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Research Paper

Investigation of the Effects of ChlorpyrifosToxicity on Movement Physiology in *Drosophila melanogaster*

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Abstract: Today, the increase in the use of pesticides, which are highly resistant to environmental conditions, gains importance by directly or indirectly affecting human and environmental health. Chlorpyrifos, a broad spectrum organophosphate insecticide with neurotoxic effect; it causes DNA damage in livingorganisms by taking it to the organism through water and food, and toxicity by providing the formation of reactive oxygen species. Organophosphorus insecticides, which are effective on the nervous system, cause changes in features such as memory, movement, climbing and courtship. For this purpose, the change in movement according to climbing performance was determined after 2 and 24 h of feeding with Chlorpyrifos (0.015–15 µg/l) added to the adult model organism (*Drosophila melanogaster* Meigen) diet. It was determined that climbing performance increased in female and male individuals in direct proportion to the amount of Chlorpyrifos added to the diet with short-term exposure. The increase in the exposure time to Chlorpyrifos causes the movements to slow down by half compared to the short-term exposure, while the movements increase compared to the control, and this change in the movement is due to the resistance of the insect.

Keywords: Environmental pollutant, Chlorpyrifos, *Drosophila melanogaster*, climbing, sex, exposure time.

Introduction

To ensure the nutritional needs of the growing population in cities, pesticides are used in plant production, against pests seen in buildings or other environments. These products, which are used unconsciously and intensively; As well as the food sector, it affects many structures of the city such as water, air and soil, causing negative effects on the environment and human health. Pesticides used in plant production to kill, control or reduce the damage of insects that will harm the crop are known as chemicals and compounds (Özdemir & Kurt, 2021). Excessive pesticide use in developing and some developed countries affect living things in the food chain by creating residues both directly and indirectly, which cause water and air pollution (Eyhorn *et al*., 2015; Amaliah *et al.,* 2019).

Organophosphates are compounds that make up more than half of the insecticide use and have a special importance due to their chemical structure (Yang *et al*., 2020). Chlorpyrifos, an agricultural and nonagricultural broad spectrum chlorinated organophosphate insecticide; It is classified as a moderately toxic substance by the World Health Organization (Özdemir & Kurt, 2021). LD_{50} value is 82–270 mg/kg; It has a sharp smell, colorless and white crystalline powder structure (Kurt *et al*., 2021). Although its solubility in water is low (0.39–2 mg/L); It is easily soluble in corn oil, benzene, dimethyl sulfoxide, methanol, acetone, xylene, methylene chloride (Smith *et al*., 2009; Ur Rahman *et al.,* 2021). Due to its low cost and high impact, it is widely used in agriculture, forest and domestic pests in many countries; It causes the death of beneficial microorganisms and environmental pollution, threatening human and animal health (Özdemir & Kurt, 2021). With pesticide exposure; It is thought that Parkinson's, Alzheimer's, some types of cancer, impaired immune system, diabetes, obesity, autoimmune diseases, hormone imbalances, reproductive problems, food allergy risk, behavioral effects and birth defects in children and cognitive development, attention deficit/hyperactivity in young people may be related (Pukkala et al., 2009; Bouchard et al., 2010; Weichenthal et al., 2010; Bouchard *et al*., 2011; Mnif *et al*., 2011; Ismail et al., 2012; Jerschow et al., 2012; Van Maele -Fabry et al., 2012; Corsini et al., 2013; Ross et al., 2013; Ntzani *et al*., 2013; Zaganas *et al*., 2013; Leu 2014; Mandrich 2014; Allsop, 2015).

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Chlorpyrifos connects to the serine amino acid in the active site of AChE enzyme by phosphate bonds and inhibits it irreversibly (Goel *et al.*, 2007). AChE is a neurotransmitter responsible for signal transmission from neurons to skeletal and smooth muscles, glands, autonomic and central nervous system (Robey & Meggs, 2004; Jortner, 2008). Chlorpyrifos; crossing the blood-brain barrier, it causes AChE inhibition at cholinergic synapses in the brain, thanks to its oxon metabolites (Timchalk, 2007). This event causes accumulation of acetylcholine in the autonomic and central nervous systems, overstimulation of presynaptic, nicotinic and muscarinic receptors, the contraction of smooth muscles, high secretion from glands, and ultimately cholinergic synaptic transmission paralysis. This situation, which is described as a cholinergic syndrome or cholinergic crisis, negatively affects the movement physiology of living things (Robey & Meggs, 2004; Goel *et al.,* 2007). Chlorpyrifos in animals in the prenatal period; It has been reported that it stops DNA synthesis, leads to deterioration in DNA replication and protein synthesis, oxidative stress, free radical formation, disruption in neuronal calcium metabolism, apoptosis, and a decrease in the number of neurons in brain regions where cholinergic stimulation is high (Myers & Davidson, 2000; Özmert, 2005). Exposure to repeat doses in the prenatal and early postnatal period; It has been stated that a decrease in acetylcholinesterase activity causes learning, movement and balance difficulties (Slotkin, 2005).

Although it is necessary to test the toxic effects of pesticides in vivo in mammalian experimental animals in detail, it is impossible to test every pesticide. For this reason, cheap and fast alternative screening methods are used to accurately detect the possible effects of a chemical. For this purpose, some non-mammalian model animal species and in vivo test systems can use in studies. *Drosophila melanogaster* (Do Amaral *et al*., 2005; Gomes *et al.,* 2020; Zamberlan et al., 2020), a model organism used to evaluate the potential of pollutants, can easily change its behavioral response depending on the pollutant present in the environment. The climbing test, which is one of the in vivo test systems, allows the determination of many neurodegenerative diseases in model animals (Triphan e*t al.,* 2010; Madabattula *et al*., 2015). By using the information obtained from model creatures, inferences are made about the possible effects on mammals.

In the study; it was evaluated the effect of experimentally induced Chlorpyrifostoxicity on the movement physiology of *D. melanogaster*.

Materials and Methods

Chlorpyrifos has been bought commercially in 2012. *D. melanogaster* (Oregon) has been cultivated in the laboratory under standard conditions (25°C, 60% humidity, and 12 h light/dark) and artificial diet since 2014 (Güneş and Büyükgüzel, 2017). Based on previous studies (Gupta *et al*., 2010; Güneş *et al*., 2021), chlorpyrifos was prepared by dissolving in corn oil; Climbing groups were formed by adding 0.015–15 µg/l to the insect food. The first group is the negative control group and consists of individuals fed with standard food (SN), while the second group is the solvent control group with dimethylsulfoxide (DMSO, 0.3%) (Table 1). In climbing experiments, 5 unmated daily 5 female/5 male individuals were taken for each group and aged within 2 h and one day with the experimental groups, then after 2 h of fasting, according to the method described in the study of Güneş (2021) climbing performance was calculated (Grover et al., 2009; Güneş, 2021; Güneş and Olcay, 2021). Thus, the insect's movement score/number of individuals according to the environment was calculated.

In the evaluation of the data; One-Way Analysis of Variance (ANOVA) was used to determine the within-group variation, and the "LSD Test" was used to determine the significance of the difference between the means. The significance of the means were evaluated at the 0.05 probability level. The experiments were repeated twice, and the mean and standard deviation was calculated.

Table 1. Experimental design for insect exposure to Chlorpyrifos

Experimental Groups	Groups	The material addition in meal
Control groups	1. group	SN
	2. group	Solvent (DMSO)
Toxicity groups	3. group	0.015 µg/l Chlorpyrifos
	4. group	0.15 µg/l Chlorpyrifos
	5. group	1.5 µg/l Chlorpyrifos
	6. group	15 µg/l Chlorpyrifos

Results and Discussion

Chlorpyrifos, which is widely used for various purposes today; damage to invertebrates living in water and soil, environmental impact and residue generation are of concern for biota. Chlorpyrifos are a neurotoxic substance (Slotkin, 2006), as well as inducing diseases such as Parkinson's in individuals (Deveci & Karapehlivan, 2018); It is necessary to understand the amount and the effect of its use not only for humans but also for other living things. In the study, which is based on the investigation of the effects of nervous stimuli and diseases on the ability to move, changes in the movements of female/male individuals in the 2-hour and 1-day experimental groups were determined. To this situation; It is thought that motor behaviors such as climbing, direction finding, learning, memory, addiction and aggression are reflected in the movements of the fly in relation to many factors such as nutrition, nutrient content, age and gender (Güneş, 2021). Because of the increased stress on the flight muscles of the insect, depending on the environment or nervously, aggression and climbing behavior may increase (Güneş & Olcay, 2021), and movement may decrease due to toxicity. While the nervous systems of male and female insects cause different movements on food due to both flight and courtship-reproduction, the observation of these movements is also used effectively in Parkinson's models (Shaltiel-Karyo *et al*., 2012). In the study, it was determined that female individuals were a little more sedentary than male individuals (Figure 1. A and B). Although this result is associated with more locomotor disorders in males (Baker *et al.,* 2001; Gomes et *al*., 2020), it is inevitable that males are athletic, and those male movements are high due to mating and courtship.

When female individuals first encountered increased Chlorpyrifos, it was determined that their movements were statistically significantly increased depending on the dose (Figure 1. A; $p<0.05$). However, it is thought that after being fed with Chlorpyrifos at the same rates for one day; the movement increased up to three times compared to the control, and the decrease in movement compared to 2 h decreased the aggressive behavior due to the resistance developed against increased stress (Gupta *et al*., 2010). Parkinson's-like aggressive behavior is also associated with fruit flies' ability to taste, smell, and hear (Versteven *et al*., 2017). Climbing decreases in flies that get used to the substance in the environment and start producing antioxidant enzymes. It can be said that the effect seen in females is similar in terms of chlorpyrifos and food adaptation in movement experiments with male individuals (Figure 1. B; $p<0.05$). The fact that there is a direct proportionality in movement in males and females supports studies in terms of courtship and continuation of generation (Manoli and Baker, 2004; Manoli, 2005; Shaltiel-Karyo *et al*., 2012; Akpa et al., 2021). The increase in climbing performance observed in both genders due to increased stress has led us to think that environmental pesticide exposure may have a similar effect in terms of diseases. Although flies show resistance to stress, the occurrence of antioxidant enzyme deficiency; it suppresses vitality, the ability to move accelerates but becomes irregular and the mortality rate increases (Fernandez-Ayala *et al*., 2009).

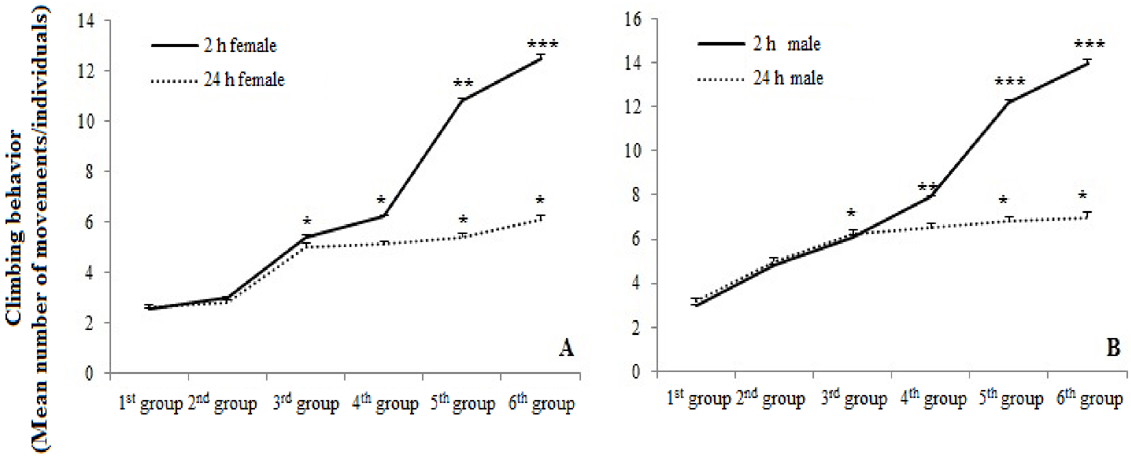

Figure 1. Climbing variability between groups of female (A) and male (B) fly exposed to chlorpyrifos (C). Statistically, The difference between the groups after 2 and 24 h of exposure of male and female individuals was interpreted. *differ from**, ** differ than *** ($p < 0.05$)

Conclusion

As a result, it was determined that the negative effect induced by Chlorpyrifos on *Drosophila* females and males was reflected in the action, and that hyperactivity may continue to increase somewhat depending on exposure to the active substance. For this reason, it has been supported that to prevent/minimize the damage to humans and the environment in the use of pesticides, it is necessary to pay attention to both their agricultural and domestic use.

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