Original Article

Toxicological Evaluation of a New Lepidopteran Insecticide, Flubendiamide, in Non-Target *Drosophila melanogaster* Meigen (Diptera: Drosophilidae)

*Saurabh Sarkar[1], Prem Rajak[2], Sumedha Roy[*3]*

Received: 25.10.2017 *Accepted: 11.12.2017*

ABSTRACT

Background: Flubendiamide, comparatively a new pesticide designed to eradicate lepidopteran insect pests is known to have low risk to birds, mammals, fish, algae, honey bees, non-target arthropods, earthworms, soil macro- and micro-organisms, non-target plants as well as sewage treatment organisms; however, the risk assessment for aquatic invertebrates from metabolite could not be finalized with available data.

Methods: Different concentrations of flubendiamide (TATA TAKUMI®, Rallis, India) were introduced to larvae, pupae, and adult flies. A wide range of comparatively higher concentrations was selected for acute LC_{50} than chronic LC_{50} due to their exposure duration. Furthermore, relatively lower concentrations were introduced to larvae for assessment of emergence.

Results: At chronic exposure, the effect-concentration relationship exhibited a linear response when adult emergence was considered in *Drosophila melanogaster*. When acute LC_{50} of flubendiamide in 3rd instar larvae was compared with the chronic LC_{50} then it was seen to be approximately 21 fold higher whereas chronic LC_{50} for adult flies was nearly 19 times less than the adult acute LC_{50}. Similarly, adult emergence was seen to lower by 91.95% at 1500 µg/mL concentration. The chronic LC_{50} of the flubendiamide in *Drosophila* was approximately 170303 times more than the reported No Observed Effect Concentration (NOEC).

Conclusion: Hence, the chemical, flubendiamide can induce its effects at very low concentration, far below the lethal ones. Thus, the study is of relevance for the non-target insects as well as the insect dependent organisms.

Keywords: *Drosophila Melanogaster*, Emergence, Flubendiamide, LC_{50}.

IJT 2018 (3): 45-50

INTRODUCTION

Lethal concentration 50 or LC_{50} is the concentration of any chemical substance, which on application causes 50% death of individuals in a population. Evaluation of LC_{50} is the pioneer step in toxicological assessment of any chemical. It helps to select sub-lethal concentrations to carry out several toxicity tests. Therefore, this knowledge is essential for exploring impacts of any chemical on physical and physiological status of exposed organisms. The present study was focused on a newly formulated lepidopteran insecticide flubendiamide [1], vastly used in India in rice and cotton fields [2]. Additionally, other fruiting vegetables, grapes, and tobacco are also known to be benefitted from it. The maximum residual levels of flubendiamide and its metabolites are 0.2 and 1 mg/kg weight in rice and cotton respectively. According to European Food Safety Authority (EFSA) [3], the acceptable/ allowable daily intake value of flubendiamide has been reported around 0.017 mg/kg body weight.

Drosophila melanogaster (common fruit fly), a well-known dipteran model organism was chosen for this study based on its short lifespan, ease in experimental manipulations and conserved genetic and developmental mechanisms [4, 5]. Furthermore, United States Environment Protection Agency (US EPA), National Toxicology Program (NTP) and National Institute of Health Chemical Genomics Center (NCGC) jointly approved fly based experiments in modern toxicological studies [6]. Several authors [7-12] across the globe advocated the use of *Drosophila* for toxicological assessments of various chemicals.

The present study was carried out to determine the acute (24, 48, 72h) as well as chronic LC_{50} values of flubendiamide in larvae, pupae and adult flies of *D. melanogaster*. Further extension of the study was done using much lower concentrations (below LC_{50} value) of the test chemical to search its

1. Department of Zoology, The University of Burdwan, Burdwan, India.
2. Department of Zoology, ABN Seal College, Cooch Behar, India.
3. Department of Zoology, the University of Burdwan, Burdwan, India.
*Corresponding Author: E-mail: roysumedha@gmail.com

sub-lethal effects if any, on adult emergence and fecundity of fruit flies.

MATERIAL AND METHODS

Model Organism

D. melanogaster Oregon R strain was selected for the present study as model organism. Flies were cultured in environmental test chamber at 22±1 ºC and 65% relative humidity. They were allowed to feed on Standard Drosophila Medium (SDM) containing 3 qm agar-agar (Fisher Scientific, Mumbai, India), 17 qm corn meal (Victoria Foods Private Limited, Delhi, India), 15 qm sucrose (Fisher Scientific) and 9 qm yeast (Merck Specialities Private Limited) in 360 mL distilled water [12]. One mL Propionic acid and 1 mg methyl paraben (Nipagin) in rectified spirit were added as preservative and fungicide.

LC_{50} Determination

LC_{50} determination was performed following the method [13] with some modifications. Different concentrations of flubendiamide (TATA TAKUMI®, Rallis, India) were made in distilled water, mixed with SDM and poured in Petri dishes (diameter - 9 cm) for feeding and maintenance of larvae, pupae, and adult flies. Triplicate sets of untreated larvae were maintained in a standard food medium as control. A wide range of comparatively higher concentrations was selected for acute LC_{50} than chronic LC_{50} due to longer exposure duration. Furthermore, relatively lower concentrations were introduced to larvae than adult because of lesser food intake of adults in comparison to larvae [14].

Acute LC_{50} Determination

For Larvae

Different concentrations of flubendiamide (5000, 7500, 10000 and 15000 µg/mL) were prepared in distilled water and mixed with SDM. Thirty early third instar larvae were released on each plate having different concentrations of the chemical. Triplicate plates of each category were maintained for comparison. Larvae were allowed to feed and number of deaths was recorded at 24, 48 and 72 h, respectively.

For Adult Flies

Four graded concentrations of flubediamide (100000, 200000, 300000 and 500000 µg/mL) were prepared in SDM. Petri plates in triplicate sets were maintained for each treatment category. Thirty newly emerged adult flies per Petri dish were allowed to be exposed to each treatment concentration and number of dead flies was counted at 24, 48 and 72 h.

Chronic LC_{50} Determination

For Larvae

In order to determine chronic LC_{50} of flubendiamide for larvae, four increasing concentrations viz. 250, 500, 1000 and 1500 µg/mL were selected and mixed with SDM. Overall, 30 first instar larvae per Petri dish were reared in each concentration represented in triplicate sets and number of pupae formation was recorded. Number of larvae failed to pupate was considered as dead larvae.

For Pupae

Chronic LC_{50} for pupae was determined by counting the pupae that failed to emerge out as adult flies.

For Adult Flies

To evaluate chronic LC_{50} of flubendiamide for adult flies, four different concentrations (10000, 25000, 50000 and 75000 µg/mL) were prepared in distilled water and mixed with food for treatment of adult flies. Thirty newly emerged adult flies were maintained in each concentration for 5 d and the numbers of dead flies were recorded. Triplicate sets of each treatment category were maintained for statistical analysis.

Effect on Emergence of Adult Flies (Chronic Exposure)

To investigate the effects of flubendiamide on adult emergence, four graded concentrations viz. 250, 500, 1000 and 1500 µg/mL were made in SDM and to each treatment category, thirty early first instar larvae were released and reared up to their emergence as adult flies. Number of emerging flies were counted and noted. Each treatment category was maintained in triplicate along with triplicate sets of control.

Statistical Analysis

Observed and expected mortality frequencies were considered and examined through Probit analysis [15] to determine the acute and chronic lethal concentration of flubendiamide for different developmental stages of *D. melanogaster*.

RESULTS

LC_{50} Determination and Acute LC_{50} Determination for Larvae

Mean percentage of larval mortality after an interval of 24 h was found as 32.22%±2.94%, 38.88%±2.94%, 42.22%±4.0%, 52.22%±2.94%, and 56.66%±1.93% for the treatment concentrations of 5000, 7500, 10000, 12500 and 15000 µg/mL respectively (Fig. 1a). An increase in percentage mortality was noted where the values were 34.44%±2.94%, 41.11%±2.94%, 48.89%±2.94%,

58.89%±2.94% and 64.44%±2.94% after exposure of 48 h (Fig. 1b). On completion of 72 h, further elevation in percentage of larval deaths was recorded as 47.78%±2.94%, 53.33%±1.93%, 60%±3.85%, 68.89%±4.0% and 77.78%±2.94% for consecutive treatment concentrations (Fig. 1c). Probit analysis of the data indicated acute LC_{50} values of flubendiamide for larvae as 12022.64, 8317.64 and 4897.79 µg/mL after 24, 48 and 72 h of exposure (Fig. 1a, b, c).

For Adult Flies

Adult flies of *D. melanogaster* showed mean mortality of 25.56%±4.84%, 33.33%±1.93%, 48.89%±2.94% and 60%±1.92% when exposed to four graded concentrations (100000, 200000, 300000 and 500000 µg/mL) of flubendiamide for a period of 24 h (Fig. 2a). Interestingly after exposure of 48 h, percentage mortality increased up to 33.33%±1.93%, 42.22%±2.94%, 55.56%±4.0% and 63.33%±1.93% respectively (Fig. 2b). Adult flies manifested highest percentage mortality was 44.44%±2.94%, 52.22%±1.11%, 60%±1.92% and 73.33%±1.93% respectively after an exposure of 72 h (Fig. 2c). Probit analysis of the data provided the values of acute LC_{50} of flubendiamide in adult flies as 338844.16, 257039.58 and 154881.66 µg/mL that demonstrated a time-dependent declining trend in lethality (Fig. 2a, b, c). Thus, greater exposure time corresponds to lesser concentration for effective mortality ((Fig.2d).

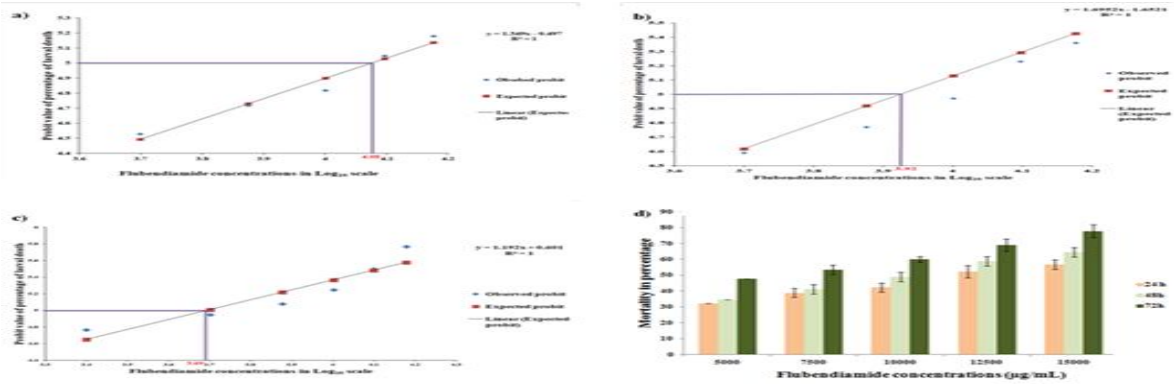


Figure 1. Probit analysis for evaluation of acute LC_{50} values of flubendiamide in larvae of *Drosophila melanogaster* at exposure periods of 24 h (a), 48h (b) and 72h (c) respectively. Scattered dots in the graphs represent observed and expected percentage of adult mortality. The dots representing the expected percentage are joined by a trend line. On Probit analysis, the concentrations representing 50% mortality values in Log_{10} scale were found to be 4.08, 3.92 and 3.69 for 3 exposure durations. Antilog of these figures provided LC_{50} values of flubendiamide as 12022.64, 8317.64 and 4897.79 µg/mL for 24 h, 48h and 72h durations respectively. Column graph (d) summaries concentration and time dependent larval mortality. Here data indicates Mean ± Standard error (SE) of triplicate observation for each treatment category.

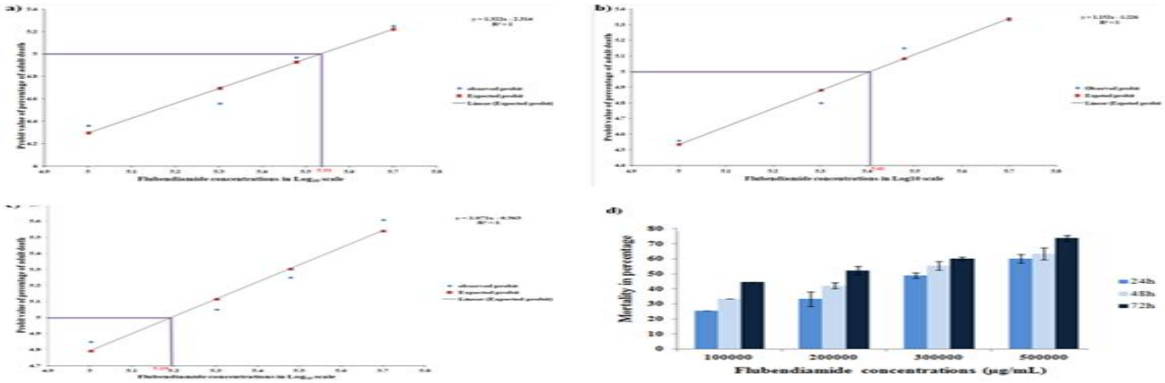


Figure 2. Probit analysis for evaluation of acute LC_{50} values of flubendiamide in adult flies of *Drosophila melanogaster* after exposure for 24 h (a), 48h (b) and 72h (c). Scattered dots in the graphs represent observed and expected percentage of adult mortality. The dots representing expected mortality are joined by a trend line and analysis of the values showing 50% mortality in Log_{10} scale were found to be 5.53, 5.41 and 5.19 for the three exposure periods. Antilog of these figures provided LC_{50} values as 338844.16, 257039.58 and 154881.66 µg/mL for 24 h, 48h and 72h respectively. Column graph (d) represents a comparative account of concentration and time dependent adult mortality. Data indicates Mean ± Standard error (SE) of triplicate observations from each treatment category.

Chronic LC_{50} Determination

For Larvae

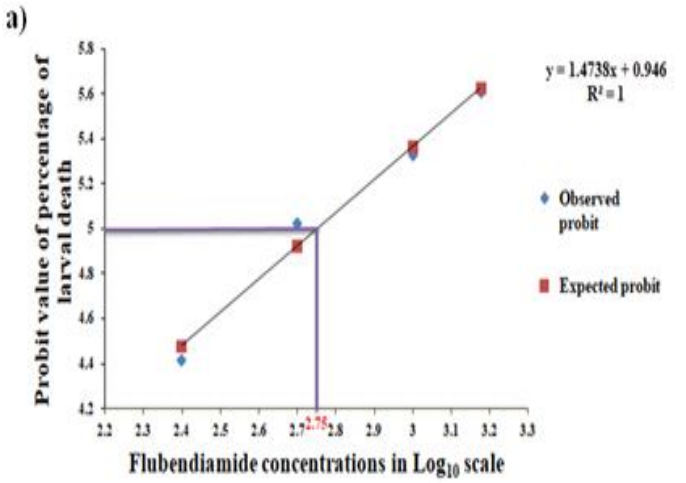
Failure in pupation is recorded as larval death. The mean mortality percentage in larvae was found as 27.78%±2.94%, 51.11%±2.94%, 63.33%±1.93% and 73.33%±1.93% following the treatment with 250, 500, 1000 and 1500 µg/mL flubendiamide (Fig. 3a). Probit analysis of the data indicated that chronic exposure to 562.34 µg/mL flubendiamide could cause 50% mortality hence it is the LC_{50} of the chemical in *Drosophila* larvae.

For Pupae

Pupae failed to emerge as adult was considered as dead ones. Therefore, number of dead pupae was counted and their mean mortality percentage was calculated as 18.46%±2.46%, 47.5%±7.47%, 63.64%±4.55% and 69.97%±6.47% respectively for the treatment concentrations of 250, 500, 1000 and 1500 µg/mL (Fig. 3b). Probit analysis presented the chronic LC_{50} of flubendiamide for pupae as 676.08 µg/mL

For Adult Flies

Freshly emerged adult flies maintained in 10000, 25000, 50000 and 75000 µg/mL of test chemical for a period of 5 d manifested concentration-dependent increase in mean percentage mortality that appeared to be 41.11%±2.94%, 53.33%±1.93%, 67.78%±2.94% and 82.22%±2.94% respectively (Fig. 3c). Thus, chronic LC_{50} of flubendiamide for adult *D. melanogaster* was found as 17782.79 µg/mL through Probit analysis.



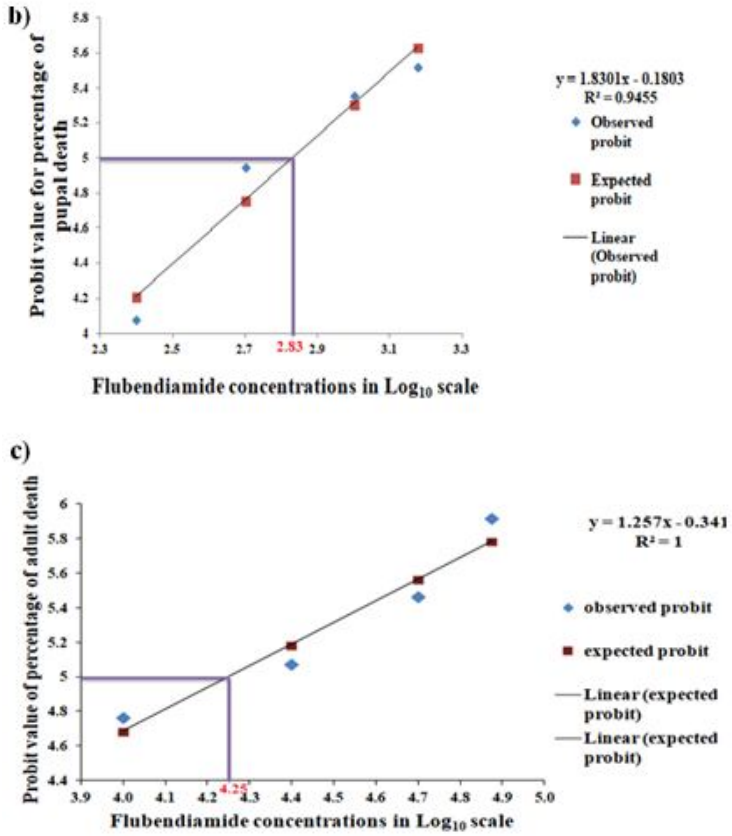


Figure 3. Probit analysis for estimation of chronic LC_{50} values of flubendiamide for three developmental stages (larva (a), pupa (b) and adult (c)) of *Drosophila melanogaster*. Scattered dots in the graphs represent observed and expected percentage of adult mortality. A trend line has been drawn joining the dots representing expected mortality. Analyzing data showing 50% mortality the LC_{50} values in Log_{10} scale were found to be 2.75, 2.83 and 4.25 for the 3 life stages. Antilog of figures provided LC_{50} values as 562.34, 676.08 and 17782.79 µg/mL for larvae, pupae and adults respectively.

Effect on Emergence of Adult Flies (Chronic Exposure)

In case of control sets, mean percentage of adult emergence was found to be 96.67±1.93 under laboratory conditions. A significant (P<0.05) reduction in emergence of adult flies was noted in case of individuals exposed to graded concentrations of flubendiamide. Individuals exposed to 250 and 500 µg/mL flubendiamide, showed adult emergence of 58.89%±2.94% and 23.33%±3.85% respectively. Interestingly, further reduction in emergence up to 13.33%±1.93% and 7.78%±1.11% was noticed in treatment categories receiving 1000 and 1500 µg/mL chemical exposure respectively (Fig. 4).

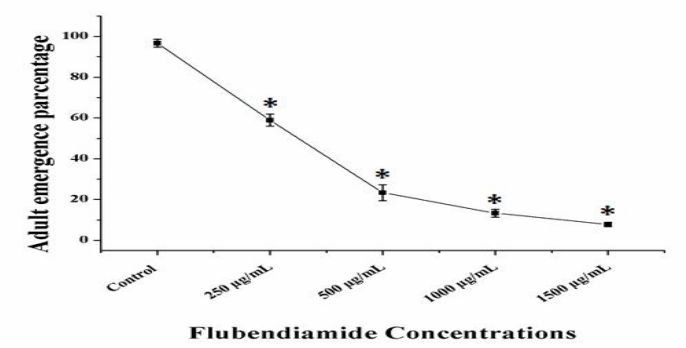


Figure 4. Variation in emergence pattern of adult flies of *Drosophila melanogaster* after exposure to different concentrations (250-1500 µg/mL) of flubendiamide at larval and pupal stages. Data represents Mean ± Standard error (SE) of three pooled determinations from triplicate sets. Statistical significance is ascribed as *P<0.05 after comparison with control.

DISCUSSION

Present study reveals acute as well as chronic LC_{50} values of flubendiamide for different developmental stages of *D. melanogaster* (Fig. 1a-d, Fig. 2a-d). The acute LC_{50} value of flubendiamide for adults was approximately 28 folds higher than the same for larval population (Fig 5). Similarly, the chronic LC_{50} value for adult flies was 32 folds greater than chronic LC_{50} value of larvae (Fig 5). A similar kind of result was also documented with another pesticide, namely imidacloprid [16]. Two plausible hypotheses can be forwarded regarding this outcome. First, one includes the voracious feeding behavior of larvae that led them to face higher amount of the test chemical leading to their death at much lower concentrations compared to adult individuals. Besides, topical diffusion of any pesticide is expected to be higher in larvae than the adults who have strong chitinous exoskeleton. These probable mechanisms, which discriminated larval and adult LC_{50} values, were earlier suggested [17].

The present study was further extended to determination of chronic LC_{50} for all the three life stages such as larval, pupal and adult forms of *D. melanogaster* (Fig. 3). In course of the determination of chronic LC_{50} values, the study also demonstrated results very close to the nature of findings on the effects of a fluoride chemical [13], where the chronic LC_{50} values for larvae, pupae, and adult flies were much lower compared to that of acute values (Fig 5). The reason behind such observation might be because higher concentrations of this chemical are essential to cause both physical and physiological damage in a short period of time which might result in death of the individuals. As the chronic LC_{50} values were found to be lower, hence these lower concentrations might have damaged the internal systems of the exposed organisms at a much slower rate and thereby taken comparatively longer period to cause lethality to 50% of exposed individuals.

Analogous to the finding [18], the present test chemical is also found to reduce percentage of adult emergence in concurrence with increase in treatment concentrations (Fig. 4). The most probable explanation behind this is that more of the exposed individuals succumb to increased tissue damage associated with impaired biochemical processes.

Thus, the present findings are suggestive of the fact that treatment with the test chemical in *D. melanogaster* causes probable sub-organismal changes that propagate towards organismal responses like reduced adult emergence with altered life cycle [19-22].

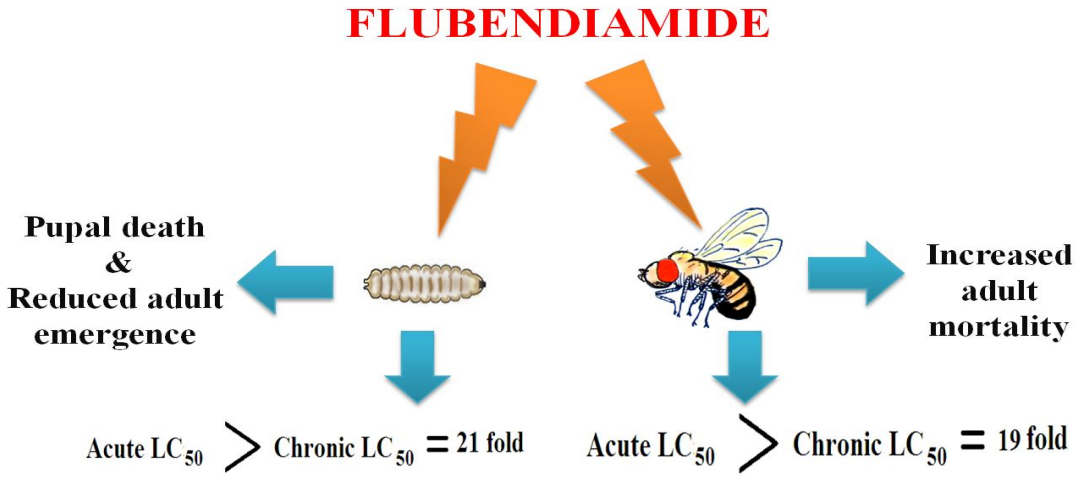


Figure 5. Graphical abstract, summarizing the entire finding of the present research work Acute LC_{50} values for both larvae and adult individuals were much higher than their chronic ones. Moreover, test chemical at sub-lethal concentration increased pupal and adult mortality in concurrence with declined adult exclusion.

CONCLUSION

Acute and chronic LC_{50} values of the chemical in the model organism provide and this would be very useful in further toxicological assessment of the chemical at molecular levels. Moreover, current findings on time- and concentration-dependent effects of the test chemical in *Drosophila* biology suggest that flubendiamide, which also appears to be an embryotoxic compound, can cause severe effects at concentrations far lower than the lethal ones. This is of serious concern regarding its effect on non-target insects and insect-dependent organisms.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, Department of Zoology (DST-FIST and UGC-DRS sponsored), The University of Burdwan, India. We also thank Prof. Abhijit Majumdar and Dr. Niladri Hazra, Department of Zoology, BU, for providing environmental test chamber facilities. This study is a part of Ph.D. thesis (Saurabh Sarkar).

The authors declare that there is no conflict of interests.

REFERENCES

1. Tohnishi M, Nakao H, Furuya T, Seo A, Kodama H, Tsubata K, et al. Flubendiamide, a novel insecticide highly active against *lepidopterous* insect pests. J Pestic Sci 2005;30(4):354-60.
2. Government of India, Ministry of Agriculture. Major use of Pesticides Registered under the Insecticides Act.1968.Available from: http://www.cibrc.nic.in/mupi.pdf.
3. European Food Safety Authority. Reasoned opinion of EFSA: Modification of the existing MRLs for flubendiamide in various food commodities; 2010.
4. Benford DJ, Hanley AB, Bottrill K, Oehlschlager S, Balls M, Branca F, et al. Biomarkers as predictive tools in toxicity testing. ATLA 2000;28(1):119-32.
5. Festing MF, Baumans V, Combes RD, Halder M, Hendriksen CF, Howard BR, et al. Reducing the use of laboratory animals in biomedical research: problems and possible solutions ATLA 1998;26:283-302.
6. Collins FS, Gray GM, Bucher JR. Transforming environmental health protection. Science (New York, NY) 2008;319(5865):906-7.
7. Das SK, Podder S, Roy S. Effect of Fungicide, Thiovit Jet on Several Life History Trait of *Drosophila melanogaster* (Diptera: Drosophilidae). J Appl Biol Sci 2010;4(3):31-6.
8. Rand MD. Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. Neurotoxicol Teratol 2010;32(1):74-83.
9. Kumar A, Nim R. Effect of bis-acrylamide on reproductive performance of *Drosophila melanogaster*. J Adv Dev Res 2012;3(1):104-7.
10. Gayathri DS, Harini BP. Effect of phenytoin on development and life-history traits of *Drosophila melanogaster*. Curr Sci 2013;104(4):508-514.
11. Rajak P, Dutta M, Roy S. Effect of acute exposure of acephate on hemocyte abundance in a non-target victim *Drosophila melanogaster*. Toxicol Environ Chem 2014;96(5):768-76.
12. Dutta M, Sarkar S, Roy S. Sodium fluoride induced alteration in lifecycle parameters and compound eye morphology of *Drosophila melanogaster* and trans-generational transmission of the altered eye architecture. JIARM 2014;2(8):247-59.
13. Podder S, Roy S. Exposure-dependent variation in cryolite induced lethality in the nontarget insect, *Drosophila melanogaster*. Interdiscip Toxicol 2014;7(1):17-22.
14. Jennings BH. *Drosophila*–a versatile model in biology & medicine. Mater Today 2011;14(5):190-5.
15. Finney DJ, Tattersfield F. Probit analysis: Cambridge University Press; Cambridge; 1952.p.318-9.
16. Frantzios G, Paptsiki K, Sidiropoulou B, Lazaridis I, Theophilidis G, Mavragani-Tsipidou P. Evaluation of insecticidal and genotoxic effects of imidacloprid and acetochlor in *Drosophila melanogaster*. J Appl Entomol 2008;132(7):583-90.
17. Charpentier G, Louat F, Bonmatin JM, Marchand PA, Vanier F, Locker D, Decoville M. Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model *Drosophila melanogaster*. Environ Sci Technol 2014;48:4096-102.
18. Rajak P, Sahana S, Roy S. Acephate-induced shortening of developmental duration and early adult emergence in a non-target insect *Drosophila melanogaster*. Toxicol Environ Chem 2013;95:1369-79.
19. Sarkar S, Dutta M, Roy S. Potential toxicity of flubendiamide in *Drosophila melanogaster* and associated structural alterations of its compound eye. Toxicol Environ Chem 2014;96(7):1075-87.
20. Sarkar S, Podder S, Roy S. Flubendiamide-induced HSP70 expression in transgenic *Drosophila melanogaster* (hsp70-lacZ). Curr Sci 2015;108(11):2044-50.
21. Sarkar S, Roy S. A mini review on heat shock proteins (HSPs): special emphasis on heat shock protein70 (HSP70). B N Seal Journal of Science 2017;9(1):130-9.
22. Sarkar S, Roy S. Monitoring the effects of a lepidopteran insecticide, Flubendiamide, on the biology of a non-target dipteran insect, *Drosophila melanogaster*. Environ Monit Assess 2017;189:557-8.