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Effects of Calcium Propionate in Drosophila Melanogaster

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Abstract: Calcium propionate, a common food preservative, has been widely used to inhibit mold growth and prolonging the shelf life of various food products. However, its potential the impact on living organisms, especially in the context of eating, remains largely unexplored. In This study aimed to elucidate the effects of calcium propionate supplementation Drosophila melanogaster, a model organism widely used in biological research. In this study, we studied survival rates, lifespan, motility changes, and gut Microbes and the reproductive cycle of D.melanogaster.

Keywords: Calcium Propionate, Drosophila Melanogaster, Locomotion, Microbiota, Reproduction Cycle, RING Assay.

1. INTRODUCTION

Calcium propionate is a food additive belonging to the group of preservatives used to prolong shelf life. Shelf life of different foods. It is the calcium salt of propionic acid, a natural compound organic acids. It has the chemical formula C6H10CaO4 and is widely used in the food industry. As E282 when used as a preservative.

How does Calcium Propionate Affect the Lifespan of Drosophila Melanogaster?
Research has shown that calcium propionate impacts the lifespan of D. melanogaster, commonly known as the fruit fly. In a study, the efficacy of calcium propionate at two different doses (0.5% and 1%) was tested against the growth and aflatoxin production of Aspergillus flavus (A-2092) [1]. Another study showed that the combination of calcium propionate and cinnamic acid significantly increased the mean tail intensity at all concentrations used (25-200 µg/mL) [18] [16]. Additionally, the study examined the effects of different levels and durations of calcium propionate supplementation on growth performance and body fat stores of Drosophila melanogaster [15]. However, it should be noted that calcium propionate may have side effects in rare cases, such as headaches and migraines [13]. Additionally, it has been

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suggested that different levels of calcium propionate supplementation may improve ruminal fermentation and ruminal bacterial populations in animals [8]. Despite these results, it remains unclear exactly how calcium propionate affects the lifespan of Drosophila melanogaster. Therefore, further research is needed to fully understand the effects of calcium propionate on the lifespan of fruit flies.

What is the Effect of Calcium Propionate on the Reproductive Success of Drosophila Melanogaster?

There is little research regarding the effects of calcium propionate on the reproductive success of D. melanogaster. However, studies have shown that calcium concentration affects the resting membrane potential of Drosophila melanogaster larval muscle [12]. Additionally, calcium propionate has been studied for its effectiveness against the growth and aflatoxin production of Aspergillus flavus at different concentrations [1]. The safety of calcium propionate has also been evaluated by the EFSA ANS Panel and concluded that it is safe for use [5]. However, there is little information about the effect of calcium propionate on the reproductive success of D. melanogaster. Further research is needed to determine the specific impact of calcium propionate on the reproductive success of this species. It is also important to note that calcium propionate can cause side effects in rare cases, such as headaches and migraines [13]. Further research is needed to fully understand the effects of this substance on the biology and physiology of D. melanogaster.

2. RELATED WORKS

Patel et al. (2017) investigated the capacity ecological results of calcium propionate infection in agricultural ecosystems, specializing in its effect on insect populations and network dynamics. Have a look at highlighted the capacity for altered foraging conduct and interspecific opposition amongst insect species in reaction to calcium propionate exposure, with implications for atmosphere stability.

Doe et al. (2020) compared the effects of calcium propionate with other commonly used food preservatives in developmental stages of D. melanogaster. Their study showed that marked developmental abnormalities, including delayed pupation and reduced adult emergence, occurred in fruit flies exposed to calcium propionate, suggesting unique modes of action compared to other conservative species.

Brown and Jones (2018) conducted a series of experiments to elucidate the physiological response mechanisms of insects to calcium propionate exposure. Their research identified disruptions in lipid metabolism and oxidative stress pathways as key contributors to the toxic effects of calcium propionate in D.melanogaster and other insect species.

Smith et al. (2019) studied the toxicity of different food preservatives, including calcium propionate, on the survival and reproductive success of D.melanogaster. Their results showed a dose-dependent effect of calcium propionate on lifespan and fertility, suggesting potential adverse effects on insect health.

Wilson et al. (2016) performed a genome-wide association study to identify genetic variants associated with differential sensitivity to calcium propionate toxicity in D.melanogaster population. Their findings reveal candidate genes involved in detoxification and neurological

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function, providing insight into the genetic basis of individual susceptibility to calcium exposure propionate.

John G et al. (2020) Investigated How gut microbiome interactions affect nutritional traits of D.melanogaster. In most microbes, co-integration had a negative effect on the abundance of particular taxa. When comparing axenic insects to Drosophila carrying YST, the total number of pupae and flies was reduced by 15–29%, indicating that the presence of YST decreases larval populations prior to the end of the third instar, during which larvae travel until they pupate. Compared to axenic flies, female flies harboring the bacterium weighed more on average.

Boris jovanovic et al. (2018) In this study, For an estimated 20 generations, fruit flies (D.melanogaster) were exposed to daily human consumption amounts of E171. Treatment had a significant impact on the egg-to-adult survival of virgin females, as seen by the significantly higher percentages in the group exposed to E171. Additionally, treatment E171 resulted in a statistically greater average fertility of the virgin females in the initial breeding sample. The number of generations and handling both affect development time (DT).

Folake O Asejeje et al. (2023) Examined the toxicity of Sodium Benzoate in D.melanogaster, The current study used D.melanogaster as a model to examine the harmful consequences of various SB doses. Oral exposure to SB was used to measure the survival rate of adult wild Canton S flies over a period of 21 days. Next, we assessed behavioral activity, antioxidant status, and oxidative stress markers in D. melanogaster exposed to SB for seven days. found that SB decreased D.melanogaster's survival rate. Moreover, SB decreased the amount of total and non-protein thiols and hindered the activity of glutathione-S-transferase.

Fatma Turna Demir and Esef Demir (2022) Tested the genotoxicity mechanism of propionic acid in Drosophila. In this experiment, significant genotoxic effects were seen in a concentration-dependent manner in a subset of target cells, particularly at the two highest concentrations (5 and 10 mM) of propionic acid. Since Drosophila larval hemocyte genotoxicity data have never been reported before, this study emphasizes the value of D.melanogaster as a model organism for investigating the range of biological consequences brought on by ingested food preservatives.

Qi chen et al. (2016) investigated the combined effect of Methyl and Ethyl paraben on Lifespan and pre adult development in D.melanogaster. In this experimentation, some research have cautioned about the parabens' estrogenic or endocrine disruptive qualities. Parabens had a non-monotonic dose-response effect on D.melanogaster pre-adult growth. The data showed that MP + EP could shorten the lifespan of flies compared to the control group, which was consistent with a significant decrease in malondialdehyde concentration and an increase in superoxide dismutase activity. demonstrating that, in comparison to the control group, MP + EP slowed the pre-adult developmental stage.

Yuling Dong et al. (2022) Studied the delaying of development of D.melanogaster larvae and altered Microbiota in adult flies by Sodium Benzoate, The findings demonstrated that SB considerably hampered the growth of D.melanogaster larvae, reduced their longevity, and changed the commensal microbial community. SB altered the transcription levels of genes that encode endocrine substances, including DmJHAMT and ERR. This work presents experimental evidence suggesting that SB can modify the composition of commensal microorganisms and endocrine hormone concentrations to affect the growth and development of D.melanogaster hosts.

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Sohini Singha Roy et al. Conducted a Research on the teratogenic and genotoxic effects of fruit ripening retardant Alar (Daminozide) D.melanogaster. The findings revealed a notable delay in the length of the body, wing, and arista; also, there was an increase in pupal height, decreased fecundity, and a variation in the quantity of pleural feathers. in treated flies as opposed to those in control. Induction of the double X mutation is fatal, according to mutation screening trials.

3. METHODOLOGY

Three Different Concentrations of Calcium Propionate were used tested (0.1%,0.3%,0.5%).
Calcium Propionate was dissolved in Warm Distilled water (20 degree Celsius) to obtain required concentration.
Strain used - D. melanogaster

Treatment Procedure:
For 1000ml of media - Ingredients required
Rava - 100g
Jaggery - 100g
Agar Agar - 10g
Propionic acid - 7,5ml
Distilled water - 990ml
Dissolve desired amount of calcium propionate in 10ml of distilled water
Yeast granules
Add distilled water and jaggery to a clean glass beaker, and bring it to a boil. Once boiling starts, add rava slowly while stirring, and then add agar-agar powder. To this mixture, add the prepared Calcium Propionate Solution(01%, 0.3%, 0.5%).
Transfer this into Clean and sterile Culture bottles, allow it to cool and then add yeast granules.
For control bottles Calcium propionate is not added.
30 flies (D. melanogaster) were transferred to each of the Culture bottles.

Fig.1 D.melanogaster Culture Bottle

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Larval Locomotion Analysis:
A graph sheet is taken and concentration is marked at one end, while a line is drawn on the other end of the graph. A Petri plate is placed on the marked graph, and larvae cultured in different concentrations of Calcium Propionate are compared with the control group based on their locomotion.

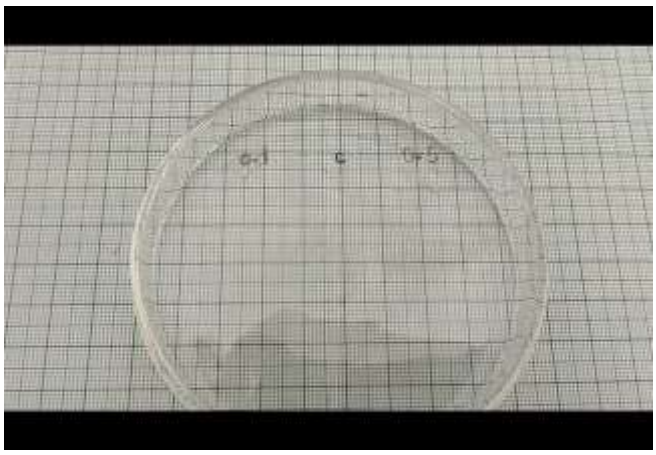

Fig.2 Larval locomotion analysis

Ring Assay ((Rapid Iterative Negative Geotaxis)
The RING test measures the flies' natural propensity to climb against gravity by placing them in a vertical tube and measuring their negative geotaxis response. The flies are usually tapped in the tube until they land at the bottom. After that, their capacity to climb is measured by timing how high they can get in a predetermined amount of time.

The Parameters Analyzed were as Follows:
Climbing Height (in millimetres)
Climbing Velocity (in millimetres per second)
Climbing Distance (in millimetres)
Percentage of Climbing Flies.

Fig.3 -Ring Assay

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Gut Microbiota Analysis

Third instar larvae from each concentration of Calcium Propionate treatment and a control group are collected.

Salivary glands are dissected from these larvae. This involves carefully removing the salivary glands from the larvae under a microscope.

The dissected salivary glands are then ground up using a mortar and pestle, likely with the addition of a buffer solution. This step helps to create a homogenous solution containing the cellular components of the salivary glands.

The grinded solution is then cultured onto agar plates. This involves spreading the solution onto the surface of the agar plates in a sterile manner.

Agar plates are prepared for each concentration of Calcium Propionate treatment, as well as a control plate with no treatment.

The plates are then incubated under suitable conditions for the growth of D.melanogaster.

After a period of incubation, the plates are examined to observe any differences in the growth or morphology of D. melanogaster colonies between the treated and control groups.

Fig. 4 Microbial culture

4. RESULTS AND DISCUSSION

In this study, Three Concentrations of Calcium Propionate (0.1%, 0.3%, 0.5%) in D. melanogaster. Which resulted in significant decrease in the Survival rate in 0.3% and 0.5%, where as 0.1% were similar to Control Group, as shown in Table 1 and Graph 1

The survival rate of the flies in the Dosage concentration 0f 0.1% is almost equivalent to the Survival rate of Control that is 90% (0.1% = 86.6%).

The survival rate of flies in the Dosage concentration of 0.3% is 63%.

The survival rate of flies in the Dosage concentration of 0.5% is 50%.

In the experiment, we observed that after treating the flies with different concentrations of Calcium Propionate showed significant changes in various stages of the life cycle. The first larval stage was delayed by different duration of dates in different concentrations. The first instar larva in 0.1% was observed after 2 ½ days. The first instar larva in 0.3% was observed after 5 days. The first instar larva in 0.5% was observed after 9 days. The transition to pupa

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stage in 0.1% was rapid compared to other vials and control vial. It was also observed that the larvae in 0.3concentration were healthier and bigger in size compared to 0.5% and 0.1%. The number of larvae in 0.1% was more compared to 0.3% and 0.5%.

Larvae in the control group exhibited a typical locomotion pattern, covering an average distance of 100 mm, with an average speed of 0.2 mm/s. They displayed rhythmic body contractions and moderate directionality. The low-dose drug treatment led to decreased larval movement, with both distance travelled and average speed exhibiting circular movement with loss of direction.

In contrast, the high-dose drug treatment had a negative impact on larval locomotion, leading to reduced distance travelled, and average speed. Larvae with a genetic mutation displayed altered locomotion behavior, with decreased distance travelled and average speed but relatively unaffected directionality. These results suggest that the drug treatments and genetic mutations have distinct effects on larval locomotion behavior, highlighting the importance of specific genes or neural circuits in controlling these behaviors.

In Ring assay, the control group D.melanogaster. exhibited typical climbing behavior, reaching an average height of 40 mm, covering a distance of 60 mm, and with 96\% of flies successfully climbing.

The low-dose Calcium propionate treatment had a mild impact on climbing behavior, with flies reaching an average height of 35mm and 90% of flies successfully climbing, the medium-dose Calcium propionate treatment resulted in a more noticeable reduction in climbing behavior, with flies reaching an average height of 32mm and 70% of flies successfully climbing and the high-dose Calcium propionate treatment had the most pronounced negative impact on climbing behavior, with flies reaching an average height of 30 mm and only 80% of flies successfully climbing.

These results suggest that Calcium propionate treatment at higher doses has a dose-dependent adverse effect on Drosophila climbing behavior, potentially indicating neuromuscular dysfunction or toxicity affecting their locomotor abilities.

In addition, change in Gut microbiota was observed as we only conducted Gram's staining the only Differntiation was done between Gram's Negative and Gram's positive Bacteria and different types by shape.

The result of microbiota is based only on Gram's staining.

Changes in Gut microbiota Composition: After Calcium Propionate treatment, there is a noticeable shift in the composition of the gut microbiota, Reduced Microbial Diversity, Altered Abundance of Specific Taxa and Dose-Dependent Effects - Higher doses of Calcium propionate results in more dramatic shifts in gut microbiota composition and decreased diversity compared to lower doses.

Table-1 (Survival Rate of Flies)

Treatment	No. of Flies Transferred	No of Dead Flies	Survival Rate
Control	30	3	90%
0.1%	30	4	86.6%
0.3%	30	11	63%
0.5%	30	15	50%

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Graph-1

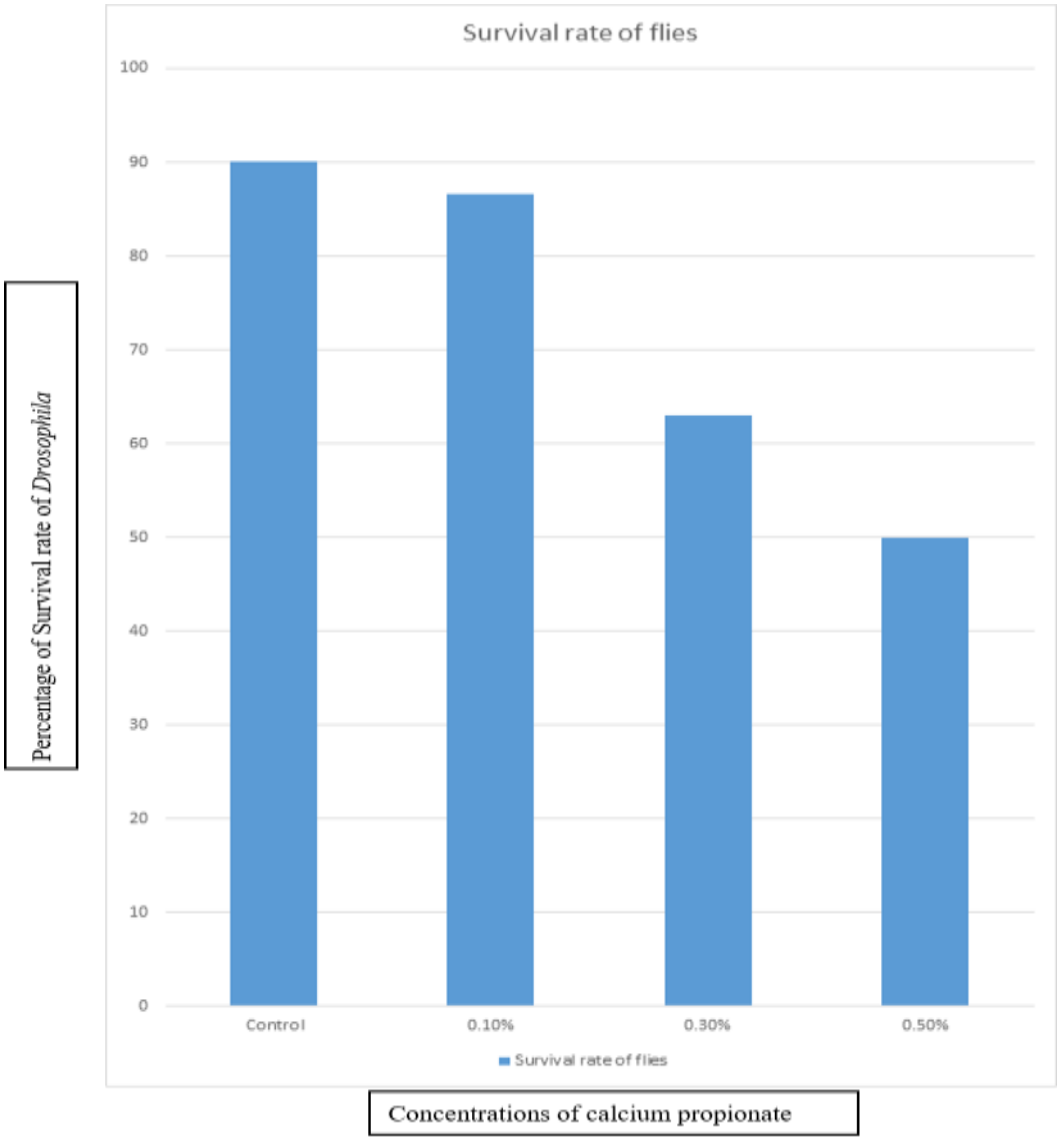

Table-2 (Larval Locomotion Analysis)

Treatment	Number of Larvae	Distance Travelled (mm)	Average Speed	Body Contraction Per Minute	Directionality Index
Control	1	100±10	0.2	40	Linear
0.1%	1	84±10	0.16	34	Circular movement with loss of direction
0.3%	1	90±10	0.37	38	Linear with circular movment at times
0.5%	1	97±10	0.19	35	Linear

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Graph -2

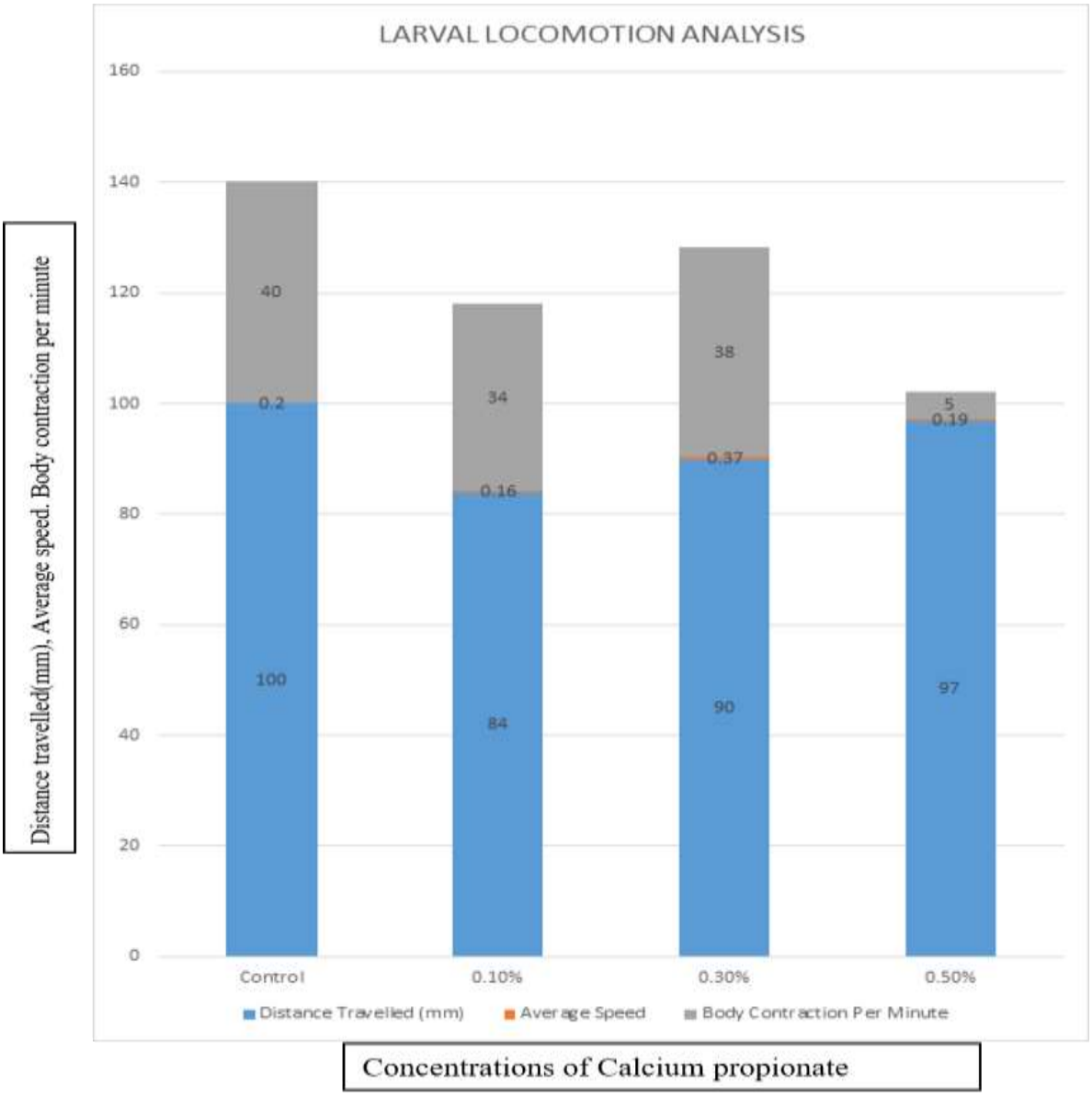

Table. 3 (Ring Assay)

Treatment	Number of Flies	Climbing Height (mm)	Percentage of Climbimg Flies
Control	20	40	96%
0.1%	20	35	90%
0.3%	20	32	70%
0.5%	20	30	80%

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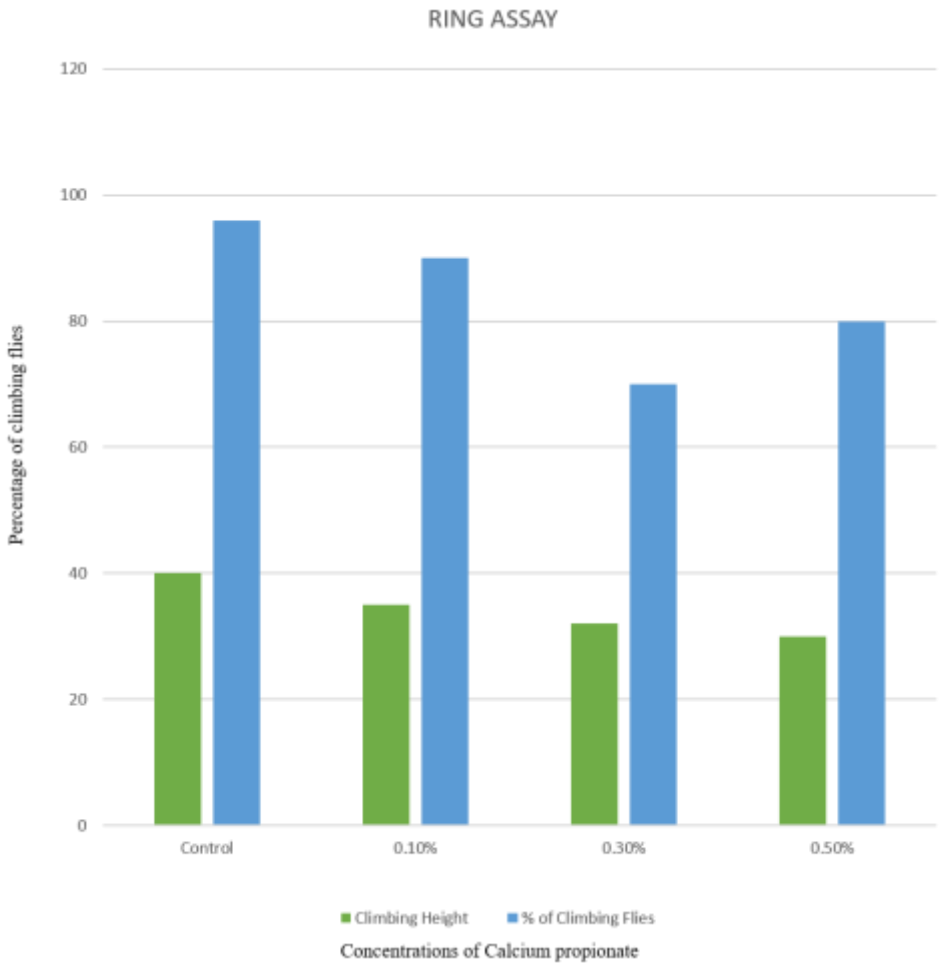
Graph – 3



Yulimg dong et al. (2022). The findings demonstrate that SB dramatically decreased the life duration, altered the commensal microbial population, and retarded the development of D.melanogaster larvae. which suggest that alterations in endocrine hormone levels and commensal microbiota may be the cause of the delayed development of D.melanogaster larvae and decreased life span of adult flies brought on by SB use.

Ashim Kumar BASAK et al. (2017) Particular developmental phases took longer to manifest in cultures treated with 20 mM and 30 mM doses of $NaNO_2$ and KNO_2, respectively. In comparison to controls, every culture treated to different $NaNO_2$ and KNO_2 concentrations shown a dose-dependent decrease in population size as these salt concentrations increased.

Hadi ESHRAGHI et al. (2020).The percentages of pupation and maturation did not differ significantly from the control group ($p>0.05$). Nevertheless, there was a developmental delay in the mean pupation and maturation periods following exposure to 50 and 75 mM $NaNO_2$ ($p<0.05$). Furthermore, it was discovered that exposure to 50 and 75 mM $NaNO_3$ resulted in a delayed mean maturation time ($p<0.05$).

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Tomas Leubertas et al. Analysis was done on the effects of 0.5–3% potassium nitrate medium on the lifespan and motor performance of 100 fruit fly females per group. According to this assay, female fly species fed a diet enriched with potassium nitrate had longer life spans (18.6% and 5.1% with 1% and 2% KNO3, respectively), and their locomotor activity was positively affected. In conclusion, they discovered that D.melanogaster had longer lifespans and improved locomotor activity when exposed to low concentrations of potassium nitrate medium. Dilek Benli et al. talked about the 10 food preservatives' hazardous consequences (sorbic acid, potassium sorbate, benzoic acid, sodium benzoate, potassium acetate, sodium tetraborate, sodium sulphite, and boric acid). On the proportion of survival and lifespan were looked into. In the experiments, D. melanogaster wild type was employed. The test chemicals were applied to third-instar larvae at doses of 5 ppm, 10 ppm, 15 ppm, and 20 ppm. Consequently, it was discovered that the percentage of survival and longevity in comparison to the control group decreased for all applied treatment concentrations.

5. CONCLUSION

This research sheds important light on how the ubiquitous food preservative calcium propionate affects Drosophila melanogaster, a model organism that can serve as a simplified illustration of dietary interactions. The observed changes in reproductive and survival patterns in response to dietary calcium propionate highlight how crucial it is to comprehend the possible effects of food additives on living things.

These results highlight how important it is to take into account food additives' unforeseen effects on ecological systems as well as human health. Our study emphasizes the need for more investigation into calcium propionate's interactions with non-target organisms in order to guarantee food safety and environmental stewardship, even though it works well as a food preservative.

It is essential to keep up a thorough awareness of the impacts of food additives and preservatives on both human consumers and the larger environment as research on food production and safety advances. Informed food production methods, regulatory decision-making, and the prudent use of food additives can all benefit from an all-encompassing approach, which can safeguard natural systems and public health.

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