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Effect of *Piper longum* on cold-Induced Stress in *Drosophila melanogaster*

[1]Preethi M A, *[2]Shakunthala V

1, MSc. Zoology, Department of Studies in Zoology, Manasagangotri, University ofMysore, Drosophila stock centre, Mysuru, Karnataka, India.

*2 Professor, Department of Studies in Zoology, Manasagangotri, University ofMysore, Mysuru, Karnataka, India.

Abstract:

Insect habitats are significantly influenced by thermal constraints, with a strong correlation observed between latitudinal and elevational distributions and various indices of cold resistance. While some insects can endure sub-freezing temperatures, most succumb at temperatures above freezing due to factors unrelated to ice formation. The term "chill tolerance" encompasses a complex set of traits that enable insects to survive and function at relatively mild cold temperatures. From an evolutionary biology perspective, coping with harsh environmental conditions is a major challenge. Extreme conditions impose physiological stresses, leading to selective pressure favoring stress tolerance. Temperature is a critical abiotic factor for ectothermic animals, especially insects, potentially causing heat or cold stress. Different species thrive within specific temperature ranges conducive to their generational success. Adult *Drosophila melanogaster* experience a knockdown, or cold coma, at low temperatures, losing their ability to move and feed. Upon returning to a higher temperature, adults gradually resume normal activities, and cold treatment is often used as an alternative to CO2 or ether anesthesia. Cold exposure causes sudden structural alterations in proteins and cell membranes, leading to chill injury. Despite the hemolymph of *Drosophila melanogaster* remaining supercooled at temperatures as low as -20°C, individuals may die after an hour of exposure to -6 or -8°C. Rapid cold-hardening (RCH), a brief pre-exposure to low temperature, can temporarily enhance cold shock tolerance and reduce severe chill injury. The present study investigates the thermal stress response in *D. melanogaster* treated with *Piper longum* powder.

Key words: *D.melanogaster*, thermal stress, cold resistance, chill tolerance, *Piper longum.*

Introduction

Insect habitats are significantly influenced by thermal constraints, with a strong correlation observed between latitudinal and elevational distributions and various indices of cold resistance. While some insects can endure sub-freezing temperatures,

most succumb at temperatures above freezing due to factors unrelated to ice formation. The term "chill tolerance" encompasses a complex set of traits that enable insects to survive and function at relatively mild cold temperatures. From an evolutionary biology perspective, coping with harsh environmental conditions is considered a major challenge (Hoffmann and Parsons, 1991; Bijlsma and Loeschcke, 1997). Extreme conditions impose physiological stresses, leading to selective pressure favoring stress tolerance.

Temperature is a critical abiotic factor for ectothermic animals, especially insects, potentially causing heat or cold stress. Different species thrive within specific temperature ranges conducive to their generational success. For example, within the Drosophilid family, it is impossible to rear the tropical species *Drosophila* ananassae below 16°C (Morin et al., 1997), while for other species, 25°C is the upper lethal limit. Cold tolerance has been measured by the survival duration at non-freezing temperatures, such as -1°C (Parsons and Stanley, 1981).

Adult *Drosophila melanogaster* experience a knockdown, or cold coma, at low temperatures, losing their ability to move and feed (Somme and Block, 1982; Schenker, 1984). Upon returning to a higher temperature, adults gradually resume normal activities, and cold treatment is often used as an alternative to CO2 or ether anesthesia (Ashburner, 1989). Cold exposure causes sudden structural alterations in proteins and cell membranes, leading to chill injury. Despite the hemolymph of *Drosophila melanogaster* remaining supercooled at temperatures as low as -20°C, individuals may die after an hour of exposure to -6 or -8°C (Czajka and Lee, 1990; Kelty and Lee, 1999). Rapid cold-hardening (RCH), a brief pre-exposure to low temperature, can temporarily enhance cold shock tolerance and reduce severe chill injury (Meats, 1973; Chen et al., 1987; Lee et al., 1987a; Czajka and Lee, 1990; Powell and Bale, 2004). The present study the thermal stress has been observed in *D. melanogaster* treated with *Piper langam* Powder.

For insects that are susceptible to freezing, ion balance is lost in the winter and must be regained upon rewarming. Insects that are chilled gradually lose their equilibrium of water and ions (Overgaard et al., 2017; Kostal et al., 2004). The renal organs, the rectum and the Malpighian tubules, generally use active ion pumping to maintain the equilibrium of ions and water at permitted temperatures. However, in colder temperatures, active transport slows down (Zachariassen, et al., 2004). Water and Na+ leak into the gut along their own concentration gradients, but passive ion loss cannot be made up for by active transport in the cold, therefore K+ is concentrated in the residual hemolymph (Kostal et al., 2006; MacMillan et al., 2011).

It Is believed that an insect's recovery period from a chill coma depends on its capacity to restore K+ homeostasis and the extent to which homeostasis is compromised in the cold. This is because high haemolymph [K+] promotes muscle cell depolarization (Andersen, et al., 2019; Kostal, et al., 2004; MacMillan, et al., 2012; MacMillan, et al., 2014). The degree to which homeostasis is lost in the cold is also related to survival: elevated hamolymph [K+] is toxic and can cause cellular Ca2+ overload, which can result in chilling injury and death (Bayley, et al., 2020; MacMillan, et al., 2015).

Materials and methodology:

The *Drosophila melanogaster* experimental stock was acquired from the University of Mysore's Manasagangotri stock Center. Among all the model organisms, *Drosophila melanogaster* is one of the most used and understood. Its brief, straightforward reproduction cycle, which lasts between 8 and 14 days, is impacted by ambient temperature. The collected flies were redistributed and raised in several culture bottles filled with wheat cream agar media (cooked in 1000 ml of double-distilled water with 100 g of jaggery, 100 g of wheat powder, and 10 g of agar agar). Finally, 7.5 milliliters of propionic acid were added. Twenty flies (10 males and 10 females) were placed in culture bottles and kept in the dark for 12 hours at 22 °C and 70% relative humidity. The virgin flies were isolated at the pupa stage and cultured in test media. Test media contain 10 mg/l and 20 mg/l of long pepper media, respectively. flies grown in normal wheat agar media were used as controls. The test media contained pepper concentrations of 10 mg/L and 20mg/L respectively. Media containing *Piper longum* is referred to as tested media, and the flies grown in normal wheat agar media were used as control. The test media piper longum powder was taken by Government Ayurveda Medical College and Hospital, Mysore. The test media *Piper longum* is administrated of *Drosophila* through wheat agar media in different concentration 10mg 20mg per 1000ml of distilled water.

Piper longum media 10%: treated *Piper longum* media was prepared from 10g of *Piper longum* powder, 100g of jaggery, 100g of wheat cream rava, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

Piper longum media 20%: 1% *Piper longum* media was prepared from 20g of *Piper longum* powder, 100g of jaggery, 100g of wheat cream rava, 10g of agar boiled in 1000ml distilled water and 7.5ml of propionic acid added to it. The *Drosophila melanogaster* cold resistance experiment was studied using the flies that emerged from the wheat cream agar media and other experimentally treated media under the identical laboratory settings as earlier showed.

Experimental procedure:

In order to investigate the fly's resistance to cold, *Drosophila* flies were cultivated in both wheat cream agar and *Piper longum* media (10mg/l, 20mg/l). Five-day-old mated male and female *Drosophila* flies were obtained from the cultured media [from control media and *Piper longum* treated media] etherised by using ether, vial, five male and five female flies were added and plugged with cotton. These vials were kept at 0°C in a refrigerator for continuous cold. The flies were then monitored every ten and twenty minutes until they recovered. The data were subjected to two way ANOVA and Tukey's post hoc test using SPSS package 29 version.

Statistical analysis:

The data obtained were analysed using SPSS version Mean, standard error, one way ANOVA and Tukey's Post-hoc test were carried out for the data for cold resistance assay. A graph of media v/s Mean cold resistance of mated males and females *Drosophila melanogaster* flies was plotted.

Results:

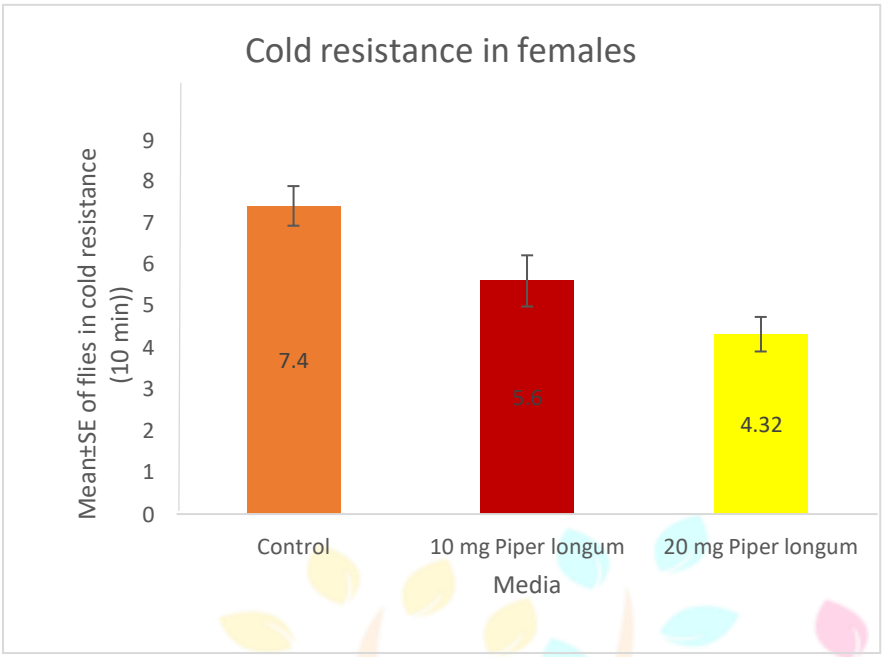

Figure 1: Effect of *Piper longum* on 10 minutes cold resistance in mated females of *Drosophila melanogaster.*

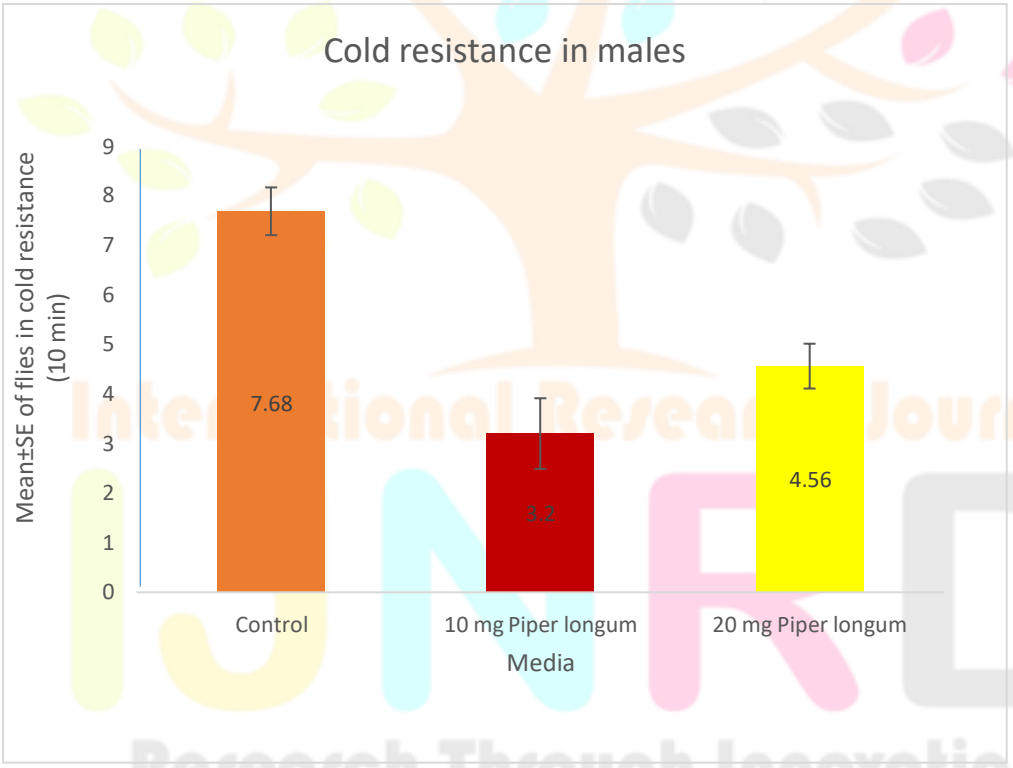

Figure 2: Effect of *Piper longum* on 10 minutes cold resistance in mated males andfemales of *Drosophila melanogaster*.

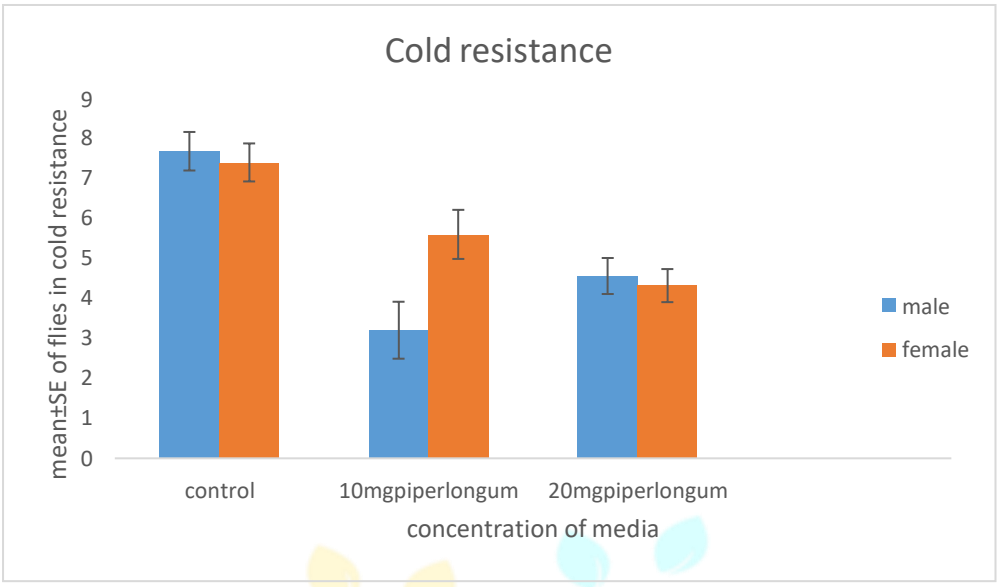

Figure 3: Effect of *Piper longum* on 10 minutes cold resistance in mated males andfemales of *Drosophila melanogaster*

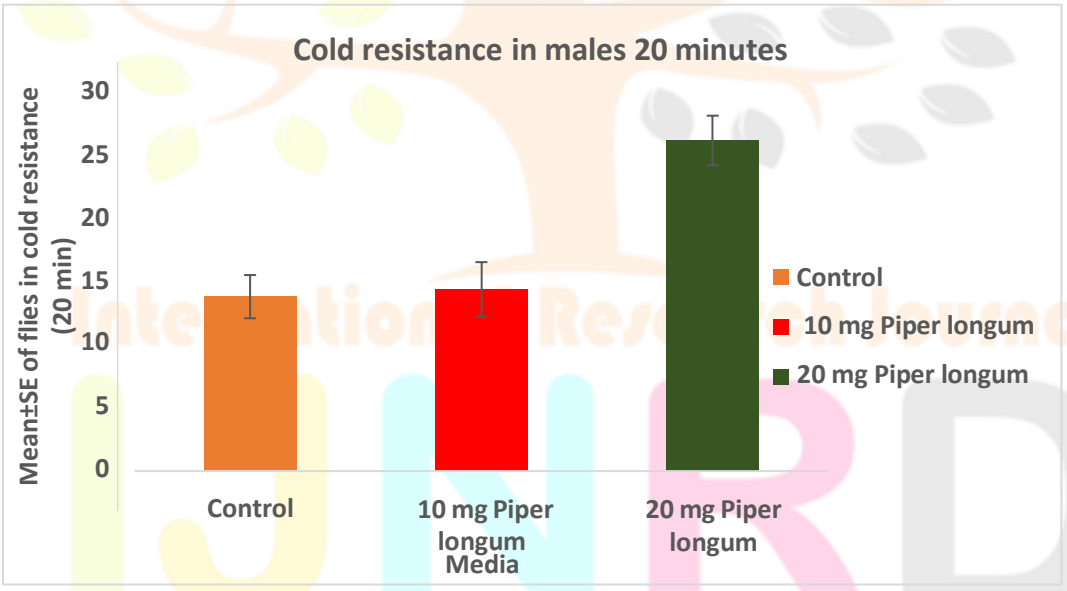

Figure 4: Effect of *Piper longum* on 20 minutes cold resistance in mated males of *Drosophila melanogaster*

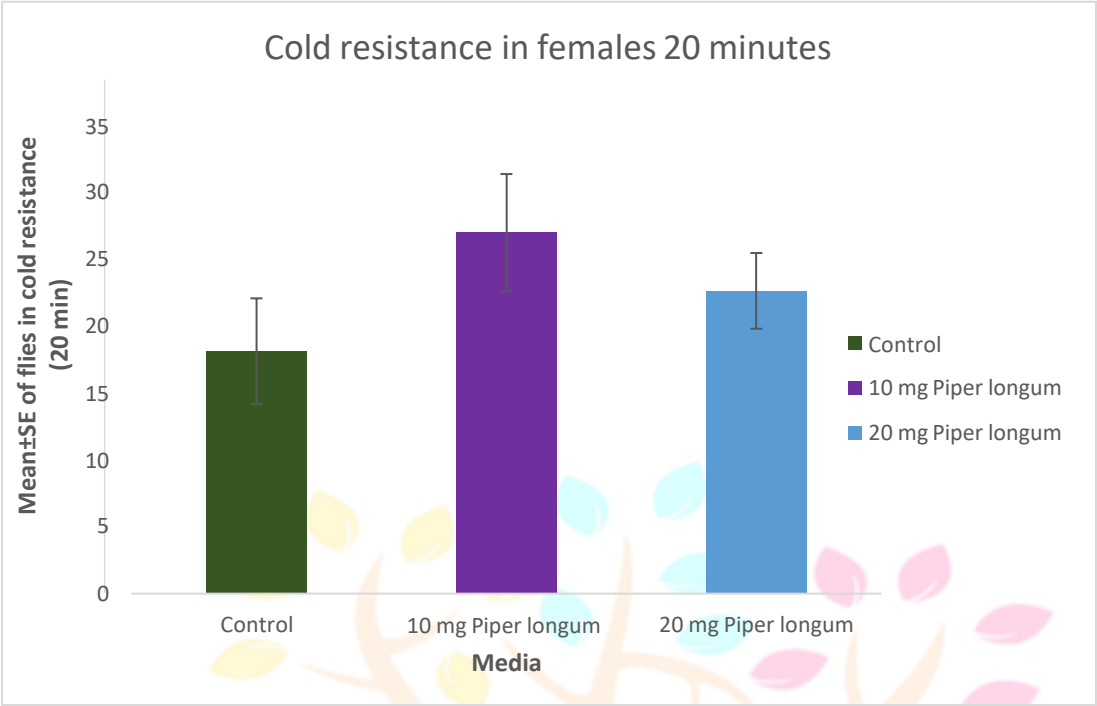

Figure 5: Effect of *Piper longum* on cold resistance in mated males and females of *Drosophila melanogaster*

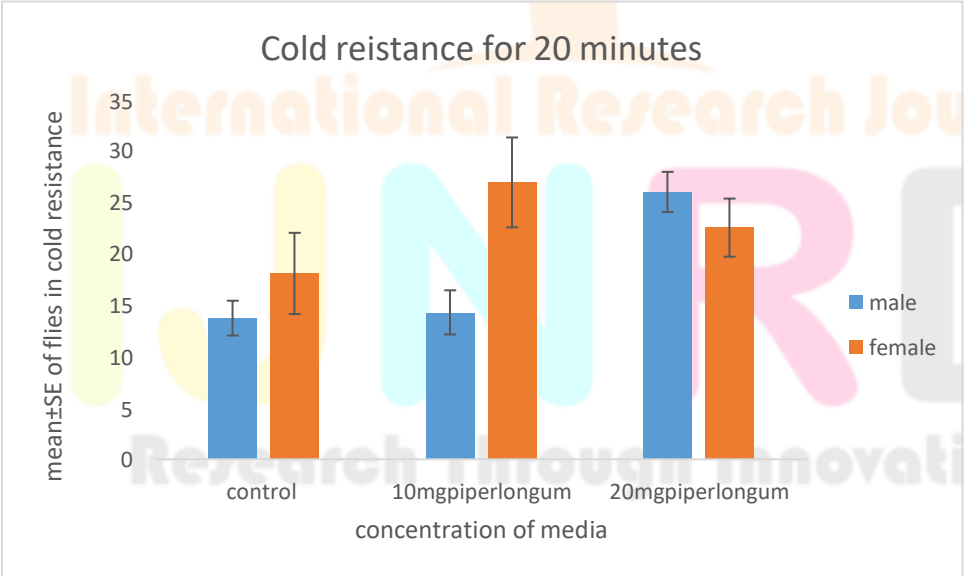

Figure 6: Comparative effect of *Piper longum* on cold resistance in mated males andfemales of *Drosophila melanogaster* in 20 minutes

Effect of *Piper longum* on 10-Minute Cold Resistance in Mated Male and Female *Drosophila melanogaster*

Figure 1 Shows the impact of *Piper longum* on the cold resistance of 5-day-old male *Drosophila melanogaster* fed with control and treated media. The recovery time was faster in *Drosophila* fed with 20 mg *Piper longum* treated media compared to the control. Within the concentration groups, the recovery time was faster in *Drosophila* fed with 10 mg treated media, which is statistically significant ($P < 0.05$, $df = 2$, $F = 9.259$).

Figure 2 Illustrates the effect of *Piper longum* on the cold resistance of 5-day-old female *Drosophila melanogaster*. The recovery time was found to be faster in females fed with 10 mg *Piper longum* treated media compared to the control. Within the concentration groups, the recovery time was faster in *Drosophila* fed with 20 mg treated media, showing significant results ($P < 0.05$, $df = 2$, $F = 16.790$).

Figure 3 Compares the cold resistance between male and female *Drosophila melanogaster*. Males fed with control media exhibited the longest recovery time. Among the treated groups, males fed with 10 mg *Piper longum* showed faster recovery, while those fed with 20 mg *Piper longum* had the fastest recovery. However, the difference was not statistically significant ($P > 0.05$, $df = 2$, $F = 4.118$).

Effect of *Piper longum* on 20-Minute Cold Resistance in Mated Male and Female *Drosophila melanogaster*

Figure 4 Represents the impact of *Piper longum* on the cold resistance of 5-day-old male *Drosophila melanogaster* after 20 minutes of cold exposure. The recovery time was faster in *Drosophila* fed with control media compared to those fed with 20 mg *Piper longum*. Within the concentration groups, the recovery time was longer in *Drosophila* fed with 10 mg treated media, which is statistically significant ($P < 0.05$, $df = 2$, $F = 12.778$).

Figure 5 Shows the effect of *Piper longum* on the cold resistance of 5-day-old female *Drosophila melanogaster* after 20 minutes of cold exposure. The recovery time was faster in females fed with control media compared to those fed with treated media. Within the concentration groups, females fed with 20 mg treated media recovered faster, although this difference was not statistically significant ($P > 0.05$, $df = 2$, $F = 1.376$).

Figure 6 Compares the 20-minute cold resistance between male and female *Drosophila melanogaster*. Males fed with control media had the longest recovery time. Among the treated groups, males fed with 10 mg *Piper longum* recovered faster, and those fed with 20 mg *Piper* longum recovered the fastest. This difference was not statistically significant ($P > 0.05$, $df = 2$, $F = 3.587$).

Discussion:

The results indicate that *Piper longum* affects cold resistance in *Drosophila melanogaster*, with variations based on sex, exposure duration, and concentration. In general, males and females fed with 10 mg *Piper longum* showed faster recovery

times after 10 minutes of cold exposure compared to those fed with control media. However, for 20-minute cold exposure, *Drosophila* fed with control media showed faster recovery times compared to those fed with treated media.

Proteins and cell membranes undergo sudden structural alterations when exposed to cold, which is thought to be the cause of chill injury. Although the hemolymph of the fruit fly *Drosophila melanogaster* may remain supercooled at temperatures as low as 20^0 C, individuals of the species may perish after being exposed for one hour to 6 or 8^0 C (Czajka and Lee, 1990; Kelty and Lee, 1999). Rapid cold-hardening (RCH), in which a brief pre-exposure to low temperature temporarily enhances the cold shock tolerance, may help reduce resistance to severe chill injury (Meats, 1973; Chen et al., 1987; Lee et al., 1987a; Czajka and Lee, 1990; Powell and Bale, 2004). Insects vulnerable to chilling lose ion balance in the cold and must restore it upon rewarming. Chilled insects gradually lose their water and ion homeostasis (Overgaard et al., 2017; Kostal et al., 2004). Normally, the Malpighian tubules and rectum, acting as renal organs, maintain ion and water balance through active ion pumping at favorable temperatures. However, in the cold, active transport slows down (Zachariassen et al., 2004). Passive ion leakage cannot be compensated for by active transport in the cold, resulting in K+ concentration in the remaining hemolymph as water and Na+ leak into the gut along their concentration gradients (Kostal et al., 2006; MacMillan et al., 2011). Further, It is believed that an insect's recovery period from a chill coma depends on its capacity to restore K+ homeostasis and the extent to which homeostasis is compromised in the cold. This is because high hemolymph (K+) promotes muscle cell depolarization (Andersen, et al., 2019; Kostal, et al., 2004; MacMillan, et al., 2012; MacMillan, et al., 2014). The degree to which homeostasis is lost in the cold is also related to survival: elevated hemolymph (K+) is toxic and can cause cellular Ca2+ overload, which can result in chilling injury and death (Bayley, et al., 2020; MacMillan, et al., 2015). Hence in the present findings suggest that while *Piper longum* can enhance cold resistance at lower concentrations and shorter exposure times, its efficacy may decrease with longer exposure durations. Further research is needed to understand the mechanisms behind these effects and to explore optimal dosages and exposure times for maximizing cold resistance in *Drosophila melanogaster*.

Conclusion

This study explored the effects of *Piper longum* on the cold resistance of *Drosophila melanogaster*, focusing on different concentrations and exposure durations. The findings reveal that *Piper longum* can enhance cold resistance in *Drosophila melanogaster*, with notable differences based on sex, concentration, and exposure time. For 10-minute cold exposure, both male and female *Drosophila* fed with 10 mg *Piper longum t*reated media showed significantly faster recovery times compared to those fed with control media. This suggests that lower concentrations of *Piper longum* are effective in improving cold tolerance for short durations. In contrast, for 20- minute cold exposure, *Drosophila* fed with control media exhibited faster recovery times than those fed with *Piper longum* treated media. This indicates that the efficacy of *Piper longum* in enhancing cold resistance diminishes with longer exposure durations. The study highlights the importance of considering concentration and exposure time when using *Piper longum* to enhance cold resistance in insects. While lower concentrations and shorter exposures show promise, longer exposures may

require adjustments in treatment strategies to maintain efficacy. Overall, these findings contribute to a better understanding of how *Piper longum* can be utilized to enhance cold resistance in *Drosophila melanogaster*, potentially aiding in the development of more robust strategies for managing insect populations under varying thermal conditions. Further research is recommended to elucidate the underlying mechanisms and to optimize treatment protocols for different environmental scenarios.

Results:

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