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Life Table Evaluation of Synthetic and Botanical Insecticides on *Drosophila melanogaster*: Comparative Effects of Chlorpyrifos, Cypermethrin, and *Azadirachta indica* Leaf Extract

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ABSTRACT
The effects of chlorpyrifos, cypermethrin, and *Azadirachta indica* leaf extract on *Drosophila melanogaster* have been quantified in the present study "Life Table Evaluation of Synthetic and Botanical Insecticides on *Drosophila melanogaster*: Comparative Effects of Chlorpyrifos, Cypermethrin, and *Azadirachta indica* leaf extract," which also provides insightful information of their impact on the life stages and mortality rates of this model organism. This study demonstrated that Drosophila undergoes age-specific mortality, with mortality rates frequently rising in later stages of their development. Drosophila were subjected to varying doses of cypermethrin, chlorpyrifos, and neem leaf extract for 2 hours. They were then transferred to a fresh medium and reared for a generation. The various life table parameters were analysed and quantified. The research demonstrated that *Azadirachta indica* leaf extract emerged as a promising replacement for synthetic pesticides like cypermethrin and chlorpyrifos, especially at higher dosages. The research also exhibited a clear dose-dependent effect, with larger dosages of cypermethrin, chlorpyrifos, and neem leaf extract resulting in pronounced reductions in Drosophila survival. This shows how important it is to carefully assess pesticide dose and choice when employing them in agricultural approaches to reduce the influence on organisms that are not intended targets.

Keywords: *Drosophila melanogaster*, Cypermethrin, Chlorpyrifos, age-specific mortality rate, age-specific survival rate, life expectancy.

Introduction

Lifetable studies are a cornerstone of population ecology, providing valuable data for understanding the fundamental dynamics of populations and informing critical decisions related to conservation, management, and ecosystem health. It also helps understand how populations change over time. By examining birth and death rates across different age classes, one can identify factors influencing population growth or decline. This information is crucial for predicting future population trends and understanding the factors affecting population stability. Numerous research on population ecology, including those on the population biology of exotic species, conservation tactics, demographic ecotoxicology, harvesting theory, and the timing of pest control methods have used life tables to quantify the data. (Lin, 2014).

Drosophila, more commonly known as fruit flies, are a diverse and fascinating group of small insects belonging to the genus Drosophila, a part of the larger Drosophilidae family. Most Drosophila spp. is small, about 2–4 mm long, but some are larger than a house fly. They are typically pale yellow to reddish brown or black and possess transverse black rings across the abdomen with brick red eyes. Many species have distinct black patterns on the wings with plumose and arista antennae. The duration of the lifecycle of *Drosophila melanogaster* species is variable and depends on various factors. However, it increases with the increase in temperature. Moreover, the shortest lifecycle from egg to adult, 7 days, is achieved at 28°C (82°F). While under crowded conditions, the lifecycle is prolonged with the emergence of smaller flies. However, their average life span is 35–45 days. (Perveen, 2018)

Cypermethrin is a synthetic pyrethroid used as a pesticide in agriculture, household and animal husbandry, mainly for controlling pest insects. Cypermethrin also cause many health hazards resulting in physiological impacts, neurotoxicity, reproductive toxicity, molecular toxicity, etc. (Sharma et.al, 2018)

Chlorpyrifos is a chemical compound that belongs to a class of organophosphate insecticides. It has been widely used for pest control in agriculture, as well as for residential and commercial purposes to manage various pests like insects and mites. Chlorpyrifos is a crystalline white solid at room temperature. It works by inhibiting an enzyme called acetylcholinesterase in the nervous system of insects. Acetylcholinesterase is responsible for breaking down acetylcholine, a neurotransmitter. By inhibiting this enzyme, chlorpyrifos leads to an accumulation of acetylcholine, causing overstimulation of the nervous system in insects, which eventually leads to their death. According to research, chlorpyrifos persists in the body.

The native tree, *Azadirachta indica*, also known as neem contains a number of biologically active substances, including azadiractin, salanin, nimbin, and nimbidin which have pronounced insecticidal properties. Azadirachtin is the most studied and potent of these compounds. Neem-based insecticides act as growth disruptors, repellents, and feeding

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deterrents, affecting various stages of insect development. They are effective against a wide range of pests, including aphids, whiteflies, caterpillars, mites, and more. Neem-based products are considered biopesticides because they are derived from natural sources and have low toxicity to non-target organisms. Neem disrupts the hormonal balance in insects, affecting their feeding, growth, and reproduction. It can interfere with moulting, feeding, and the development of insect eggs, leading to reduced pest populations. Neem also has properties that promote plant growth and strengthen the plant's natural defence mechanisms. Neem-based products are biodegradable and do not persist in the environment, reducing the risk of long-term ecological damage compared to synthetic chemical pesticides. However, while neem-based products offer many advantages, it is essential to consider their proper application and potential side effects. Excessive and indiscriminate use of neem-based pesticides can harm beneficial insects, soil microorganisms, and other non-target organisms. Integrated pest management practices, which involve combining various pest control methods and using neem-based products judiciously, are crucial for effective and sustainable pest management. (Anjum et al., 2010).

Literature Review

Mukhopadhyay et al. (2004) contributed to the understanding of the genotoxic effects of cypermethrin using the alkaline Comet assay in Drosophila melanogaster. By systematically presenting their methodology, results, and interpretations, the authors provided valuable insights into the potential risks associated with pesticide exposure.

Gupta et al (2010) conducted a study on the effects of Chlorpyrifos exposure on Drosophila, shedding light on the mechanisms by which this pesticide triggers apoptosis and DNA damage through the generation of reactive oxygen species. It underscores the importance of addressing pesticide-induced cellular damage and oxidative stress, contributing to the ongoing dialogue surrounding sustainable agricultural practices and pesticide regulation.

Buneri et al. (2011) conducted a study on the determination of LC50 of Chlorpyrifos and Neem Extract on the third instar larvae of houseflies and their effect on fecundity. By determining the LC50 values and examining the impact on fecundity, the researchers have illuminated the complex interactions between pesticides and insect biology.

Alvarenga et al (2012) sheds light on the potential of neem seed cake as a biopesticide against Ceratitis capitata larvae. The research not only confirms the concentration-dependent toxicity of neem seed cake but also highlights its selective impact on the target pest compared to its parasitoid. As agricultural practices shift towards sustainable alternatives, further exploration of neem seed cake's efficacy, mechanisms of action, and ecological implications is vital for informed and effective pest management strategies.

Yee and Alston (2012) studied the behavioural responses, mortality rates, and oviposition patterns of the western cherry fruit fly when exposed to malathion, zeta-cypermethrin, and spinetoram. The article focuses on the practical application of insecticides and their effects on pest behaviour and reproduction.

Syed et al., (2013) conducted the toxicological evaluation of Chlorpyrifos and Neem Extract (Biosal B) against 3rd instar larvae of Drosophila melanogaster. The study showcased the differential effects of chlorpyrifos and neem extract.

Qing et al (2014) in the study on "Comparative Developmental Times and Laboratory Life Tables for *Drosophila suzukii* and *Drosophila melanogaster* (Diptera: Drosophilidae)" quantified and contrasted the life histories of these species. The study reveals important differences in the developmental times and life tables of *D. suzukii* and *D. melanogaster*. Comparative analyses likely uncovered distinct patterns in egg-to-adult development, survival rates, and reproductive characteristics. These insights contribute to a better understanding of the two species' ecological niches and interactions with their environments.

Methodology

A standard culture medium (Potato agar medium) suitable for *D. melanogaster* was prepared, containing essential nutrients for their growth and development. Male and female flies were collected from a healthy stock culture maintained in the laboratory. The required minimum concentrations of Chlorpyrifos (0.03175% and 0.0635%), Cypermethrin (0.01225 % and 0.0245%) and neem leaf extract (0.6% and 1.2%) were prepared using standard procedures. Distilled water was used in the control vials. The vials containing the medium were saturated with 0.25 ml of different concentrations of Chlorpyrifos, Cypermethrin and Neem. A set of triplicates was used for each concentration. Control vials were also maintained. Fixed numbers of male and female flies were introduced into the vials and removed after two hours. They were transferred to fresh media without the chemicals.

A few of them died immediately after the treatment. The live ones were allowed to reproduce for a generation and then assessed for further research and comparison of their life histories and stages of development. The following parameters were calculated for the construction of lifetables.

Number of survivors in a particular age interval (n_x).

Age-specific death in a particular age interval (d_x) is number of deaths that occurred within each age interval.

Age-specific survival rate(lx)is the proportion of individuals surviving from birth to a specific age x. ($l_x = n_x/n_0$)

Age-specific mortality rate (q_x) is the proportion of individuals dying at each age interval.

$q_x = d_x/n_x$

Life expectancy (e_x) indicates the expected remaining lifespan of individuals in each age interval, on average. ($e_x = T_x/n_x$)

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Results and Discussion

Table 1. Comparative analysis of lifetable parameters exhibiting the effects of chlorpyrifos, cypermethrin and *A. indica* leaf extract on *D. melanogaster*

ADULT	n_x (number of individuals alive at age x)	d_x (age- specific death)	q_x (age-specific mortality rate)	l_x (age specific survival rate = n_x/n_0)	e_x (life expectancy = T_x/n_x)
Control	101.5	15.5	0.1527	0.8601	3.3546
Cypermethrin (0.01225)	24	22.5	0.9375	3.2	2.9687
Cypermethrin (0.0245)	7.5	31	4.1333	1.1538	1.1
Chlorpyrifos (0.03175)	36.5	9	1.0735	0.2465	3.9452
Chlorpyrifos (0.0635)	21	15.5	0.9333	0.738	5.0119
Neem (0.6)	125	0	0	2.5	2.118
Neem 1.2	88	8.5	0.0965	1.6146	2.7642

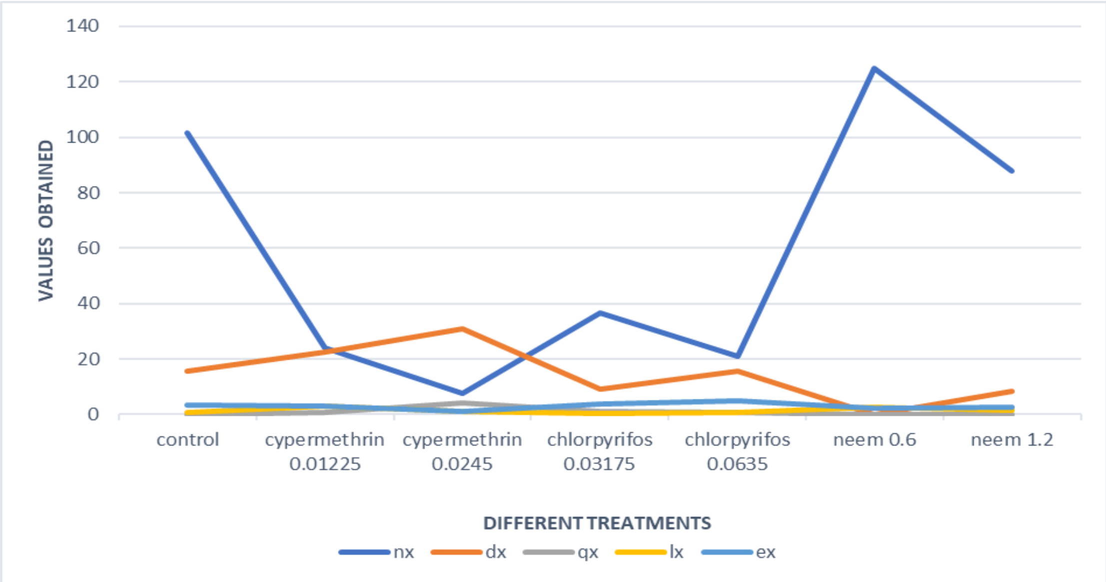

Figure 1. Comparative analysis of lifetable parameters exhibiting the effects of chlorpyrifos, cypermethrin and *A. indica* leaf extract on *D. melanogaster*

The impacts of various concentrations of the insecticides chlorpyrifos and cypermethrin as well as a naturally derived extract of neem leaves were assessed in the current study using a set of lifetable variables. The findings indicates that Drosophila exposed to Cypermethrin and Chlorpyrifos concentration have higher death rates as they age, which causes lower life expectancy (e_x) values and lower l_x values in later stages. Age-specific mortality (q_x) typically climbs as Drosophila progress through their life stages, with higher mortality rates in later stages. A higher risk of dying is also indicated by the fact that the age-specific mortality rate (q_x) tends to increase as the Drosophila get older. The death risk, which normally increases with age, is represented by the q_x numbers. The age-specific survival rate (l_x) values decrease from one age interval to the next, demonstrating that as mortality increased with age, the l_x values decreased. Overall, the results point to a population having a range of mortality rates and life expectancies throughout several life stages, with adulthood being the most affected stage by cypermethrin and chlorpyrifos exposure.
Neem extract, on the other hand, displayed a varied pattern of effectiveness. In comparison to chlorpyrifos and cypermethrin, neem leaf extract appears to be effective but requires larger dosages to produce outcomes that are equivalent to them.

Conclusion

Comparative laboratory life tables analysis of the effects of Chlorpyrifos, Cypermethrin and neem leaf extract on *Drosophila melanogaster* reveals insightful information on their influence on the life stages and death rates of this model organism. Throughout this study, it was evident that Drosophila experienced age-specific mortality, with

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mortality rates often increasing in later stages of their development. There are notable differences in their survival patterns when exposed to Chlorpyrifos, Cypermethrin, and neem leaf extract at varying dosages. The Drosophila were treated for two hours with different concentrations of chlorpyrifos, cypermethrin, and neem leaf extracts and then reared for a generation in a fresh medium to quantify the subsequent changes in the different life table parameters. The resulting data revealed that neem leaf extract emerged as a promising substitute for synthetic pesticides like cypermethrin and chlorpyrifos, particularly at higher concentrations. The study also demonstrated a definite dose-dependent effect, with higher doses of cypermethrin, chlorpyrifos, and neem extract causing more drastic reductions in Drosophila survival. This demonstrates how crucial it is to carefully examine the pesticide dose and choice when using them in agricultural techniques in order to reduce the influence on organisms that are not intended targets.

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