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Research Article

Impact of pomegranate extracts on longevity and wing fluctuating asymmetry in *Drosophila melanogaster*: A geometric morphometric analysis

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Abstract. Longevity plays a significant role in determining an animal's overall health. Nutrition is a key factor that can influence an animal's longevity. Studying the relationship between wing symmetry and longevity provides a useful approach to understanding how nutrition can affect both longevity and symmetry. The effect of Pomegranate Juice (PJ) and Pomegranate Peel (PP) on longevity and Fluctuating Asymmetry (FA) of wings in male and female *Drosophila melanogaster* was analysed using Geometric Morphometric Analysis (GMA). The study revealed that PJ and PP-treated flies showed increased longevity and the highest FA compared to control in both males and females. Between PJ and PP-treated flies, PJ had the highest longevity in both males and females whereas PP flies had higher FA in both sexes. Between the sexes, females had lower longevity and FA values compared to males. Overall GMA study reveals shape variation for wings and the presence of a strong FA in PJ and PP flies. Pearson correlation revealed a strong positive association between longevity and wing FA.

Keywords: Pomegranate extracts, Wing Symmetry, GMA, Lifespan, *Drosophila*

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Introduction

Ageing is a biological process that differs among species. It is time-dependent and an integral part of development that leads to a decline in physiological functions (Aunan *et al.*, 2016). Once an organism has reached its full growth and maturity, it may successfully reproduce and end in senescence (Arking, 2006). The longevity of a species reflects the extent of its biological ageing and represents the average duration of an organism living in its environment (Aguiar-Oliveira & Bartke, 2018). It is consistently linked to an organism's fitness. It is also influenced by environmental, developmental, and genetic factors (Hu *et al.*, 2022). Studies reveal that the longevity and ageing delay was enhanced in many model organisms by the use of fruit extracts, herbal compounds, mineral supplements, other chemical treatments and pesticides (Chattopadhyay & Thirumurugan, 2018; Parashar *et al.*, 2023), for instance Apple (Vayndorf *et al.*, 2013), Blueberry (Peng *et al.*, 2012), Asparagus (Kumar *et al.*, 2015), Turmeric (Rawal *et al.*, 2014), Cinnamon (Schriner *et al.*, 2014), Nectarine (Boyd *et al.*, 2011), Cocoa (Martorell *et al.*, 2011), chemical compound (Castillo-Quan *et al.*, 2016), pesticides (Marcus & Fiumera, 2016) etc. on various lower model organisms like *Saccharomyces cerevisiae*, *Caenorhabditis elegans,* and *Drosophila melanogaster*.

Pomegranate is a nutrient-rich fruit that contains high amounts of bioactive molecules that have been mostly used in traditional medicine (Chinese, Unani and Ayurvedic) for treating several diseases (Zhao & Yuan, 2021; Asgharpour & Alirezaei, 2021; Ciccone *et al.*, 2023; Wang *et al.*, 2023). Pomegranate Juice (PJ) and Pomegranate Peel (PP) are known for their strong antioxidant properties due to their abundant polyphenols including anthocyanins, tannins, flavonoids, flavanols, phenolic acids, and lignans possess anti-ageing properties (Riaz and Khan, 2016; Fathy *et al.*, 2021; Giri *et al.*, 2023). According to Balasubramani *et al.,* (2014), PJ-treated *D. melanogaster* flies showed increase in their longevity. There are no reports on the effect of PP on longevity in any animal models.

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Bilateral symmetry is a condition in which similar anatomical organs are situated on the left and right sides of a median axis in which one plane can divide the organism into two identical halves. Similar to longevity, it is also dependent upon its developmental process and environmental factors (Manuel, 2009; Savriama & Klingenberg, 2011). Fluctuating Asymmetry (FA) is a process of slight random deviation from perfect bilateral symmetry (Graham *et al.*, 2010). It is a tool to measure developmental instability, where it is influenced by environmental and genetic factors on individual development (Nunes *et al.*, 2022).

The wing of *D. melanogaster* is a very vital structure for flight. It is bilaterally symmetrical, with all its venations distributed equally on either side. The wing size and shape and other dimensions such as wing length, wing width and its indices are influential in determining its reproductive success (Antson *et al.*, 2022). Morphometry of a wing can determine its size, shape, and other attributes. Wing morphology may vary when an organism's development process is exposed to environmental and nutritional stress (Hoffmann *et al.*, 2005). Geometric Morphometric Analysis (GMA) is a modern technique of analysing shape and size of any object involving geometric principles with statistical analyses and precisely quantifies shape variations and FA (Vilaseca *et al.*, 2022). GMA has been studied in different organisms like amphibians (Larson, 2005), reptiles (Gurgis *et al.*, 2019), birds (Foster, 2018), mammals (Lang & Martin, 2022), insects and other invertebrates (Li *et al.*, 2022; Chen *et al.*, 2022). GMA studies also involve wing shape in response to nutritional stress in insects i.e *Bactrocera dorsalis* and *Ceratitis capitata* reared on different fruits and nanoparticles (Cvetkovic *et al.*, 2020; Posgai *et al.*, 2011; Pieterse, 2017).

Wing FA has been studied on insects like *Argia tinctipennis* (Pinto *et al.*, 2012), the Butterfly community of the families Nymphalidae, Hesperiidae, Pieridae, Lycaenidae and Papilionidae (Henriques & Cornelissen, 2019), *D. melanogaster, D. buzzatii, D. koepferae* (Carreira *et al.*, 2008), *Chironomus tepperi, Lucilia cuprina, Epiphyas postvittana* (Hoffmann *et al.*, 2005) at various environmental and nutritional stressors and population density (Palmer & Strobeck, 1986), insecticides (Nattero *et al.*, 2019). Studies involving longevity, wing FA, and wing shape variation are very few to mention such as in *Musca domestica* (Akhmetkireeva *et al.*, 2018) and in *D. melanogaster*, where a comparison was made between wings of a normal laboratory population and ablated wing population (Harshman *et al.*, 2005). Most of the above-mentioned studies have either addressed longevity or FA separately. The main objective of the present study is to observe how a fruit extract (Pomegranate) which is already known to influence ageing in flies would affect the FA. In view of this present study, we aimed to analyse the relationship between wing FA and longevity in *D. melanogaster* using Pomegranate Juice and Pomegranate Peel.

Materials and methods

Drosophila stock culture and maintenance

D. melanogaster (Oregon-K) was obtained from the *Drosophila* Stock Centre, Department of Zoology, University of Mysore, India. The flies were cultured and maintained in the laboratory under controlled conditions of 22±1°C and 70-80% relative humidity (Shivanna *et al.*, 1996). For the experiment, ten virgin males and females were randomly chosen from the main stock culture and allowed to interbreed, forming a distinct experimental culture.

Preparation of Pomegranate Juice (PJ) and Pomegranate Peel (PP) solution

Pomegranate fruits were washed, arils were separated and ground in a grinder, the solution was filtered using Whatman No.1filter paper and the filtrate was stored in a refrigerator (Balasubramani *et al.*, 2014). Peels from the pomegranate fruit were removed, washed in distilled water and dried in oven. Later the dried peels were ground to a fine powder in a mixer, the powder was stored in an airtight bottle in cold condition. The peel powder was dissolved in distilled water and filtered (Whatman No.1), and the filtrate was used for experiments (El-Said, 2014; Kennas, 2020). The F1 larvae from the experimental culture were used for both the control and treatments involving pomegranate juice (PJ) and pomegranate peel (PP). PP extract of 10g, 15g, and 20g was dissolved in 100ml of distilled water then yeast granules were added to prepare 10%, 15%, and 20% PP solution. The longevity experiment showed the highest lifespan of flies at 15% of PP compared to control and other treatments, therefore the 15% PP was considered as a suitable concentration for treatments. The yeast was dissolved in 15% PP and 10% PJ was used for further experiments (Balasubramani *et al.*, 2014).

Longevity assay

Newly eclosed virgin male and female flies were transferred to new vials every 2-3 days and mortality was recorded every 12 hours. The total lifespan was tracked until the death of last fly. 10 replicates were maintained for each sex with one fly per vial. Longevity data were analyzed using Kaplan-Meier survival analysis, the log-rank (Mantel-Cox) test, the Gehan-Breslow-Wilcoxon test followed by one-way ANOVA, *t*-test to assess survival differences between treatments using SPSS version 21.0.

Wing preparation for Geometric Morphometry Analysis (GMA)

Adults (1 day old) of both sexes were stored in 70% ethanol (v/v), after 24 hours (Cvetkovic *et al*., 2020), thirty individual flies were dissected under a microscope with a drop of 70% ethanol, both wings were mounted on a glass slide and photographed (Alba *et al*., 2021), then measured using an Olympus phase contrast microscope (BX51) and an Olympus stereo zoom microscope (SZX16) equipped with a Magcam DC5 camera, using MagVision version x64 software.

GMA and Fluctuating asymmetry (FA) of wing

GMA was carried out to determine the differences in wing size and shape (Zelditch *et al*., 2012). Fifteen landmarks were selected for both the right and left wings following the method of Gidaszewski *et al*., (2009). These landmarks were digitized using the software TpsDig 2w64 V.2.32 (Rohlf, 2015) (Fig. 1). To analyse the variation in wing shapes, a general Procrustes analysis was performed by applying a Procrustes fit to the landmark data, as described by Klingenberg (2011). To assess measurement error (ME), both the right and left wings were digitized twice (Vilaseca *et al*., 2022). Measurement error was evaluated across all groups to prevent any errors related to the data. The results from a Procrustes ANOVA showed that the mean square (MS) values for FA (Individual*side) were higher than the MS values for error, indicating that measurement error was absent in the data.

The effect of all treatments on wing shape changes was evaluated using Procrustes ANOVA (Klingenberg *et al*., 2002). To identify morphometric differences between treatments, Canonical Variate Analysis (CVA) was performed on the Procrustes coordinates to visualize the variation between control and treatment groups. Confidence ellipses for the wing shape of each group were calculated. Simultaneously, FA of wings from control and treated flies was compared (Vilaseca *et al*., 2022). The Procrustes ANOVA conducted for wing shape was used for FA analysis (Debat *et al*., 2008). FA intensity was analysed using, i) the mean square (MS) values for the (Individual*Side) interaction from the Procrustes ANOVA and ii) a multivariate regression of shape as the dependent variable against Procrustes FA scores (the asymmetric component of the data) as described by Benitez *et al*., (2020). General Procrustes, Canonical Variate, and FA Analysis were performed using MorphoJ software (Klingenberg, 2011). To know the relationship between longevity and FA of wing among different treatments, data were subjected to the pearson correlation test using SPSS version 21.0.

Results

Longevity

The mean longevity of *D. melanogaster* treated with PP-10%, 15%, and 20% revealed that the average longevity of virgin males is 36.6 days, 43.2 days, and 42.3 days, respectively. Compared to the control, the results suggest a 10.73% and 8.63% increase in longevity for 15% and 20% and a decrease in 5.83% for 10% PP-treated flies. Among the varied concentrations, 15% treatment of PP showed an increase in longevity of 16.54% and 2.10% to 10% PP and 20% PP-treated flies. The mean longevity of females is 34.8 days, 40.6 days, and 39.0 days for 10% PP, 15% PP, and 20% PP, respectively. Compared to the control, the results suggest a 9.81% and 5.80% increase in longevity for 15% and 20% PP-treated flies and a decrease in 5.58% for 10% PP. The results suggest that there is an increase of 15.38%, 4.02% and longevity in 15% of PP than 10%, 20% PP-treated flies. The statistical analysis of longevity, log-rank $\chi 2$ (14.144) and Wilcoxon $\chi 2$ (13.646) test showed that the difference between control and treated flies in all treatment groups (Kaplan-Meier survival analysis) is found significant ($p<0.05$). One-way ANOVA showed a significant difference between the treatment groups (F=5.120, df-3, 76, $p<0.05$).

The mean longevity of *D. melanogaster* treated with 10% PJ and 15% PP revealed that the average longevity of virgin males is 45.2 days, 43.5 days, and for control is 38.80 days. Compared to the control, the results suggest a 15.23% and 11.42% increase in longevity for PJ and PP-treated flies and a decrease of 3.83% for PP when compared to PJ. In female, mean longevity is 42.1 days, 40.6 days, and 36.8 days for 10% PJ, 15% PP, and control, respectively. The results suggest a 13.43% and 9.81% increase in longevity for PJ and PP-treated flies when compared to control and a decrease of 3.62% for PP when compared to PJ (Fig. 2). The statistical analysis of longevity, log-rank $\chi 2$ (7.121), and Wilcoxon $\chi 2$ (7.861) test showed that the difference between control and treated flies in all treatment groups (Kaplan-Meier survival analysis) is found significant ($p<0.05$). *T*- test results revealed that, PJ (t-2.789, df-38, $p<0.05$) and PP (t-2.159, df-38, $p<0.05$) were found significant compared to control.

GMA and FA

Procrustes ANOVA showed a significant level of wing FA (Individual*side: $p < 0.0001$) was found in males and females of PJ and PP (Tables 1-6). Multivariate regression of Shape vs. Procrustes FA Scores revealed that FA intensity was found to be higher in PP, whereas lower for PJ-treated and control flies of both the sexes indicating an increase of FA from control to PP (Table 7 and Fig. 2). Figure 2 provides data on multivariate regression of shape showing the intensity of FA between males and females. In males, the control group and PJ group have points clustered around lower FA scores, while the PP group shows some individuals with higher FA scores. The relationship between the FA score and the regression score shows a positive trend, especially among higher FA scores, indicating that as asymmetry increases, the regression score also increases, particularly for the PP group (Fig. 2A).

In females, a narrower range of FA scores was observed, with most points clustered around lower values. The distribution of the groups is similar to males, but the overall range of FA scores is smaller. Again, there is a trend where higher FA scores are associated with higher regression scores, but the range of these scores is smaller compared to males. The PJ and PP groups appear to be more intermixed in this plot compared to males (Fig. 2B).

The influence of FA by sexes displayed that male flies from PP-treated groups showed higher levels of FA than the corresponding groups in the female flies (Table 7). CVA revealed a clear separate grouping along with their confidence ellipse between treatments and control of both male and female flies (Fig. 3).

Correlation between FA scores with Longevity

The control group has the lowest longevity in both males and females. The PJ group has the highest longevity in both sexes, noticeably higher than the control and PP groups. The PP group shows intermediate longevity, higher than the control but lower than the PJ group. FA values show an increasing trend as longevity increases across the groups. In males, the correlation between longevity and FA is r=0.66, indicating a moderate positive relationship. In females, the correlation is higher at r=0.92, indicating a strong positive relationship between longevity and FA (Fig. 4).

Table 1. Procrustes ANOVA for centroid size and shape of control male flies.

Centroid /Size/Effect	SS	MS	df	F	p	Pillai tr.	p (Param)
Individual	2576285.060938	88837.415894	29	1.08	0.4176		
Side	4882.931014	4882.931014	1	0.06	0.8091		
Ind*Side	2383137.620494	82177.159327	29	769.29	<.0001		
Error 1	6409.290220	106.821504	60				
Shape/Effect							
Individual	0.03152998	0.0000418169	754	0.99	0.5748	12.40	0.8988
Side	0.00210435	0.0000809366	26	1.91	0.0043	0.98	0.0462
Ind*Side	0.03196650	0.0000423959	754	3.12	<.0001	11.92	<.0001
Error	0.02116730	0.0000135688	1560				

Table 2. Procrustes ANOVA for centroid size and shape of PJ-treated male flies.

Centroid/Size/Effect	SS	MS	df	F	p	Pillai tr.	p (Param)
Individual	2388330.375299	82356.219838	29	0.82	0.7029		
Side	20376.685502	20376.685502	1	0.20	0.6560		
Ind*Side	2916502.150696	100569.039679	29	1376.74	<.0001		
Error 1	4382.931389	73.048856	60				
Shape/Effect							
Individual	0.04608689	0.0000611232	754	1.08	0.1579	13.38	0.2139
Side	0.00413197	0.0001589220	26	2.80	<.0001	0.84	0.6762
Ind*Side	0.04283807	0.0000568144	754	5.94	<.0001	12.94	<.0001
Error	0.01492060	0.0000095645	1560				

Table 3. Procrustes ANOVA for centroid size and shape of PP-treated male flies.

Centroid/Size/Effect	SS	MS	df	F	p	Pillai tr.	p (Param)
Individual	2553457.530697	88050.259679	29	0.77	0.7610		
Side	98559.931462	98559.931462	1	0.86	0.3620		
Ind*Side	3332092.119459	114899.728257	29	1531.23	<.0001		
Error 1	4502.254205	75.037570	60				
Shape/Effect							
Individual	0.05642466	0.0000748338	754	1.01	0.4603	13.37	0.2185
Side	0.00931720	0.0003583537	26	4.82	<.0001	0.99	0.0117
Ind*Side	0.05601632	0.0000742922	754	6.43	<.0001	11.46	<.0001
Error	0.01803384	0.0000115602	1560				

Table 4. Procrustes ANOVA for centroid size and shape of control female flies.

Centroid/Size/Effect	SS	MS	df	F	p	Pillai tr.	p (Param)
Individual	2568530.694129	88570.023935	29	2.76	0.0040		
Side	75811.737510	75811.737510	1	2.36	0.1353		
Ind*Side	931271.271121	32112.802452	29	385.74	<.0001		
Error 1	4995.046380	83.250773	60				
Shape/Effect							
Individual	0.04196942	0.0000556624	754	1.70	<.0001	13.80	0.0459
Side	0.00381288	0.0001466492	26	4.47	<.0001	0.98	0.0182
Ind*Side	0.02474024	0.0000328120	754	2.58	<.0001	11.52	<.0001
Error	0.01986604	0.0000127346	1560				

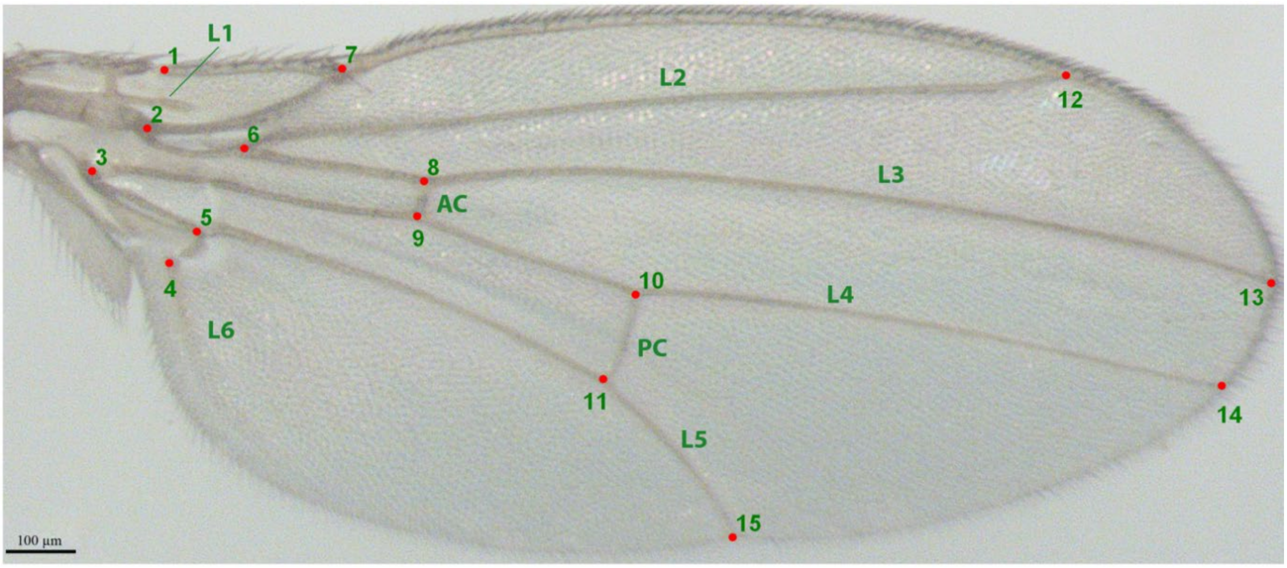

Fig. 1. The wing landmarks (1-15) for GMA with nomenclature, L-longitudinal veins (L1-L6), AC- anterior cross vein, PC- posterior cross vein. LM 1-9- proximal part of the wing; LM 10-15- distal part of the wing; LM 4,7, 12-15- edge of the wing; LM 4, 5, 11, 15- wing lobe, anal part of the wing (Gidaszewski *et al.*, 2009).

Table 5. Procrustes ANOVA for centroid size and shape of PJ-treated female flies.

Centroid/Size/Effect	SS	MS	df	F	*p*	Pillai tr.	*p* (Param)
Individual	2263516.338550	78052.287536	29	1.70	0.0799		
Side	873305.562933	873305.562933	1	19.00	0.0001		
Ind*Side	1332956.529313	45964.018252	29	1032.52	<.0001		
Error 1	2670.981379	44.516356	60				
Shape/Effect							
Individual	0.03419948	0.0000453574	754	1.03	0.3236	12.36	0.9109
Side	0.00670444	0.0002578629	26	5.88	<.0001	0.98	0.0220
Ind*Side	0.03307813	0.0000438702	754	7.90	<.0001	13.03	<.0001
Error	0.00866255	0.0000055529	1560				

Table 6. Procrustes ANOVA for centroid size and shape of PP-treated female flies.

Centroid/Size/Effect	SS	MS	df	F	*p*	Pillai tr.	*p* (Param)
Individual	1273322.218471	43907.662706	29	0.75	0.7835		
Side	108065.831869	108065.831869	1	1.83	0.1861		
Ind*Side	1708950.912549	58929.341812	29	834.35	<.0001		
Error 1	4237.741572	70.629026	60				
Shape/Effect							
Individual	0.04313834	0.0000572126	754	1.26	0.0008	13.09	0.4256
Side	0.00406150	0.0001562116	26	3.44	<.0001	0.99	0.0024
Ind*Side	0.03425300	0.0000454284	754	5.72	<.0001	12.89	<.0001
Error	0.01239305	0.0000079443	1560				

Table 7. Intensity of Fluctuating Asymmetry between treatments and each sex in *D. melanogaster* flies (N= 30) data of Procrustes ANOVA of shape.

	Male		Female	
	MS (Individual*Side)	Pillai tr.	MS (Individual*Side)	Pillai tr.
Control	0.0000423959	11.92	0.0000328120	11.52
PJ	0.0000568144	11.46	0.0000438702	13.03
PP	0.0000742922	12.94	0.0000454284	12.89

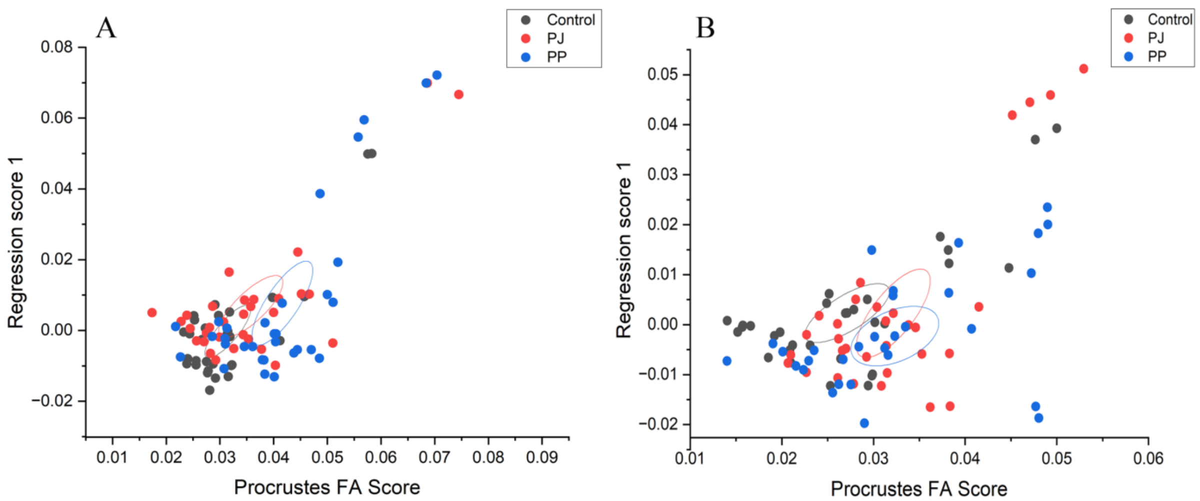

Fig. 2. Multivariate regression of Shape showing the intensity of FA between A- Male groups and B- Female treated groups.

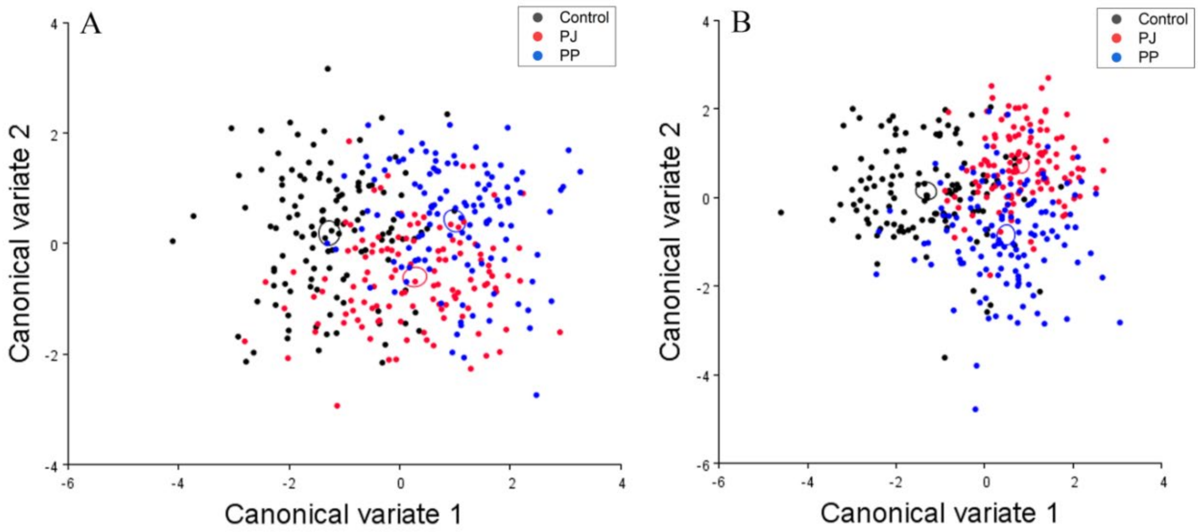

Fig. 3. Canonical variate analysis of differences in the wing shape between A- Males and B-Females treated groups.

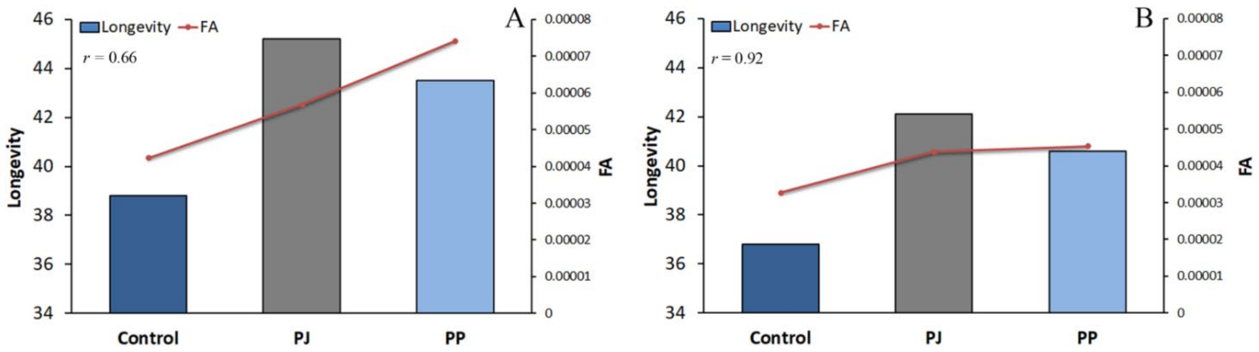

Fig. 4. Longevity and Fluctuating Asymmetry of wings of A- Males and B- Females of control and treated flies.

Discussion

Longevity and Treatments

In the present study, the results have shown an increase in the mean lifespan of *Drosophila* flies treated with PJ by 15.23% in males and 13.43% in females compared to the control group (Fig. 4). Pomegranate Juice treatment has significantly increased the median lifespan compared to control by 18.51% and 8% in male and female *D. melanogaster* flies, respectively. The increased longevity of the present study in both male and female *D. melanogaster* is on par with the findings of Balasubramani *et al*., (2014). An attempt was made to analyse the effect of PP extract on longevity. For 15% PP, the results revealed that there was an increase in lifespan of 10.73% in males and 9.81% in females when compared to the control. The longevity of flies treated with PP has slightly decreased compared to PJ i.e., 3.83% in males and 3.62% in females. Hence, between PJ and PP, it is the PJ extract that is more effective in enhancing the lifespan in *D. melanogaster* flies (Fig. 4). This may be due to high levels of antioxidants, polyphenols, and other bioactive compounds found in PJ and PP (Viuda-Martos *et al*., 2011; Almiahy & Juma, 2017; Akhtar *et al*., 2019; Yassin *et al*., 2021).

FA analysis

To provide more perspective on Procrustes FA analysis for control, PJ and PP males, overall size and shape are relatively consistent among individuals, the asymmetry between sides is both present and varies depending on the individual (Table 1-3). Similarly, control and treated females have consistent symmetry for individual and side, except PJ (significant difference between sides) for centroid size, but shape analysis has shown significant variations between individuals, sides and interaction between individual and side. This suggests that females displayed more asymmetry compared to males (Table 4-6). Vilaseca *et al.*, (2022) have reported wing centroid size and shape variation between individuals, side, and effect of side on size in different individuals in *Triatoma infestans* species among different populations of Bolivia. This study showed the importance of FA, and because of varied food conditions and the influence of insecticides on FA among different populations of intra and peridomicile regions. Significant differences in head shape and size were observed between adults of the same species fed on different food sources Nattero *et al.* (2013). This indicates genetic, environmental and nutritional factors that can influence asymmetry across the species (Graham, 2021; Lemic, 2023).

The intensity of FA (MS) between treatments and each sex has higher values for males than females, suggesting greater variability in the interaction effect in control and treatments. Similarly, Pillai's trace values show similar effect strength between sex in control and PP groups, whereas stronger effect for females in the PJ group (Table 7). Similar findings can be seen in *T. infestans* of laboratory populations, which reveal higher FA intensity for males (Vilaseca *et al.*, 2022). Nattero *et al.*, (2019) reported how sex can be influenced by wing FA by spraying insecticides. They reported that females showed lower FA values before and after insecticide spraying than males. The intensity levels of FA are further justified in multivariate regression of shape vs FA plots. The FA score is positively related to the regression score in both males and females, suggesting that as shape asymmetry increases, the effect of the treatments also increases as provided by the regression score. The control group tends to have lower FA scores, indicating less asymmetry, while PJ and PP groups show a wider distribution, particularly PP in males, which may suggest that PJ and PP are associated with increased shape asymmetry. Overall, to differentiate between male and female plots, males show a broader range and a more pronounced increase in FA with the regression score, whereas female plots show a more constrained range with less pronounced differentiation between groups (Fig. 2). The relationship between shape and FA score using multivariate regression analysis was also used by Vilaseca *et al.*, (2022). The difference among populations was clear in their analysis with laboratory populations showing lower FA values compared to insecticide treated populations.

The CV plots also reveal similar result with clear distinction of wing shape changes between control and treatments in both male and female. The confidence ellipse and very few overlapping clusters in both the plots suggest that wing shapes of males and females vary between the control and treatments (Fig. 3). In *B. dorsalis*, wing variations were significant among the different fruits with which they were treated. In *B. dorsalis*, the wing of flies reared on apple formed a clear separate grouping compared to the overlapped cluster for citrus and pear, and plum and nectarine, whereas in *C. capitata*, the wings of flies reared on apple also formed a clear separate grouping while nectarine and plum overlapped (Pieterse, 2017).

Finally, the association between longevity and FA of wings in male and female flies is addressed with a strong positive association between the two. FA often represents developmental instability (Debat & Peronnet, 2013) where higher FA indicates greater asymmetry and poorer environmental or nutritional conditions (Ivanković Tatalović *et al.*, 2020). However, the positive correlation between FA and longevity in the present study suggests a different inference. Higher FA might reflect a certain adaptive flexibility or robustness in developmental processes, whereas organisms with higher FA might also be better suited for surviving in varying conditions, thereby living longer (Libiseller *et al.*, 2020). The PJ group shows the highest longevity and FA in both plots, which might suggest that the nutritional condition that PJ represents is particularly favourable or that PJ might promote both longevity and a degree of variability. The control group, with the lowest longevity and FA could be in a condition devoid of the nutritional supplements of PJ and PP. The strong correlation between longevity and FA in females is a novel observation in the present study. In other studies, wing asymmetry of females and males also significantly influences the life span, especially for males. Wing asymmetry can probably be an indicator of events earlier in life correlated with Harshman *et al.*, (2005). The reasons may be due to various factors, such as genetic, developmental, and sex-specific pressures. These factors may combine to affect asymmetry and longevity in females, resulting in distinguishable patterns of FA between males and females. Genetic factors play a key role in determining levels of FA in both sexes. However, certain genetic expressions linked to immunological responses and overall fitness may differ between males and females (Johnson *et al.*, 2008). Overall, the plots suggest a stronger correlation in females suggesting that longevity is more strongly linked with FA, possibly indicating a more pronounced effect of the pomegranate extracts (Fig. 4).

Longevity vs. FA trade-off

There is a trade-off between lifespan and FA, as longevity increases, there is an increase in FA. This refers to the idea that there could be a balance or compromise between an organism's lifespan and its developmental stability (as indicated by FA). Typically, it is expected that high FA, which indicates developmental instability, would be negatively correlated with fitness traits like longevity (Harshman *et al.*, 2005; Nattero *et al.*, 2017, 2019). However, the present experimental data on *D. melanogaster* suggest a positive relationship between FA and longevity, prompting a discussion of a potential trade-off. When FA is quantified in these pomegranate extract-treated flies, the results are higher in PP and PJ-treated flies. This indicates that there is a certain amount of developmental instability observed among the treated flies. Developmental instability or a rise in FA can occur when there is a variation in its regular feeding pattern (Büyükgüzel *et al.*, 2020). The fitness and longevity trade-offs separately in male and female flies involve considering the different evolutionary pressures and biological roles that affect each sex. Males and females might adopt different behavioral strategies to cope with environmental fluctuations. They might modify their mating behaviors, foraging strategies, or habitat preferences based on environmental conditions, which could indirectly affect their developmental stability and lifespan (Wong & Candolin, 2015). Studies suggest that for example, females may be more stable against environmental and genetic stresses, because they carry two X-chromosomes. Deleterious recessive alleles on the X-chromosome are buffered in females than in males (Graham & Özener, 2016). The presence of a single copy of the X chromosome in males can result in exposure to recessive deleterious genes; resulting in developmental instability (Harshman *et al.*, 2005). In the present study, both the sexes have displayed a positive relationship between FA and longevity, displaying a condition of adaptive plasticity.

Usual Trend (Negative Correlation) - Traditionally, low FA is associated with high developmental stability and good overall health, which would support longer lifespans (Harshman *et al.*, 2005). In contrast, high FA would suggest developmental problems, potentially reducing lifespan (Büyükgüzel *et al.*, 2020). If an organism needs to allocate resources towards maintaining developmental stability (keeping FA low), it might have fewer resources available for other processes, such as growth, immune function, or longevity. This could lead to a trade-off where organisms that invest heavily in symmetry and developmental stability might live shorter lives due to the high energy costs (Berg & Finstad, 2008). Alternative scenario (Positive correlation) – Due to some changes in environments or nutritional change, a certain level of developmental instability (reflected by high FA) might be beneficial (Castillo & Rivas, 2023; Knierim *et al.*, 2007). This could be because these organisms are more adaptable to changing conditions, allowing them to survive longer despite (or because of) their higher FA. High FA might be a sign of organisms that can tolerate a wider range of environmental conditions. These organisms might live longer because they can survive in varied or stressful environments that might otherwise reduce longevity in more developmentally stable individuals (Minois, 2000; Møller & Manning, 2003). Organisms that do not invest as heavily in maintaining perfect symmetry might allocate those resources towards other survival-enhancing processes. This could lead to a trade-off where they tolerate higher FA but gain increased longevity.

Re-evaluating FA as a fitness indicator - The positive correlation between FA and longevity in the present study suggests that FA should not always be viewed as a negative indicator of fitness Harshman *et al.*, (2005). Instead, in certain contexts, higher FA might be associated with advantageous traits that contribute to longevity. The trade-off between FA and longevity is likely context-dependent. In some environments, the trade-off might favor low FA and high developmental stability, while in others, higher FA might be tolerated or even beneficial for longevity. This intriguing relationship warrants further investigation, potentially exploring the environmental conditions, genetic factors, and physiological mechanisms that contribute to this trade-off.

Conclusion

Overall, Both PJ and PP resulted in increased mean longevity compared to the control group, with PJ showing particularly significant enhancements in lifespan for both sexes. Furthermore, a notable correlation between longevity and FA was observed. Specifically, the results suggest that increased FA in treated flies might be linked to an adaptive strategy, whereby organisms exhibiting greater variability are better equipped to endure environmental or nutritional changes.

Author's Contributions

Vijaykumar Rajashekhar: Investigation, methodology, draft preparation, funding acquisition; **Srinath Sridhar Belgaum**: Visualization, conceptualization, supervision, review and edit, formal analysis; **Nanjaiah Shivanna**: Visualization, conceptualization, supervision, project administration, final review and edit.

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Data Availability Statement

All data supporting the findings of this study are available within the paper.

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Ethics Approval

Insects were used in this study. All applicable international, national, and institutional guide lines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare no conflict of interest.

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تاثیر عصاره انار بر طول عمر و عدم تقارن نوسان بال در مگس سرکه: یک تحلیل مورفومتریک هندسی

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چکیده: طول عمر نقش مهمی در تعیین سلامت کلی حیوان دارد. تغذیه یک عامل کلیدی است که می‌تواند بر طول عمر حیوان تأثیر بگذارد. مطالعه رابطه بین تقارن بال و طول عمر یک رویکرد مفید برای درک اینکه چگونه تغذیه می‌تواند بر طول عمر و تقارن تأثیر بگذارد، ارائه می‌دهد. در این تحقیق، تأثیر آب انار (PJ) و پوست انار (PP) بر طول عمر و عدم تقارن نوسانی (FA) بال‌های مگس سرکه نر و ماده با استفاده از آنالیز مورفومتریک هندسی (GMA) مورد تجزیه و تحلیل قرار گرفت. این مطالعه نشان داد که مگس‌های تیمار شده با PJ و PP طول عمر و بالاترین FA را در مقایسه با شاهد در نر و ماده نشان می‌دهند. بین مگس های PJ و PP تیمار شده، PJ بالاترین طول عمر را در هر دو جنس نر و ماده داشت در حالی که مگس های PP در هر دو جنس FA بالاتری داشتند. در بین جنس‌ها، ماده‌ها طول عمر و مقادیر FA کمتری نسبت به نرها داشتند. مطالعه کلی GMA تنوع شکل بال‌ها و وجود FA قوی در مگس‌های PJ و PP را نشان می‌دهد. همبستگی پیرسون ارتباط مثبت قوی بین طول عمر و بال FA را نشان داد.

کلمات کلیدی: عصاره‌ی انار، تقارن بال، GMA، طول عمر، مگس سرکه

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