Pfeifer S. Response of puffing pattern to in vivo treatments with organomercurials in *Drosophila melanogaster*. Hereditas. 1973; 74(1):89–102. PMid: 4202374. https://doi.org/10.1111/j.1601-5223.1973.tb01107.x

21. Vasudev V, Krishnamutrhy NB. Preliminary studies on the effect of cadmium chloride on. *D. melanogaster*. Dros Inf Serv.1981; 56:153–4.
22. Vasudev V, Krishnamurthy NB. Utilization of *Drosophila* to understand the toxicity and mutagenicity of Baygon. Environ Pollut Resources Land Water. 1991; 205–13.
23. Bastock M, Manning A. The courtship of *Drosophila melanogaster*. Behaviour. 1955; 1:85–111. https://doi.org/10.1163/156853955X00184
24. Spieth HT. Courtship behavior in *Drosophila*. Annu Rev Entomol. 1974; 19(1):385–405. PMid: 4205689. https://doi.org/10.1146/annurev.en.19.010174.002125
25. Nazari M, Hegde SN. Effect of fluoxetine on the courtship latency, mating latency and copulation duration of *Drosophila melanogaster*. J Postgrad Med Inst. 2006; 20(1):58–63.
26. Nazari M. Effect of fluoxetine on the sexual behaviour of *Drosophila melanogaster*. J Postgrad Med Inst. 2011; 25(4):298–303.
27. Roy SS, Ghosh S. Effects of fruit ripening Retardant Alar (Daminozide) on behaviour of *Drosophila melanogaster*. Proc Zool Soc. 2019; 73(3):296–301. https://doi.org/10.1007/s12595-019-00316-3
28. Eastwood L, Burnet B. Courtship latency in male *Drosophila melanogaster*. Behav Genet. 1977; 7(5):359–72. PMid: 411472. https://doi.org/10.1007/BF01077449
29. Hegde SN, Krishna MS. Size-assortative mating in *Drosophila malerkotliana*. Anim Behav. 1997; 54(2):419–26. PMid: 9268474. https://doi.org/10.1006/anbe.1996.0485
30. Markow TA. Reproductive behavior of *Drosophila melanogaster* and *D. nigrospiracula* in the field and in the laboratory. J Comp Psychol. 1988; 102(2):169–73. PMid: 3135147. https://doi.org/10.1037/0735-7036.102.2.169
31. Ejima A, Griffith LC. Measurement of courtship behavior in *Drosophila melanogaster*. Cold Spring Harb Protoc. 2007; 1:10. PMid: 21356948. https://doi.org/10.1101/pdb.prot4847
32. Markow TA, Quaid M, Kerr S. Male mating experience and competitive courtship success in *Drosophila melanogaster*. Nature. 1978; 276(5690):821–2. https://doi.org/10.1038/276821a0
33. Hegde SN, Krishnamurthy NB. Studies on mating behaviour in the *Drosophila bipectinata* complex. Australian Journal of Zoology. 1979; 27(3):421–31. https://doi.org/10.1071/ZO9790421
34. Spieth HT. Evolutionary implications of sexual behavior in *Drosophila*. Evolutionary Biology. Boston, MA: Springer; 1968; 157–93. https://doi.org/10.1007/978-1-4684-8094-8_4
35. Spiess EB. Mating propensity and its genetic basis in *Drosophila*. Essays in Evolution and genetics in honor of Theodosius Dobzhansky. Boston, MA: Springer; 1970; 315–79. https://doi.org/10.1007/978-1-4615-9585-4_12
36. Guruprasad BR, Hegde SN, Krishna MS. Positive relation between male size and remating success in some populations of *Drosophila bipectinata*. Zool Stud. 2008; 47(1):75. http://zoolstud.sinica.edu.tw/Journals/47.1/75.pdf
37. Roy SS, Begum M, Ghosh S. Exploration of teratogenic and genotoxic effects of fruit ripening retardant Alar (Daminozide) on model organism *Drosophila melanogaster*. Interdiscip Toxicol. 2018; 11(1):27–37. PMid: 30181710 PMCid: PMC6117819. https://doi.org/10.2478/intox-2018-0004
38. Spieth HT, Ringo JM. Mating behavior and sexual isolation in *Drosophila*. The genetics and biology of Drosophila. 1983; 3:223–84.
39. Spieth HT. Courtship patterns and evolution of the *Drosophila adiastola* and *planitibia* species subgroups. Evolution. 1978; 1:435–51. PMid: 28563728. https://doi.org/10.1111/j.1558-5646.1978.tb00658.x
40. Scott D, Richmond RC, Carlson DA. Pheromones exchanged during mating: A mechanism for mate assessment in *Drosophila*. Anim Behav. 1988; 36(4):1164–73. https://doi.org/10.1016/S0003-3472(88)80075-7
41. Tompkins L, Gross AC, Hall JC, Gailey DA, Siegel RW. The role of female movement in the sexual behavior of *Drosophila melanogaster*. Behav Genet. 1982; 12(3):295–307. PMid: 6812562. https://doi.org/10.1007/BF01067849
42. Taylor CE, Kekic V. Sexual selection in a natural population of *Drosophila melanogaster*. Evolution. 1988; 42(1):197–9. PMid: 28563853. https://doi.org/10.2307/2409128
43. Markow TA, Bustoz D, Pitnick S. Sexual selection and a secondary sexual character in two *Drosophila* species. Anim Behav. 1996; 52(4):759–66. https://doi.org/10.1006/anbe.1996.0220