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The Effect of Multivitamins Supplementation on the Rate of Development and Percentage of Hatchability in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster is a widely used model organism for studying developmental biology, where traits such as developmental rate and hatchability serve as important indicators of pre-adult fitness. Nutritional supplementation plays a crucial role in influencing developmental and physiological processes in organisms. In the present study, flies were reared on wheat cream agar medium (control) and multivitamin powder supplemented media containing T1 (6.75g) and T2 (13.5g) concentrations to investigate the effect of multivitamin supplementation on the rate of development and percentage of hatchability from larva to pupa and pupa to adult in D. melanogaster.

The group receiving T2 (13.5g) multivitamin supplementation exhibited the shortest developmental duration from larva to pupa, followed by the T1 (6.75g) treatment group, whereas the control group showed the slowest larval development. In contrast, during the pupa-to-adult transition, the control group displayed the fastest developmental rate, while flies reared on T1 (6.75g) multivitamin supplementation showed delayed development, followed by the T2 (13.5g) treatment group. These findings indicate that multivitamin supplementation significantly influences developmental timing in *D. melanogaster* depending on the developmental stage and concentration used.

Hatchability analysis revealed that larva-to-pupa survival percentage was highest in flies supplemented with T1 (6.75g) multivitamin powder, followed by the T2 (13.5g) treatment group, while the control group showed the lowest hatchability. Similarly, pupa-to-adult hatchability was significantly increased in the T1 (6.75g) supplemented group compared to both the control and T2 (13.5g) groups. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed significant differences among the experimental groups.

Overall, the findings suggest that multivitamin supplementation enhances pre-adult fitness parameters in *D. melanogaster*, particularly by improving hatchability and accelerating larval development at optimal concentrations. The study highlights the importance of dietary micronutrient supplementation in regulating developmental physiology and survival in model organisms.

Keywords: *Drosophila melanogaster*, multivitamin supplementation on development rate and percentage of hatchability.

Introduction

Drosophila melanogaster has long been recognized as one of the most important model organisms in genetics, developmental biology, physiology, and nutritional studies because of its short life cycle, easy laboratory maintenance, and high reproductive capacity. Developmental traits such as rate of development and percentage of hatchability are considered reliable indicators of pre-adult fitness and are strongly influenced by environmental and nutritional factors. Nutritional quality directly affects growth, metabolism, reproductive success, survival, and stress resistance in insects *(Hoffmann and Parsons, 1991; Lee et al., 2008; Simpson and Raubenheimer, 2012)*. Since food serves as the primary source of energy and essential nutrients, any variation in dietary composition can profoundly alter the physiological and developmental processes of organisms *(Sterner and Schulz, 1998; Taylor et al., 2005)*.

Environmental and dietary factors play a crucial role in shaping the life-history traits of insects by influencing nutrient allocation toward growth, maintenance, and reproduction *(Raubenheimer and Simpson, 1999; Chown and Nicolson, 2004)*. Proper nutritional balance is essential for successful development, while deficiencies or imbalances in proteins, carbohydrates, lipids, vitamins, and minerals can negatively affect developmental timing, survival, and reproductive fitness *(Bauerfeind and Fischer, 2005; Wang and Clark, 1995)*. In *D. melanogaster*, dietary composition has been shown to regulate larval growth, pupation, adult emergence, fertility, and lifespan *(Markow et al., 1999; Markow et al., 2001)*. Protein-rich and nutritionally balanced diets are known to accelerate developmental progression and improve egg-to-adult viability, whereas nutritionally poor diets often delay development and reduce survival *(Mattson, 1980; Hendrichs et al., 1991; Rodrigues et al., 2015)*.

Several experimental studies have demonstrated the influence of natural and artificial dietary supplements on pre-adult fitness in *D. melanogaste*r. Organic fruits such as chikku and watermelon significantly enhanced developmental rate and

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hatchability in *Drosophila* species *(Geeth and Krishna, 2015*). Similarly, yogurt and natural energy drinks improved larval survival and developmental progression, suggesting that nutrient-rich supplements positively influence physiological fitness *(Alexander and Krishna, 2018; D'Souza and Krishna, 2015)*. Commercial nutritional supplements have also been investigated extensively in recent years. Whey protein supplementation promoted favorable developmental conditions and improved developmental consistency in *D. melanogaster (Manaswini D. Kashyap et al., 2024)*. Mass gainer supplementation enhanced developmental speed and hatchability at optimal concentrations *(Aysha Barira H. M. et al., 2024),* while spirulina supplementation improved larval viability and pupation success *(Shreejani H.K. et al., 2023)*. Likewise, Ensure® supplementation increased hatchability percentages despite causing slight developmental delays, indicating that nutritional supplementation may affect developmental rate and survival differently *(Suma S. et al., 2024)*.

Vitamins are essential micronutrients required for numerous biochemical and physiological processes, including cellular metabolism, enzymatic activity, energy production, tissue growth, immune regulation, and oxidative stress management. Multivitamin supplements contain a combination of water-soluble and fat-soluble vitamins along with essential minerals that collectively support metabolic homeostasis and developmental processes. Vitamins such as B-complex vitamins are directly involved in carbohydrate, protein, and lipid metabolism, while vitamins A, C, D, and E contribute to tissue differentiation, antioxidant defense, and cellular protection *(Combs, 2012; Ball, 2006)*. Adequate vitamin supplementation has been associated with improved growth, metabolic efficiency, and developmental performance in various organisms. Antioxidant vitamins, in particular, help reduce oxidative stress generated during rapid cellular growth and differentiation *(Finkel and Holbrook, 2000)*.

Previous studies have shown that micronutrient supplementation can positively influence developmental physiology and reproductive success in insects. Dietary vitamin enrichment improves larval growth and enhances stress tolerance by supporting enzymatic and metabolic pathways necessary for normal development *(Simpson and Raubenheimer, 2012)*. Since D. melanogaster is highly sensitive to nutritional variation, it serves as an ideal organism for evaluating the biological effects of dietary supplements, including multivitamins, on developmental parameters. However, despite extensive studies on proteins, fruits, algae, and commercial supplements, very limited information is available regarding the effect of multivitamin supplementation on developmental rate and hatchability in *D. melanogaster*.

Therefore, the present study was undertaken to investigate the effect of multivitamin powder supplementation on the rate of development and percentage of hatchability from larva to pupa and pupa to adult in *D. melanogaster*. The study specifically aimed to evaluate whether different concentrations of multivitamin supplementation, namely T1 (6.75g) and T2 (13.5g), could alter developmental timing and pre-adult viability when compared to the control group maintained on standard wheat crea agar media.

Materials and Methods

Multivitamin tablets of the MuscleBlaze brand were purchased from Apollo Pharmacy (Mysore, Karnataka). The tablets were finely powdered using a clean mortar and pestle to obtain a uniform mixture. The powdered multivitamin was used for preparing experimental diets at different concentrations.

Establishment of Stock

For the present investigation, the experimental Oregon K strain of *Drosophila melanogaster* was procured from the *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore, Mysore.

The stock culture was maintained in culture bottles containing wheat cream agar medium. The medium was prepared by boiling 100 g of jaggery, 100 g of wheat cream rava, and 10 g of agar in 1000 ml of distilled water, followed by the addition of 7.5 ml of propionic acid. The flies were reared under controlled laboratory conditions at a temperature of 22 ± 1°C, 70% relative humidity, and a 12 : 12 h light-dark cycle. The above cultured flies were used to establish the experimental flies with different treatment media.

Control Media/Wheat Cream Agar Media

This media was prepared by adding 100g of jiggery, 100g of wheat rava powder, 10g of Agar in 1000ml of boiling distilled water. To avoid fungal growth, 7.5ml of Propanoic acid was added.

Experimental Procedure

To assess the effect of multivitamin powder supplementation on developmental parameters in *Drosophila melanogaster*, approximately 20 adult flies (10 males and 10 females) reared on standard culture medium were selected. These flies were transferred separately into culture bottles containing either control medium or multivitamin powder supplemented media consisting of T1 (6.75g) and T2 (13.5g). The flies were allowed to remain in the bottles for 3 hours to facilitate egg laying. Following oviposition, the adult flies were removed carefully, and the culture bottles were left undisturbed for 24 hours to permit the eggs to hatch into first instar larvae.

Rate of Development Analysis

Thirty first instar larvae were collected separately from each experimental group by gently scooping them from the culture medium. The larvae were then transferred into fresh vials containing their respective media: control, T1 (6.75g), and T2 (13.5g) multivitamin powder supplemented media. The larvae were monitored regularly to record the exact duration required for their transformation from larva to pupa. Observations were continued until adult emergence from the pupal stage, and the total developmental duration from larva to pupa and pupa to adult was recorded in hours for all experimental groups.

Hatchability Percentage Assessment

Another batch of thirty first instar larvae from each treatment group (control, T1 (6.75g), and T2 (13.5g) multivitamin powder supplemented groups) was introduced separately into culture vials containing their corresponding media. The larvae were continuously observed until pupation, and the total number of larvae successfully transformed into pupae was recorded in percentage. Subsequently, the number of pupae that successfully emerged into adult flies was also recorded in percentage. The experiment was conducted independently and continuously for both control and treated groups under uniform laboratory conditions.

Result

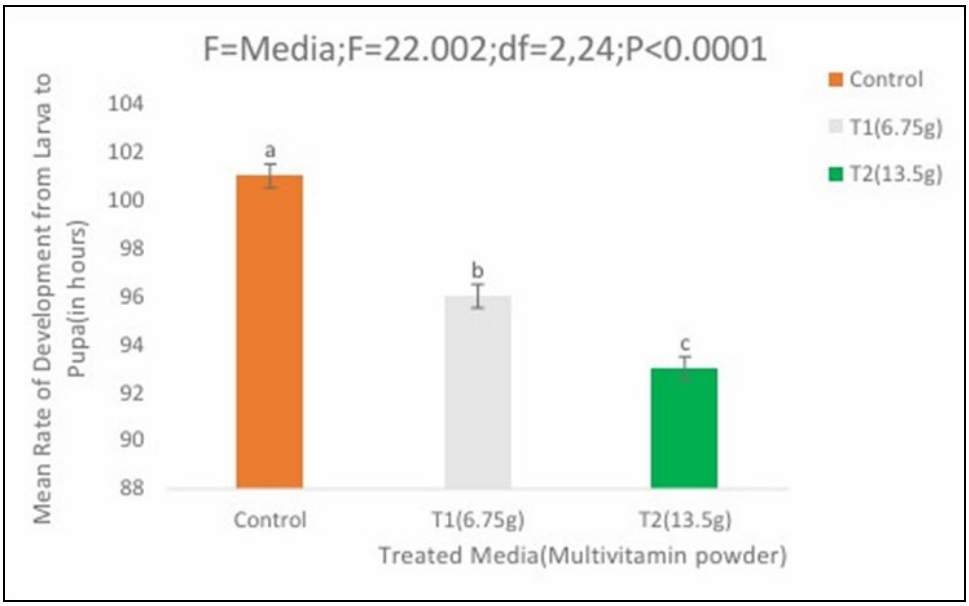

Fig 1: Effect of multivitamin powder supplemented diets on the rate of development from larva to pupa in *Drosophila melanogaster.*

Distinct letters on the bar graph represent statistically significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Larva to Pupa

Figure 1 shows the mean and standard error values of larva-to-pupa development rate in *D. melanogaster* reared on diets with different multivitamin powder concentrations (Control, T1 (6.75g), and T2 (13.5g)). According to the data, the development was slowest in flies fed with the control diet, followed by the T1 (6.75g) multivitamin supplemented diet, while faster development was observed in the T2 (13.5g) multivitamin supplemented diet, which showed the shortest developmental duration.

The experimental data were analyzed using one-way ANOVA followed by Tukey's post hoc test, which revealed statistically significant differences in the rate of development among the different diet groups. A significant difference was observed between the control group and both treatment groups, while the T1 (6.75g) group also showed a significant difference when compared with the T2 (13.5g) group by Tukey's post hoc test.

Thus, according to Tukey's post hoc analysis, larvae fed with the T2 (13.5g) multivitamin supplemented diet exhibited the fastest larva-to-pupa developmental rate compared to the control and T1 (6.75g) groups.

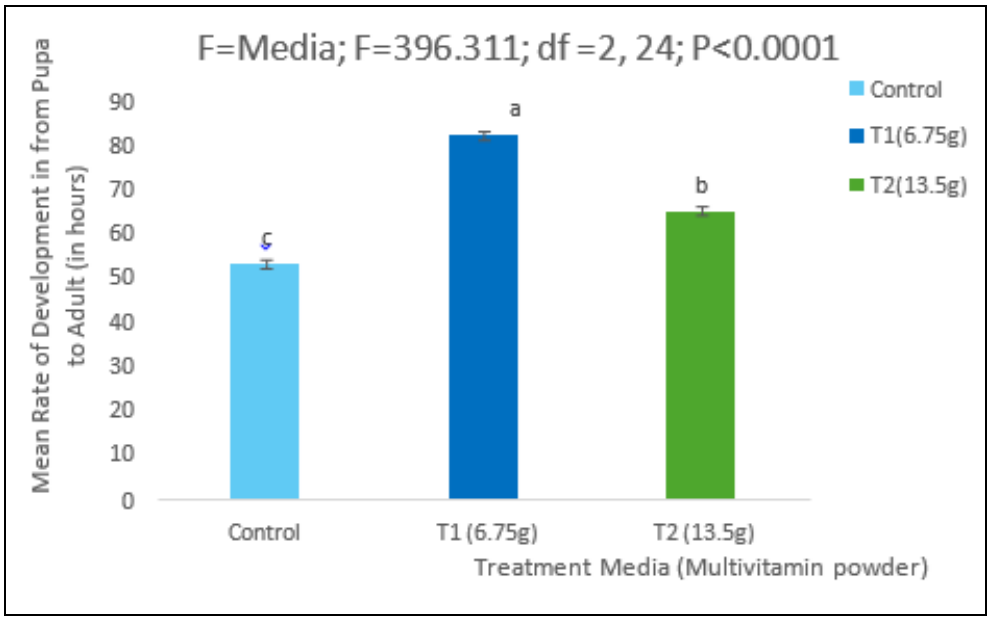

Fig 2: Effect of multivitamin powder supplemented diets on the rate of development from pupa to adult in *Drosophila melanogaster.*

Distinct letters on the bar graph represent statistically significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Pupa to Adult

Figure 2 showed the mean and standard error value of pupa-to-adult rate of development in *D. melanogaster* flies cultured in different diets (Control, T1 (6.75g), and T2 (13.5g)). The

flies maintained on the control diet showed the fastest development, followed by the T2 (13.5g) multivitamin supplemented diet, whereas flies fed with the T1 (6.75g) supplemented diet exhibited the slowest development.

The results were analyzed using one-way ANOVA followed by Tukey's post hoc test. A significant difference was observed between the control group and the T1 (6.75g) and T2 (13.5g) groups. Similarly, the T1 (6.75g) group showed significant variation when compared with the T2 (13.5g) group.

Overall, the findings suggest that multivitamin powder supplementation influenced the rate of development from pupa to adult in *D. melanogaste*r, with the control group exhibiting the shortest developmental duration.

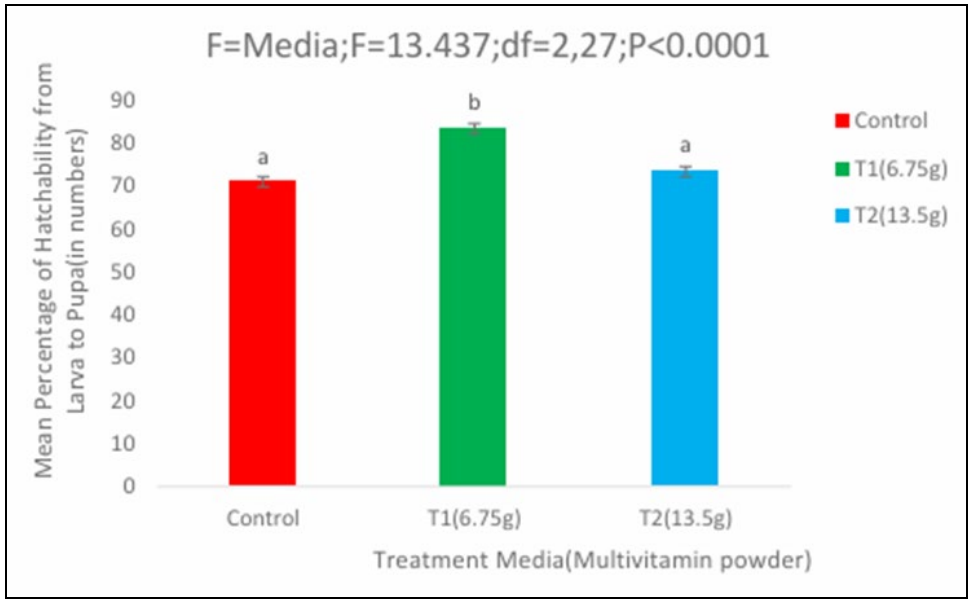

Fig 3: Effect of multivitamin powder supplemented diets on the % of hatchability from larva to pupa in *Drosophila melanogaster.*

Distinct letters on the bar graph represent statistically non-significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Larva to Pupa

Figure 3 illustrates the effect of multivitamin powder supplemented diets on the percentage of hatchability from larva to pupa in *D. melanogaster.* The flies were raised on three different diets: a standard control diet and diets supplemented with T1 (6.75g) and T2 (13.5g) multivitamin powder. Among these, the T1 (6.75g) supplemented group showed the highest percentage of larva-to-pupa hatchability, followed by the T2 (13.5g) group, whereas the control group exhibited the lowest hatchability percentage.

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed statistically significant differences among the experimental groups. The T1 (6.75g) group showed significantly higher larva-to-pupa hatchability when compared with the control group, whereas the T2 (13.5g) group showed moderate improvement in hatchability percentage.

Overall, the results indicate that multivitamin powder supplementation, particularly at T1 (6.75g) concentration, positively influenced larva-to-pupa hatchability and pre-adult viability in *D. melanogaster.*

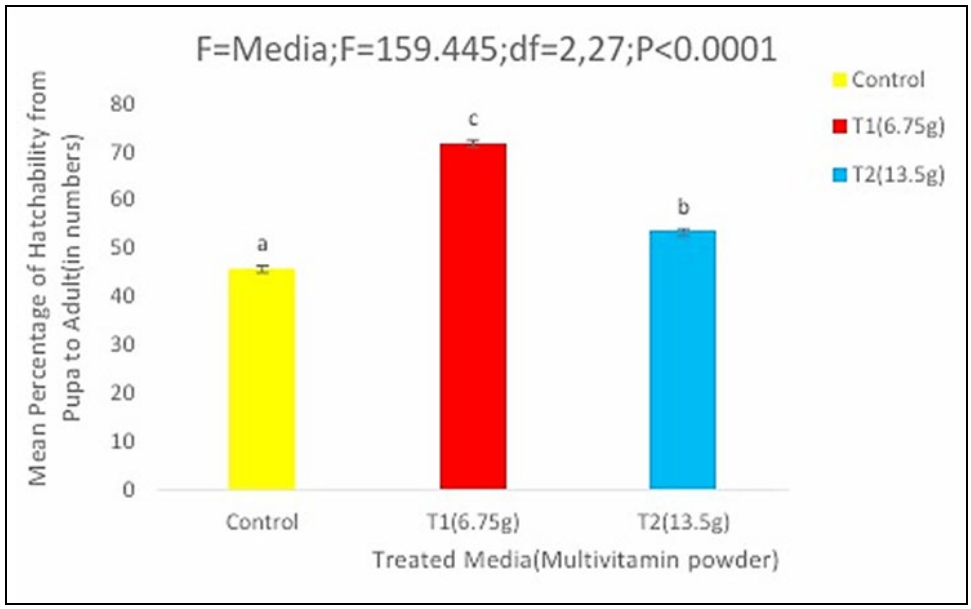

Fig 4: Effect of multivitamin powder supplemented diets on the % of hatchability from pupa to adult in *Drosophila melanogaster.*

Distinct letters on the bar graph represent statistically non-significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Pupa to Adult

Figure 4 illustrates the effect of multivitamin powder supplemented diets on the percentage of hatchability from pupa to adult in *D. melanogaster.* The flies were raised on three different diets: a standard control diet and diets supplemented with T1 (6.75g) and T2 (13.5g) multivitamin powder. Among these, the T1 (6.75g) supplemented group showed the highest percentage of pupa-to-adult hatchability, followed by the T2 (13.5g) group, whereas the control group exhibited the lowest hatchability percentage.

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed statistically significant differences among the experimental groups. The T1 (6.75g) group showed significantly higher pupa-to-adult hatchability when compared with the control group, whereas the T2 (13.5g) group showed moderate improvement in hatchability percentage.

Overall, the results indicate that multivitamin powder supplementation, particularly at T1 (6.75g) concentration, positively influenced pupa-to-adult hatchability and pre-adult viability in *D. melanogaster*.

Discussion

The Effect of Multivitamin Powder on the Rate of Development in *Drosophila melanogaster.*

The nutritional environment is a major determinant of several life history traits in developing organisms, influencing developmental timing, survival, reproduction, metabolic activity, stress resistance, and physiological fitness *(Simpson and Raubenheimer, 2012)* [35]. The quality and quantity of nutrients available during development play an essential role in providing energy and biochemical substrates necessary for cellular growth and differentiation. In insects, dietary composition strongly regulates larval growth, pupation, and adult emergence, ultimately affecting organismal fitness and reproductive success *(Hoffmann and Parsons, 1991; Lee et al., 2008; Sterner and Schulz, 1998)*. In the present study, we investigated the effects of different concentrations of multivitamin powder supplementation on pre-adult fitness parameters, specifically developmental rate and hatchability percentage in *D. melanogaster*.

Multivitamin supplements are rich sources of essential vitamins and minerals required for numerous metabolic pathways, enzymatic reactions, antioxidant defense systems, tissue differentiation, and energy metabolism. Vitamins such as B-complex vitamins are directly involved in carbohydrate, lipid, and protein metabolism, whereas vitamins A, C, D, and E contribute to cellular protection and oxidative stress regulation *(Ball, 2006; Combs, 2012).* Since rapidly developing organisms require substantial nutritional support for proper growth and differentiation, multivitamin supplementation may influence developmental physiology and survival outcomes in *D. melanogaster*.

Experimental groups were maintained on diets supplemented with T1 (6.75g) and T2 (13.5g) multivitamin powder along with the control wheat cream agar medium. As shown in Fig. 1, larvae reared on T2 (13.5g) supplemented diet exhibited the fastest larva-to-pupa development, followed by the T1 (6.75g) group, while the control group showed delayed development. However, Fig. 2 demonstrated variation in the pupa-to-adult developmental stage, where the control group exhibited the shortest developmental duration, while T1 (6.75g) supplementation resulted in comparatively slower development. Fig. 3 and Fig. 4 revealed that multivitamin supplementation positively influenced hatchability percentage, with the T1 (6.75g) group showing the highest larva-to-pupa and pupa-to-adult survival compared to the control and T2 (13.5g) groups. These observations suggest that multivitamin supplementation significantly affects developmental physiology depending on both concentration and developmental stage.

Several earlier studies have demonstrated the importance of nutritional supplementation in improving developmental fitness in *D. melanogaster. Rodrigues et al. (2015)* [32] reported that balanced nutritional conditions reduced developmental duration and improved larval progression. *Geeth and Krishna (2015)* [15] showed that natural fruit supplementation accelerated pre-adult development and improved survival in *Drosophila* species. Similarly, *D'Souza and Krishna (2015)* [13] observed enhanced developmental performance in flies supplemented with natural energy drinks. *Alexander and Krishna (2018)* [2] reported that yogurt supplementation improved larval-to-pupal transitions due to enhanced nutritional availability and gut health. Commercial supplements such as whey protein, spirulina, and mass gainer were also found to positively influence developmental progression and hatchability in *D. melanogaster (Manaswini D. Kashyap et al., 2024; Aysha Barira H.M. et al., 2024; Shreejani H.K. et al., 2023).*

In the present study, the accelerated larva-to-pupa development observed in the T2 (13.5g) supplemented group may be attributed to the availability of essential vitamins and minerals required for energy metabolism and cellular growth. Multivitamins support several enzymatic pathways involved in ATP production, nutrient utilization, and tissue differentiation, thereby enhancing developmental progression. Vitamins also function as co-factors in metabolic reactions and may improve physiological efficiency during active larval growth. Similar findings were reported by *Suma S. et al. (2024)* [37], where Ensure® supplementation influenced developmental timing and hatchability in *D. melanogaster.*

However, the pupa-to-adult developmental pattern observed in Fig. 2 suggests that excessive or altered micronutrient balance may influence metamorphic processes differently during later developmental stages. The comparatively slower development in T1 (6.75g) supplemented flies may indicate that nutrient utilization varies between larval and pupal stages. Earlier investigations by *Layalle et al. (2008)* [22] demonstrated that nutritional regulation significantly affects hormonal signaling and developmental timing in *Drosophila*. Similarly, *Guler and Kaya (2014, 2015)* [16, 17] reported that dietary imbalance and altered protein availability prolonged developmental duration and influenced pupation dynamics. Therefore, stage-specific physiological responses to multivitamin supplementation may account for the developmental variations observed in the present study.

The present study also demonstrated that multivitamin supplementation positively influenced hatchability and pre-adult viability. As observed in Fig. 3 and Fig. 4, the T1 (6.75g) supplemented group exhibited the highest hatchability percentages during both larva-to-pupa and pupa-to-adult transitions. This suggests that moderate multivitamin supplementation provides optimal micronutrient availability necessary for survival during critical developmental stages. Vitamins and minerals play important roles in cellular

maintenance, membrane stability, antioxidant defense, and enzymatic activity, all of which contribute to improved organismal viability. Similar improvements in hatchability and developmental survival have been reported in flies supplemented with nutrient-rich diets and natural food sources *(Sisodia and Singh, 2015; Geeth and Krishna, 2015; D'Souza and Krishna, 2015)*.

Conversely, the comparatively lower hatchability observed in the control group indicates that standard wheat cream agar medium may provide limited micronutrient support for optimal developmental success. The slightly reduced hatchability in the T2 (13.5g) group compared to T1 (6.75g) suggests that excessive supplementation may not always enhance survival outcomes and could potentially alter metabolic balance. Previous studies have similarly reported that excessive or imbalanced nutrient concentrations may negatively affect developmental physiology and survival in *Drosophila (Rodrigues et al., 2015; Kaya et al., 2016).*

Overall, the present study demonstrates that multivitamin powder supplementation significantly influences developmental rate and hatchability in *D. melanogaster.* The findings indicate that optimal concentrations of multivitamin supplementation, particularly T1 (6.75g), enhance hatchability and pre-adult survival, while higher supplementation levels such as T2 (13.5g) accelerate larval development. Since the experiments were conducted under controlled environmental conditions with uniform temperature and photoperiod, the observed differences can be attributed primarily to dietary variation. These findings emphasize the importance of micronutrient supplementation in regulating developmental physiology and pre-adult fitness in model organisms.

Conclusion

In conclusion, the findings of the present study suggest that multivitamin powder supplementation significantly influences the rate of development and percentage of hatchability in *Drosophila melanogaster*. The results demonstrated that higher supplementation levels, particularly T2 (13.5g), accelerated larva-to-pupa development, whereas T1 (6.75g) supplementation showed improved hatchability and pre-adult viability. These observations indicate that multivitamin supplementation, rich in essential vitamins and minerals, can enhance developmental physiology and survival in *D. melanogaster*. Thus, multivitamin powder may serve as an effective dietary supplement for improving pre-adult fitness parameters in model organisms under controlled laboratory conditions.

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