Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1504-1509 | ISSN:2251-6727

Evaluation of Resistance to the Organophosphate Chlorpyrifos Through LC_{50} and Life History Trait Analysis in Two Siblings: *Drosophila Melanogaster* and *Drosophila Simulans*

Srishti Sharma[1], Dr. Sujata Mohanty[1*]

[1] Department of Biotechnology, Jaypee Institute of Information Technology, Noida, U.P

***Corresponding author:** Prof. Sujata Mohanty

Professor, Department of Biotechnology, Jaypee Institute of Information Technology, Noida, U.P

(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

KEYWORDS

Insecticide-resistance; Chlorpyrifos; Neurotoxic; Non-target species; health risk.

ABSTRACT:

Introduction: Insecticide resistance and its management has become a major challenge in agriculture and medical health field. Chlorpyrifos (CP) known for its neurotoxic property to the target species, however, due to its extensive application it becomes the second most detected insecticide in food and water causing potential health risks to the non- targets.

Objectives: In the present study, resistance to chlorpyrifos was evaluated through lethal dose and various traits analysis in two non- target species i.e., *Drosophila melanogaster* and *Drosophila simulans*.

Methods: The LC_{50} was calculated initially after exposing the flies to six different concentrations of CP. Based on the mortality rate, two sub lethal concentrations of CP (0.3ppm and 0.5ppm) were used further to study the effects on fecundity, developmental stage duration and pupation height in F_1 and after a consistent exposure for 10 generations(F_{10}). The LC_{50} was calculated for F_{10} and compared with the F_0 generation. In all experiments, the results control/F_0, F_1 and F_{10} flies were compared to evaluate its adverse effect and resistance developed by the flies.

Results: The fecundity rate and pupation height were significantly reduced (~50-75%) on CP-exposure in F_1. The delayed development is observed in F_1 CP treated, i.e., egg to larva emergence, larval, pupation and pupation to adult duration, however, the larval stage was found to be most significantly affected. Similarly, the recovery rate of all stages was faster than the larval duration in F_{10} generation. The higher resistance factor (RF) ratio (F_{10} to F_0) observed in *D. melanogaster* CP-exposed flies evidenced its ability to develop faster resistance than *D. simulans*.

Conclusions: The findings will help to understand how important is to monitor insecticide-resistance for its management and regulation with a view to prevent health risk of other non-targets including human.

1. Introduction

Insecticide resistance and regular increase in doses of insecticides becomes a common practice for managing agricultural productivity and controlling disease-causing vector populations. A very recent report indicates about more than 500 insect and mite species developing resistance towards various insecticide classes, which is of global concern [1]. The inappropriate application of insecticides and lack of knowledge on developing insecticide-specific resistance restricts their effective uses, rather increase their residues in the environment leading to potential toxicity to living-beings [2,3]. Thus, it is crucial to maintain the insecticide-specific resistance and the mechanism behind for implementing necessary management strategy.

Resistance issues persisted with the introduction to modern insecticides such as organophosphates, carbamates, and pyrethroids [4]. Chlorpyrifos (CP) belongs to an organophosphate class of insecticides and has wide application both in agriculture (soyabean and fruits farming) and household purposes against a number of insect species (cockroaches, termites, fleas etc.) and it

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1504-1509 | ISSN:2251-6727

is neurotoxic in nature [5-8]. The first report on insecticide resistance was published more than 100 years ago for lime sulphur on *Quadraspidiotus perniciosus* (San Jose scale) [9,10]. According to Arthropod Pesticide Resistance Database, 2021 reported that the cotton leaf worm, *Spodoptera. littoralis* has developed resistance even to 31 chemicals, including organophosphorus, pyrethroid, and indoxacarb [11]. *Drosophila suzukii* is one of the most important invasive pests of soft- skinned fruits globally [12]. Although, *Drosophila melanogaster and Drosophila simulans* is not a significant crop pest or a prominent target for commercial insecticide applications but it is closely related species.

Therefore, it has been an ideal model for studying toxicity as well as resistance due to its similarity with target insect-pest species of agriculture and medical importance and a popular eukaryotic model [13]. In addition, its short lifespan allows us to access many generations in limited period of time in both field and laboratory condition [14].

2. Objective

The present study aims to understand the development of resistance against chlorpyrifos insecticide in two sibling species *D. melanogaster* and *D. simulans* using various life history traits as parameters.

3. Materials and Methods

Drosophila Strain

Two sibling species *D. melanogaster* (isofemale line DL 36) and *D. simulans* (isofemale line DL 42) collected from West Delhi, India in 2022 are used in this study. The flies are maintained on standard yeast-molasses media at 25°C, 60-70% relative humidity, and a 12-hour light/dark cycle in a BOD incubator.

Chlorpyrifos and Doses

Commercially available chlorpyrifos with 20% EC (emulsifiable concentrate) was used in the present study (Tractor Brand manufactured in RIICO Industrial area, Bhiwadi, Rajasthan). A stock solution of 1:1 ratio with acetone was prepared and used throughout. Initially the flies were exposed to different concentrations of CP (details provided in LC_{50} analysis) and based on their survivability, two sub-lethal concentrations of CP (0.3ppm and 0.5ppm) were selected for experimental purpose.

The *Drosophila* flies of both species were cultured and maintained in standard media and CP- treated food media with each sublethal concentrations up to 10 generations.

LC_{50} Analysis

4-day old 5 virgin males and females were exposed to six different concentrations (0.5ppm, 0.7ppm, 0.9ppm, 1ppm, 3ppm and 5ppm) of CP- treated media for a duration of 24, 48, and 72 hours and the number of dead flies were noted. Six replicates were performed for each concentration. The LC_{50} was calculated by taking flies mortality of all the concentrations as mentioned above through probit analysis method using the formula [15].

$$Log(c\%) = \frac{Test\ value - Intercept\ value}{Slope};$$

$$LC_{50} = 10\text{^}(Log\ (c\%)$$

To monitor resistance, the LC_{50} value was also calculated after CP- exposure (0.5ppm) of 10 generations. The resistance factor (RF) ratio between the susceptible (F_0 generation) and resistance (F_{10} generation) flies of both the species were calculated as [16]:

$$Resistance\ Factor\ (RF) = \frac{LC_{50}\ of\ resistance\ flies\ (F_{10})}{LC_{50} of susceptible\ \ flies\ (F_0)}$$

Traits for resistance study

Fecundity

Equal number of 20 virgin males and 20 virgin females of 4 day old were permitted to mate in vials for 24 hours. After that the flies were transported to a fecundity chamber for egg laying and the number of eggs laid in next 48 hours was recorded. The fecundity chamber is made up of glass which is closed by a cover and has dimensions of 13 x 13 x 6 cm. The same procedure was followed for each control and CP-treated (0.3ppm, 0.5ppm) for F_1 and F_{10} generation flies with two replicates.

Developmental Stages

The duration of each developmental stages of both species in each concentration of CP- treated and control from egg to 1st instar, 1st – 3rd instar, 3rd instar –pupa and

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1504-1509 | ISSN:2251-6727

pupa – adult (Table 1) was observed and recorded with six replicates. All the developmental stages were observed by seeing their first appearance from egg to adult. The egg to 1st instar and 1st – 3rd instar was observed by placing the plates under the stereomicroscope.

Pupation Height

The average pupation height of each species was calculated from 40 randomly chosen pupae in both control and CP- treated food vails in each concentration. The pupae were marked and their distance (in cm) from the food surface of vial was measured by placing ruler scale.

Statistical Analysis

The results of all life history traits were analysed individually for variance using the ANOVA single factor method (with $p < 0.05$*, $p < 0.01$**, and $p < 0.005$***) and for comparison multivariate analysis was done by two-way ANOVA factor with replication method [17]. The software Origin 2023b was used to generate the figures.

4. Results

Initially, the F_0 flies were acutely exposed to different concentrations of CP (see methodology) and the LC_{50} values after 24, 48 and 72hrs were found to be 0.77, 0.63 and 0.62 ppm in *D. melanogaster* and 0.80, 0.72 and 0.64 ppm in *D. simulans* species respectively. Two sub-lethal concentrations of CP (0.3ppm and 0.5ppm) were used to expose *Drosophila* for ten generations to study the initial harmful effects (F_1) and recovery (F_{10}) on various life-history traits, e.g., fecundity, development stages duration and pupation height. In each case an increased detrimental effect was observed with increased concentration of CP. The LC_{50} was calculated with exposed higher concentration of CP i.e., 0.5ppm after 10 generation which is further treated with five different concentrations (0.7ppm, 0.9ppm, 1ppm, 3ppm and 5ppm). The LC_{50} of 0.5ppm of CP in F_{10} generation was found to be 1.31ppm, 1.11ppm and 0.99ppm in *D. melanogaster* and 1.15ppm, 1.05ppm and 0.99ppm for *D. simulans* after exposure of 24, 48 and 72hrs respectively. The resistance factor (RF) ratio between the susceptible (F_0 generation) and resistance (F_{10} generation) flies of CP in *D. melanogaster* was found to be 1.70, 1.76 and 1.59 and for *D. simulans* it was 1.43, 1.45 and 1.54 respectively (Table 1).

The average number of eggs laid by 20 females of *D. melanogaster* was 252±8 in control, which was reduced to 102±6 (0.3 ppm) and 67±3 (0.5 ppm) in F_1 treated flies, but again increased to 169.5±8.5 (0.3 ppm) and119.5±9.5 (0.5 ppm) in F_{10} treated flies, which evidenced the development of its resistance to CP. Similar results were observed in case of *D. simulans* (Figure 1, Table 1). The egg laying rate in *D. simulans* was found to be significantly lower than in *D. melanogaster*, which coincides with literature [18, 19].

A significant delaying was marked in all four developmental stages duration, when F_1 and control group results were compared, but the larval duration (1st – 3rd instar) was found to be most affected. The larval duration was much slower than other developmental stages when F_1 with F_{10} generations of CP-treated flies were compared (Figure 2, Table 1).

The average distance which was travelled by pupae of *D. melanogaster* and *D. simulans* in control was 2.0±0.1 and 1.40±0.1. In *D. melanogaster* F_1 (CP- treated), it was reduced to 0.9±0.1 (0.3ppm) and 0.6±0.1 (0.5ppm) and in F_{10} again rises up to 1.7±0.1 and 1.4±0.1 in respective concentrations. The average height of *D. simulans* 0.3ppm CP- treated pupae in F_1 and F_{10} generation was 0.7±0.1 and 1.3±0.1 and with 0.5ppm it was 0.4±0 and 1.2±0.1 respectively (Figure 3, Table 1). The variation of all parameters used among control, F_1 and F_{10} were analysed for their statistical significance using ANOVA single factor method (Supplementary Table). Multivariate analysis was performed for fecundity, developmental stage durations and pupation height of control and CP-treated F_1 and F_{10} generations with the help of ANOVA two factor replication method. The p-value and F critical value showed significance difference between control v/s F_1 generation, F_1 v/s F_{10} generation. Comparatively less variation was observed between control v/s F_{10} generation (Table 1).

5. Discussion

Insecticide resistance is widely seen as a threat to vector-borne diseases in medical health sectors and for target pests in agricultural field. Extensive use of synthetic organic insecticides such as DDT, cyclodienes, and organophosphates (OPs), there was a rise in the number of incidences of insecticide resistance which demands

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1504-1509 | ISSN:2251-6727

dosage- increase to kill target species. Due to which, there is accumulation of insecticide- residues in environment impacting negatively to non-target organisms [20,21]. When it comes to insects that are crucial to agriculture, very little is known about resistance mechanisms and the reason behind [22]. It is generally known that resistance does not evolve at same rate in every species or population [23].

Organophosphates are neurotoxic and widely used in agriculture, however, only 0.1% of organophosphates reach their intended target as estimated [8, 24]. Chlorpyrifos, an organophosphate was first registered as an insecticide in the United States in 1965 [25].

The fruit fly *Drosophila* has been an important tool in studying insecticide resistance as it represents both target and non-target insect species. During this study, we have used two sibling species *D. melanogaster* and *D. simulans* to determine the effect of the sublethal concentration of CP on various life history traits, which infers how much the nontarget species are being affected. Furthermore, by studying its resistance, it can be deduced how quickly the target species can develop resistance against this insecticide, based on which its effective application might be regulated. After an exposure, it was revealed that CP has detrimental effect on fecundity (50-75% reduction) and development period (nearly two-fold from egg to adult) including pupation height (50-70% decrease), whereas after 10 generations (nearly six months' time period), the flies were developed resistance and the effects becomes minimal, even though the recovery rate was not reverting back completely to normal in any parameters studied. The effect of CP was found to be more in larval time period of *Drosophila* life cycle. The reason behind is poorly understood as it is not clear how, the neural-targeted organophosphate CP regulates the growth hormone ecdysone, responsible for development period such as molting and metamorphosis. Even in female *Drosophila* and other insects, this hormone signalling is involved with oogenesis regulation, which is also reflecting in fecundity of present study [26].

In F_0 generation *D.simulans* flies were shown to be more susceptible than *D. melanogaster* in CP-exposed flies. After subsequent exposure of F_{10} generations, the LC_{50} value was found to be higher as compared to F_0, which confirms their resistance in both the species. Earlier reports also say about the slowed development (by 6 days) and a reduction in the growth of *Brachionus koreanus* population on chlorpyrifos exposure [27], but no effect was observed in the growth of springtail *Folsomia candida*, even at a higher concentration that had a significant impact on survival and reproduction [28]. Exposure to 4 g/l chlorpyrifos retarded male maturation, lowered fecundity, and impaired in the *Nothobranchius furzeri* fish [29].

Pupation height in F_1 progeny decreased significantly (from 50% to 70%) in treated *D. melanogaster* and *D. simulans* groups compared to controls, which could be attributed to the larva's slow mobility. The change in pupation height may affect the niche-sharing among closely related species and coevolution [30]. To achieve a healthy toxic-free environment, the substitute for synthetic insecticides (Biopesticides), insecticide-degrading bacterial strains or smart strategy of insecticide application (through rotation of different insecticides) may be explored and implemented [31,32]. However, finding species-specific resistance against commonly used insecticides is crucial for effective implementation of management and regulation of insecticide-use.

6. Conclusion

The negative impacts caused by insecticides on non-targets enforce us to rethink on food security as well as a natural balanced ecosystem. It is indispensable to identify and understand the target species and their biology before applying the insecticides, which may prevent non-targets especially the beneficial insects from their harmful effects and the target species from developing resistance. The recent findings show that pesticide resistance is widespread and this problem is likely to worsen unless a robust resistance management plan is implemented. Apart from regulating and restricting the use of insecticides, it may be worthwhile to investigate alternative pest management strategies such as biopesticides and biocontrol agents.

Acknowledgements

The authors thank the Vice Chancellor, JIIT for providing infra-structure facilities for conducting this work.

Conflict of Interests

The author declares there is no competing of interests.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1504-1509 | ISSN:2251-6727

References

1. Siddiqui J.A., Fan R., Naz H., Bamisile B.S., Hafeez M., Ghani M.I., Chen X., 2023. Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. Front Physiol. 13, 1112278.
2. Brogdon W.G., McAllister J.C., 1998. Insecticide resistance and vector control. Emerg Infect Dis. 4(4), 605.
3. Khan M.A., Khan Z., Ahmad W., Paul B., Paul S., Aggarwal C., Akhtar M.S., 2015. Insect pest resistance: an alternative approach for crop protection. Crop production and global environmental issues, 257-282.
4. Hemingway J., Ranson H., 2000. Insecticide resistance in insect vectors of human disease. Annu Review Entomol. 45(1), 371-391.
5. Timchalk C., Nolan R.J., Mendrala A.L., Dittenber D.A., Brzak K.A., Mattsson J.L., 2002. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicol Sci*. *66*(1), 34-53.
6. Ware G.W., Whitacre D.M., 2004. An introduction to insecticides. *The pesticide book, 6.*
7. Hites R.A., 2021. The rise and fall of chlorpyrifos in the United States. *Environ Sci Technol*. *55*(3), 1354-1358.
8. Tefera Y.M., Gaskin S., Thredgold L., Pisaniello D., 2022. The role of formulation co-ingredients in skin and glove barrier protection against organophosphate insecticides. *Pest Manag Sci*. *78*(1), 177-183.
9. Forgash A.J., 1984. History, evolution, and consequences of insecticide resistance. *Pestic Biochem Phys*. *22*(2), 178-186.
10. Sparks T.C., Nauen R., 2015. IRAC: Mode of action classification and insecticide resistance management. *Pestic Biochem Physiol*. *121*, 122-128.
11. Fouad E.A., Ahmed F.S., Moustafa M.A., 2022. Monitoring and biochemical impact of insecticides resistance on field populations of Spodoptera littoralis (Boisd) (Lepidoptera: Noctuidae) in Egypt. *Pol J Entomol*. *91*(3), 109-118.
12. Disi J.O., Sial A.A., 2021. Laboratory selection and assessment of resistance risk in Drosophila suzukii (Diptera: Drosophilidae) to spinosad and malathion. *Insects*. *12*(9), 794.
13. Khanna R., Mohanty S., 2017. Whole genome sequence resource of Indian Zaprionus indianus. *Mol Ecol Resour*. *17*(3), 557-564.
14. Ogienko A.A., Omelina E.S., Bylino O.V., Batin M.A., Georgiev P.G., Pindyurin A.V., 2022. Drosophila as a Model Organism to Study Basic Mechanisms of Longevity. *Int J Mol Sci. 23*(19), 11244.
15. Kubitz J.A., Goodrum P., Bohrmann T., 2017. The dose-response hill slope used in the aquatic toxicity module of oil spill models should be much steeper. *Int oil spill Conf Proc*. (1), 940-958.
16. Kranthi K.R., Jadhav D.R., Kranthi S., Wanjari R.R., Ali S.S., Russell D.A., 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Prot. 21*(6), 449-460.
17. Sokal R.R., Rohlf F.J. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd Ed., WH Freeman and Co. *New York*, 1995.
18. Barker J.S.F., 1973. Adult population density, fecundity and productivity in *Drosophila melanogaster* and *Drosophila simulans*. *Oecologia*. *11*, 83-92.
19. David J.R., Allemand R., Capy P., Chakir M., Gibert P., Petavy G., Moreteau B. Comparative life histories and ecophysiology of *Drosophila melanogaster* and *D. simulans*, *Contemporary Issues in Genetics and Evolution*, 2004. pp. 151-163.
20. Riyaz M., Shah R.A., Sivasankaran K. Pesticide residues: impacts on fauna and the environment. *Biodegradation technology of organic and inorganic pollutants*, 2021.
21. Sparks T.C., Storer N., Porter A., Slater R., Nauen R., 2021. Insecticide resistance management and industry: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. *Pest Manag Sci*. *77*(6), 2609-2619.
22. Van Leeuwen T., Dermauw W., Mavridis K., Vontas J., 2020. Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests. *Curr Opin Insect Sci*. *39*, 69-76.
23. Sarwar M., Salman M., 2015. Insecticides resistance in insect pests or vectors and development of novel

strategies to combat its evolution. *IJBBE*. *1*(3), 344-351.

24. Neylon J., Fuller J.N., Van der Poel C., Church J.E., Dworkin S., 2022. Organophosphate insecticide toxicity in neural development, cognition, behaviour and degeneration: insights from Zebrafish. *J Dev Biol*. *10*(4), 49.
25. John E.M., Shaike J.M., 2015. Chlorpyrifos: pollution and remediation. *Environ Chem Lett*, *13*, 269-291.
26. Ishimoto H., Sakai T., Kitamoto T., 2009. Ecdysone signaling regulates the formation of long-term courtship memory in adult *Drosophila melanogaster*. *Proc Natl Acad Sci*. *106*(15), 6381-6386.
27. Kim R.O., Kim B.M., Jeong C.B., Lee J.S., Rhee J.S., 2016. Effects of chlorpyrifos on life cycle parameters, cytochrome P450S expression, and antioxidant systems in the monogonont rotifer *Brachionus koreanus*. *Environ Toxicol Chem*. *35*(6), 1449-1457.
28. Jager T., Crommentuijn T., Van Gestel C.A., Kooijman S.A., 2007. Chronic exposure to chlorpyrifos reveals two modes of action in the springtail *Folsomia candida*. *Environ Pollut*. *145*(2), 452-458.
29. Philippe C., Thoré E.S., Verbesselt S., Grégoir A.F., Brendonck L., Pinceel T., 2022. Combined effects of global warming and chlorpyrifos exposure on the annual fish *Nothobranchius furzeri*. *Ecotox Environ Saf*. *248*, 114290.
30. Polechova J., Storch D., 2008. Ecological niche. *Encycl Ecol*. *2*, 1088-1097.
31. Chowanski S., Kudlewska M., Marciniak P., Rosiński G., 2014. Synthetic Insecticides--is There an Alternative?. *Pol J Environ Stud*, *23*(2).
32. Mali H., Shah C., Raghunandan B.H., Prajapati A.S., Patel D.H., Trivedi U., Subramanian R.B., 2023. Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. *J Environ Sci*. *127*, 234-250.