



Effect of food material on imaginal disc and adult morphometry in *Drosophila melanogaster*

Dr. Deepali Sangale, Kartik Kekan, Dr. Sandip Chordiya, Dr. Vitthal Nale, Sana Sayyad

Department of Zoology, Tuljaram Chaturchand College of Arts, Science & Commerce, Baramati, (Autonomous), Savitribai Phule Pune University, Pune Maharashtra, India

Abstract

The imaginary disc of the fruit fly plays an important role in deciding the future fate in the larval stage after the formation of body parts and organs in adults it derived from the invagination of ectoderm the research found that larval and adult morphometry measuring dimensions in both stage 3rd instar and adult. With the help of using image j software, the correlation between larval and adult morphometry in this study revolves using different fruit culture rearing at 20-25°C studying in which one type of food material affects both stages of structure larval and adult using 6 types of collecting photographs with the help of mobile and measuring dimension of image j software initiating of measuring setting scale in 1000 micrometer in this study focus on what is the effect on which one type of food material or fruit culture in the imaginary disc and adult body part extremely growth simultaneously studying in the correlation between larval and adult stage, in fig observing graphical representation illustrate that in which one stage imaginary disc and adult body organ extremely and lower growth in different fruit culture.

Keywords: Imaginary disc, fruit culture, adult morphometry, dimension, ImageJ software

Introduction

Recent studies indicate that fruit fly comprise approximately 1600 described species (O'Grady & DeSalle, 2018) [1] and genetic research has extensively examined most commonly used species in model organism for biological science. Many genes regulate different aspects the genetic model of fruit fly is well develop and has a small genome The fruit fly has been extensively used as a model organism studying development biology and genetics, molecular genetics. (Tolwinski, 2017) [2] studies of *Drosophila* advance in the regulation of development, including the identification of genes that regulate different aspects of development and the discovery of homologies between development genes in *Drosophila* and other organisms, including human, has provide important fundamental mechanism development and even human disease. In fruit flies many research and studies in evolutionary biology and genetics are limited by the rate at which external appearance information can be acquired *Drosophila* wing has been vastly used to study the genetics of developmental morphometrics and evolution therefore much interest in the assessment of wing structure fruit fly for organ shape derived phenotypic divergence tissue size and shape and final adult proportion must be altered across generation nature selection can promote such changes by acting on the heritable counterpart phenotype variation for target size and shape existence of veritable in subordinate trait lower level of biological institution (Garland & Kelly, 2006) During the development of larvae, each imaginary disc makes several critical decisions about shape and size, fate (Spratford & Kumar 2014) [19]

The categories of cell groups in the larva are organized into sac-like epithelial structures found inside the larva, also known as the imaginary disc (Shymala, 1999). The imaginary disc aside during embryogenesis; the insect that undergoes metamorphosis, the larva turns into a pupa. All larval tissue degrades at this time, the imaginary disc turns into an external structure, head, thorax, limb, and genitalia.

There are 19 discs in the total epidermis of the head, thoracic, and limb genitalia. These disc undergoes process of invagination the development and patterning of imaginary disc leg and wing disc studies to understand mechanism of organ formation and regeneration, fate map formation of patterning and role life cycle of fly. Imaginary discs of *Drosophila* play a crucial role in regulating and developing the timing and transition to metamorphosis. The growth of the developing disc is influenced by nutrition and the nutritional status of larvae. Larvae feed on standard food. The imaginary disc develops at a fixed rate. Overall, the imaginary disc of fruit flies plays an important role in coordinating and developing the timing of growth of adult structure, and this process is closely linked to nutritional status.

The morphogenesis of the imaginary disc of *Drosophila* the managing of body and organ and organ size is a vital development process that has important consequences for organism fitness and function (Edgar, 2006; Nijhout et al. 2006; Bradley C. Striper, Development biology Elsevier) transform insect adult body size it definite by final larval stage L1, L2, L3 where larval tissue sustain existing increase the process regulate circumstance or environmental factor. Body size and the relative ratio of organs and characteristics features that divide one species from the next. (Stieper et al. 2008) [4]

fruit fly wing has been vastly used as a model for such study given some experimental advantage to another organ like the adult wing essential dimension which facilitates the characterization and illustration of its morphological variation (Mathe & Klaczko 1999) The regulation of food intake and post-ingestion mechanisms are crucial aspects in ensuring optimal growth and development in animals. Insects, such as *Drosophila melanogaster*, have demonstrated remarkable adaptability to different dietary conditions through various morphological and functional responses. (Bonelli et al., 2020) [6] Studies have shown that

Drosophila populations can exhibit significant behavioral, physical, and demographic changes in response to dietary restriction, with variations in lifespan, fertility, and activity levels. (Bross et al., 2005) [8] Furthermore, the quality of the available diet has been found to play a key role in affecting life-history components, imaginary disc including stress resistance and reproductive abilities. (Chen et al., 2017) [9]

Material and method

Fly stock culture: in this research pre pupa larva of drosophila, we are rearing on six different fruit cultures banana, papaya, kitchen garbage, custard apple, and apple. During studies, all culture media keep rearing on at 25°C. essential for the survival and breeding of drosophila

Fruit fly larva phenotype viability growth life span morphology of 3rd instar larva during study using 3rd instar semi-translucent prepupae larva collecting from different all food material one by one at time single for dissection larva ideally fly rearing and handled in room temperature (25°C) maintaining room temperature between (20-25°C)

Preparation of imaginary disc slide: Dissecting of the imaginary disc was done manually at room temperature at 25°C using 100ml 0.9% saline solution for dissection and using a light microscope initially dissecting the imaginary disc requiring to reduce movement of drosophila using 70% diethyl ether for stabilizing reducing aggregation rearing larva dissect out an imaginary disc from prepupa 3rd instar larva in sequence wise at the time only one culture initially take banana after papaya, kitchen garbage, custard apple guava, apple in every culture at the time one culture using dissecting imaginary disc different region head and thoracic region in the head region collecting eye antenna thoracic region collecting wing disc, leg disc, halter disc using 0.9% saline solution.

Measuring the different imaginary discs: dorsally, ventrally, and ventral lateral to lateral using ImageJ software with the help of mobile clicking photograph setting scale 1000 µm.

Adult morphometry: the morphology of various parts of insects is associated with function, as insects generally have no change in their exoskeleton, and their morphometry in studying different regions, lengths, and widths for adults. using the same culture at the time use previous during larva rearing using the dissecting of fruit fly adults in every culture dissecting of body parts in different regions Initially taking the adult flies on a slide horizontally observing and clicking the photograph on the head region eye after taking photograph in antenna separated head capsule after measuring size in head region present eye and antenna after dissecting wing leg halter and leg in thoracic region preparation of slide without mounting media for observation of adult morphometry and imaginary cell using light microscopy for imaginary disc observed used 10x objective adult stage used 4x in objective

Measurement of body dimensions

Measurement of antenna dimension: The antenna dimension of adult fruit fly was taken from the anterior region, the pedicel to the posterior region, funicular without dissecting the head capsule for measuring using Image J software, measure the dimension length and width using mobile photographs.

Measurement of eye dimension: Drosophila eye reddish in color, convex compound eye. We use software to measure the eye area, dorsal and ventral eye area, and measure the eye image by drawing a line measuring the distance.

Measurement of wing dimension: enlarge on the thorax and wing data reported by (Lack, 2016) [10] to examine the wing dimension initiated from the wing epithelium dorsal longitudinal muscle DLM of adults. We keep control at a temperature of 25. Wing collecting from 3 to 6 days old of an adult, taking photographs using a mobile and measurement of wing dimension using Image J software

Measurement of leg dimension: measurement of leg dimension from coxa to epitarsus dorsally and ventrally using Image J software.

Measurement of halter dimension: we gather 3-6 days old laboratory-reared Drosophila flies we anesthesia by using diethyl ether then prepared slide fruit flies without dissecting the halter measuring the halter length and width from base stock to knob (globular region) of the halter with help of photographs using image j software.

Results

The goal of these studies was to compare the development of which type of food material increases imaginary disc size drosophila and in the adult stage of drosophila which type of food material increases length of body organ many food materials available when fly consume food material we initiate study that effect of food feeding and media composition in increasing dimensions in both larval and adult stage.(Photo plate No.1,2,3,4,5,6,7,8,9 and 10)

Table 1: larval stage morphometry

Sr. No	Food Material	Region	Measurement ((µm))
1	Banana	Antenna	Length:612, Width:396
		wing	Length:200, Width:140
		halter	Length:138, Width:91
2	Papaya	Antenna	Length:432, Width:318
		Wing	Length:527, Width:419
		halter	Length:316, Width:224
3	Custardapple	Antenna	Length:156, Width:129
		Wing	Length:540, Width:328
		Leg	Length:150, Width:128
		Halter	Length:389, Width:254
4	Kitchen garbage	Antenna	Length:98, Width:137
		Wing	Length:429, Width:289
		Leg	Length:253, Width:162
5	Apple	Antenna	Length:211, Width:179
		Wing	Length:91, Width:48
		Halter	Length:194, Width:108
6	Guava	Antenna	Length:260, Width:231
		Leg	Length:284, Width:184
		Halter	Length:195, Width:112


Table 2: Drosophila adult morphometry

Sr. No	Food material	Region	Measurement (µm)
1	Banana	Antenna	Length: 90, Width: 30
		Eye	Length:219, Width:218
		Leg	Length: 127, Width: 184
		Wing	Length: 430 Width: 218
		Halter	Length: 55 Width: 39
2	Papaya	Antenna	Length: 101, Width: 42
		Eye	Length:203, Width: 231
		Leg	Length:406, Width: 26
		Wing	Length:370, Width: 223
		Halter	Length: 76, Width: 47
3	Custard apple	Antenna	Length: 76, Width: 53
		Eye	Length: 172, Width: 144
		Leg	Length: 177, Width: 18
		Wing	Length:297, Width: 210
		Halter	Length: 80, Width: 47
4	Kitchen garbage	Antenna	Length: 80, Width: 33
		Eye	Length:157, Width:129
		Leg	Length:651, Width:44
		Wing	Length:279, Width:122
		Halter	Length: 57, Width: 45
5	Apple	Antenna	Length:73, Width:49
		Eye	Length:214, Width:99
		Leg	Length:53, Width:25
		Wing	Length:380, Width:185
		Halter	Length:94, Width:47
6	Guava	Antenna	Length:103, Width:42
		Eye	Length:229, Width:126
		Leg	Length:403, Width:21
		Wing	Length:337, Width:182
		Halter	Length:59, Width:45


Table 3: comparative measurements of the larval and adult stage of Drosophila

Sr No.	Food material	Larval stage	Measurement (µm)	Adult Stage	Measurement (µm)
1	Banana	Antenna	Length:612 Width:396	Antenna	Length:90 Width:30
		Wing	Length:200 Width:140	Wing	Length:430 Width:218
		Leg	Length:127 Width:184	Leg	Length:127 Width:184
		Halter	Length:138 Width:91	Halter	Length:35 Width:39
		Antenna	Length:432 Width:318	Antenna	Length:101 Width:42
2	Papaya	Wing	Length:527 Width:419	Wing	Length:370 Width:223
		Leg	Length:259 Width:196	Leg	Length:406 Width:26
		Halter	Length:316 Width:224	Halter	Length:76 Width:46
		Antenna	Length:156 Width:129	Antenna	Length:76 Width:53
		Wing	Length:540 Width:328	Wing	Length:297 Width:210
3	Custard apple	Leg	Length:150 Width:128	Leg	Length:177 Width:18
		Halter	Length:389 Width:254	Halter	Length:80 Width:47
		Antenna	Length:98 Width:137	Antenna	Length:80 Width:33
		Wing	Length:429 Width:289	Wing	Length:279 Width:122
		Leg	Length:253 Width:162	Leg	Length:651 Width:44
5	Apple	Antenna	Length:211 Width:179	Antenna	Length:73 Width:49


		Wing	Length:91 Width:48	Wing	Length:380 Width:185
		Halter	Length:194 Width:108	Halter	Length:94 Width:47
6	Guava	Antenna	Length:260 Width:231	Antenna	Length:103 Width:42
		Leg	Length:284 Width:184	Leg	Length:403 Width:21
		Halter	Length:195 Width:112	Halter	Length:59 Width:45




10x scale for larval morphometry




Culture medium :-Banana
Region :-head
Name:- antenna
Size (µm):- L. 612W. 396




Culture medium :-banana
Region :- thoracic
Name :- wing disc
Size (µm) :-L. 200W. 140



Culture medium :- banana
Region :- thoracic
Name :- halter
Size (µm) :-L. 134 W. 83




Culture medium :- papaya
Region :- head
Name :- antenna
Size (µm) :- L. 432W. 318




Culture medium :- papaya
Region :- thoracic
Name :- wing
Size (µm) :-L. 527W. 419


Photo plate No.1 Larval imaginal disc Morphometry




Culture medium :- papaya
Region :- thoracic
Name :- halter disc
Size (µm) :-L. 316W. 224




Culture medium :- papaya
Region :- thoracic
Name :- leg disc
Size (µm) :-L. 259W. 196



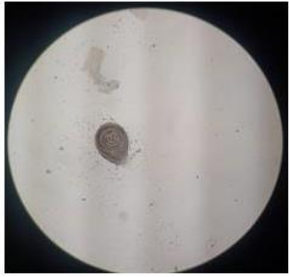
Culture medium:- custard apple
Region :- head
Name :- antenna disc
Size (µm) :-L. 156W. 129



Culture medium :- :- custard apple
Region :- thoracic
Name :- leg disc
Size (µm) :- L. 150W. 128


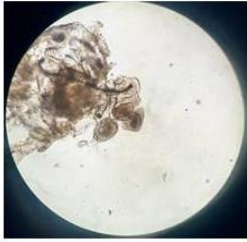



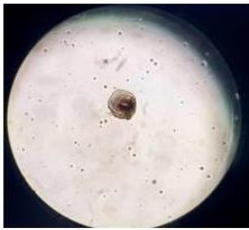


Culture medium :- custard apple
Region :- thoracic
Name :- wing
Size (µm) :-L. 540W. 328



Culture medium :- kitchen garbage
Region :- head
Name :- eye antenna disc
Size (µm) :- L. 98W. 137

Photo plate No.2 Larval imaginal disc Morphometry

			Photo plate No.3 Larval imago disc Morphometry
Culture medium:- kitchen garbage Region :- thoracic Name :- wing Size (µm) :- L. 429W. 289	Culture medium :- kitchen garbage Region :- head Name :- antenna Size (µm) :- L. 253 W. 162	Culture medium :- apple Region :- head Name :- antenna Size (µm) :- L. 211 W. 179	
			
Culture medium :- apple Region :- thoracic Name :- wing disc Size (µm) :- L. 91W. 48	Culture medium :- apple Region :- thoracic Name :- halter Size (µm) :- L. 194W. 108	Culture medium :- guava Region :- head Name :- antenna Size (µm) :- L. 260W. 231	

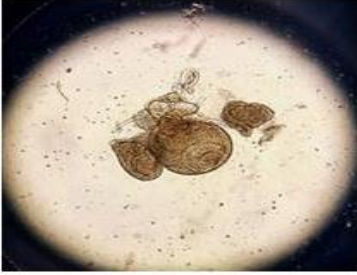

	
Culture medium :- guava Region :- thoracic Name :- leg Size (µm) :- L 284. W. 184	Culture medium :- guava Region :- thoracic Name :- halter Size (µm) :- L. 195W. 112

Photo plate No.4 Larval imago disc Morphometry

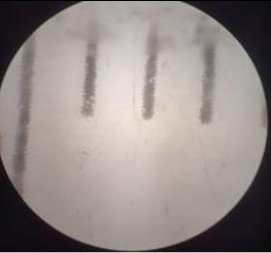



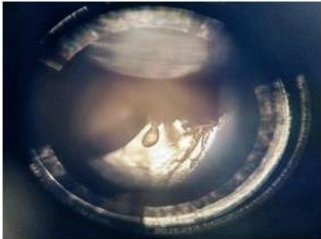

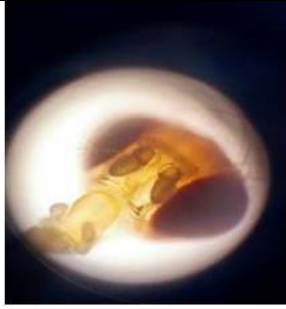
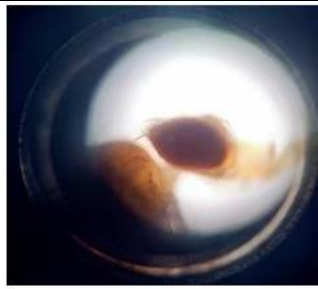
			Photo plate No.5 Adult Morphometry
Scale 4x for adult morphometry	Culture medium :- Banana Region :- head Name :- antenna Size (µm) L. 90W. 30	Culture medium :- Banana Region :- head Name :- eye Size (µm) :-L. 219W. 218	
			
Culture medium :- Banana Region :- thoracic Name :- leg Size (µm):- L. 127W. 184	Culture medium :- Banana Region :- thoracic Name :- halter Size (µm) :- L. 35 W. 39	Culture medium :- Banana Region :- thoracic Name :- wing Size (µm) :- L. 430W. 218	

Photo plate No.6 Adult Morphometry



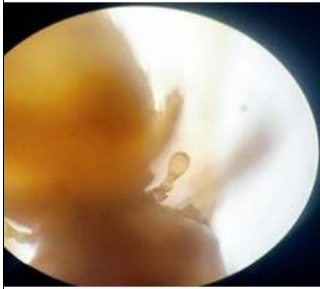
Culture medium :- papaya
Region :- head
Name :- antenna
Size (µm) :- L. 101 W. 42



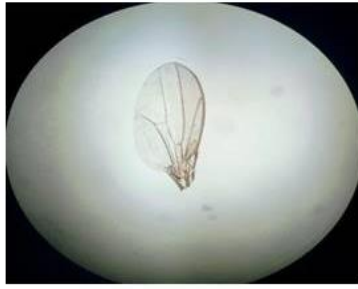
Culture medium :- papaya
Region :- head
Name :- eye
Size (µm) :- L. 203W. 231



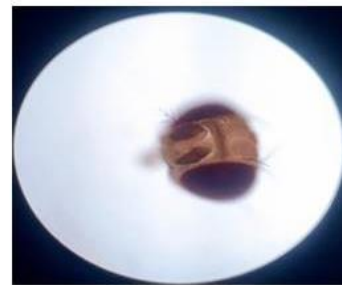
Culture medium :- papaya
Region :- thoracic
Name :- leg
Size (µm) :- L. 406W. 26



Culture medium :- papaya
Region :- thoracic
Name :- halter
Size (µm) :- L. 76 W. 46



Culture medium :- papaya
Region :- thoracic
Name :- wing
Size (µm) :- L. 370W. 223



Culture medium :- kitchen garbage
Region :- head
Name :- antenna
Size (µm) :- L. 80 W. 33

Photo plate No.7 Adult Morphometry



Culture medium :- kitchen garbage
Region :- head
Name :- eye
Size (µm) :- L. 157W. 129



Culture medium :- kitchen garbage
Region :- thoracic
Name :- leg
Size (µm) :- L. 651W. 44



Culture medium :- kitchen garbage
Region :- thoracic
Name :- halter
Size (µm) :- L. 57W. 45



Culture medium :- kitchen garbage
Region :- thoracic
Name :- wing
Size (µm) :- L. 279W. 122



Culture medium :- custard apple
Region :- head
Name :- antenna
Size (µm) :- L. 76W. 53




Culture medium :- custard apple
Region :- head
Name :- eye
Size (µm) :- L. 172W. 144



Culture medium :- custard apple
Region :- thoracic
Name :- leg
Size (µm) :- L. 177W. 18



Culture medium :- custard apple
Region :- thoracic
Name :- halter
Size (µm) :- L. 180W. 47



Culture medium :- custard
Region :- thoracic
Name :- wing
Size (µm) :- L. 297W. 210



Culture medium :- guava
Region :- head
Name :- antenna
Size (µm) :- L. 103W. 42



Culture medium :- guava
Region :- head
Name :- eye
Size (µm) :- L. 229W. 126



Culture medium :- guava
Region :- thoracic
Name :- leg
Size (µm) :- L. 400W. 90

Photo plate No.8 Adult Morphometry



Culture medium :- guava
Region :- thoracic
Name :- leg
Size (µm) :- L. 403W. 21



Culture medium :- guava
Region :- thoracic
Name :- halter
Size (µm) :- L. 59W. 45



Culture medium :- guava
Region :- thoracic
Name :- antenna
Size (µm) :- L. 337W. 182



Culture medium :- apple
Region :- head
Name :- antenna
Size (µm) :- L. 73W. 49



Culture medium :- apple
Region :- head
Name :- eye
Size (µm) :- L. 214W. 99



Culture :- medium apple
Region :- thoracic
Name :- leg
Size (µm) :- L. 353W. 25

Photo plate No.9 Adult Morphometry

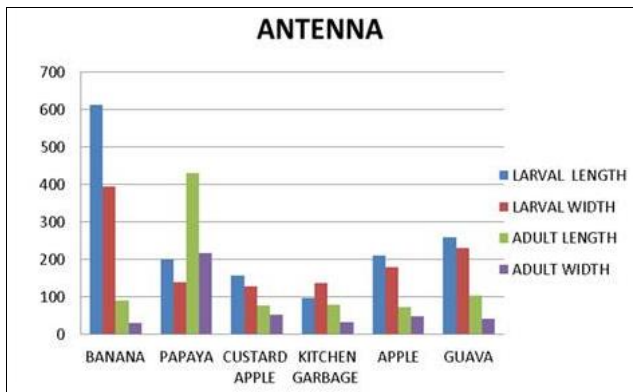


Figure 1. graphical representation show that comparative correlation between in antenna larval and adult development in given culture

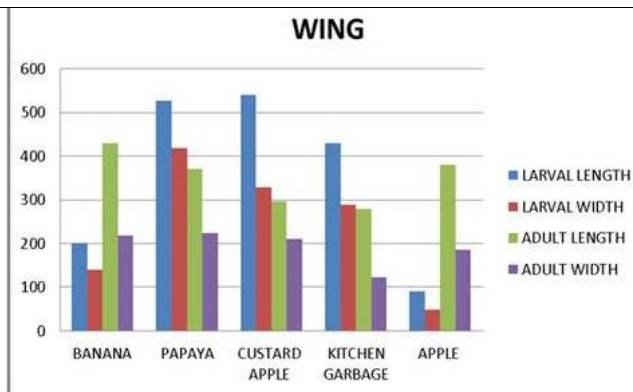


Figure 2. graphical representation show that comparative correlation between in wing larval and adult development in given culture

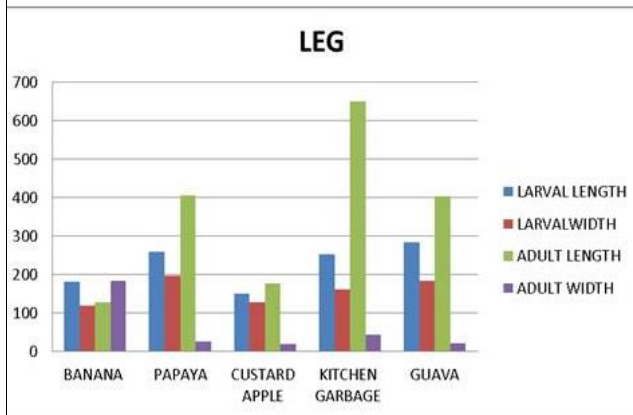


Figure 3. graphical representation show that comparative correlation between in leg larval and adult development in given culture

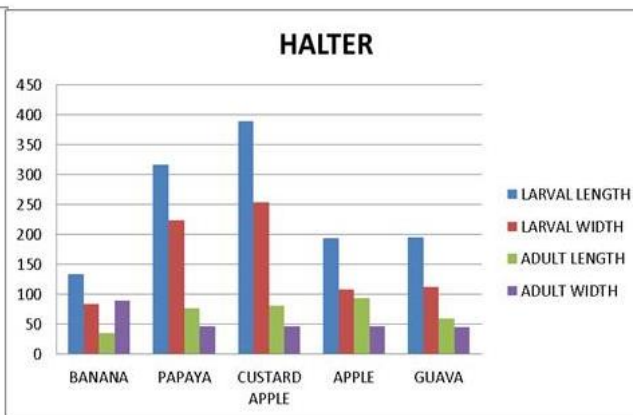


Figure 4. graphical representation show that comparative correlation between in halter larval and adult development in given culture

Discussion

The analysis of the imaginary disc when providing fruit media, increasing the dimension of eye antenna disc, wing disc, halter disc, and leg disc, the imaginary disc is identified in 3rd instar larva observation in light microscopy without staining (Table No.1). Varying in size same imaginary disc, different fruit culture, different fruit cultures these studies indicated the correlation between larvae and adult morphometry and to effect of food material on imaginary disc in both stages (Fig. no. 1,2,3, and 4).

The food material's different aspects affected the dimensions of both stages of Drosophila in the larval stage, affecting the imaginary disc and the body organ was affected in the adult stage (Table No.2). In papaya culture, larva leg disc medium growing than adult leg structure in the adult leg structure grew extremely. In the kitchen garbage, the media moderate size leg, but the adult stage structure is growing. Compared to all cultures, media is growing extremely well in guava culture. We observed that guava and kitchen garbage have a small quantity change, but in the graphical presentation,

similar dimensions. The adult stage is extremely long, but the width is very small in size. (Table No.3) The Custard apple increases in size, largely in the larval stage. Halter discs reduced in size in observing in apple, and guava food material varies width but similar length during this studies we found that antenna disc growth in extremely growing in banana culture adult antenna size very minus compared to all media in papaya culture in wing disc highly growing in larval in wing disc highly growth in adult wing structure extremely grown in banana media in leg disc largest size found that in papaya culture for adult leg part. Kitchen garbage media halter disc observing that growing custard apple in the adult stage, morphometrically larger in the apple culture media.

Conclusion

During these studies, we concluded that banana and papaya culture media are the best culture media for antenna imaginary disc development in the larval stage and in the adult stage, respectively. The papaya, Custard apple, and kitchen garbage culture media are shown in a larger size of the wing imaginary disc than the other culture media. An increased adult wing development was observed in the banana and apple culture media. The kitchen garbage media showed leg development in the adult stage study. For the study of halteres in the larval stage papaya and custard apple, the media is a good media, in such a way, for the morphometric and imaginary disc study of the antenna, wings, legs, and halteres in *Drosophila*, we can use different culture media.

References

- O'Grady PM, DeSalle R. Phylogeny of the genus *Drosophila*. *Genetics*,2018:209(1):1-25.
- Tolwinski NS. Introduction: *Drosophila*—A model system for developmental biology. *Journal of developmental biology*,2017:5(3):9.
- Beira JV, Paro R. The legacy of *Drosophila* imaginal discs. *Chromosoma*,2016:125:573-592.
- Stieper BC, Kupershtok M, Driscoll MV, Shingleton AW. Imaginal discs regulate developmental timing in *Drosophila melanogaster*. *Developmental biology*,2008:321(1):18-26.
- Shyamala BV, Chopra A. *Drosophila melanogaster* chemosensory and muscle development: identification and properties of a novel allele of scalloped and of a new locus, SG18.1, in a Gal4 enhancer trap screen. *Journal of Genetics*,1999:78:87-97.
- Bonelli M, Bruno D, Brillì M, Gianfranceschi N, Tian L, Tettamanti G, et al. Black soldier fly larvae adapt to different food substrates through morphological and functional responses of the midgut. *International Journal of Molecular Sciences*,2020:21(14):4955.
- Qiu S, Wang S, Xiao C, Ge S. Assessment of microalgae as a new feeding additive for fruit fly *Drosophila melanogaster*. *Science of the Total Environment*,2019:667:455-463.
- Bross TG, Rogina B, Helfand SL. Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging cell*,2005:4(6):309-317.
- Chen J, Xue J, Ruan J, Zhao J, Tang B, Duan R. *Drosophila* CHIP protects against mitochondrial dysfunction by acting downstream of Pink1 in parallel with Parkin. *The FASEB Journal*,2017:31(12):5234-5245.
- Lack JB, Yassin A, Sprengelmeyer QD, Johanning EJ, David JR, Pool JE. Life history evolution and cellular mechanisms associated with increased size in high-altitude *Drosophila*. *Ecology and evolution*,2016:6(16):5893-5906.
- McKean KA, Lazzaro BP. The costs of immunity and the evolution of immunological defense mechanisms. *Mechanisms of life history evolution*,2011:299-310.
- Buratovich MA, Bryant PJ. Enhancement of overgrowth by gene interactions in lethal (2) giant discs imaginal discs from *Drosophila melanogaster*. *Genetics*,1997:147(2):657-670.
- Li C, Meinertzhagen IA. Conditions for the primary culture of eye imaginal discs from *Drosophila melanogaster*. *Journal of neurobiology*,1995:28(3):363-380.
- Schwarz S, Durisko Z, Dukas R. Food selection in larval fruit flies: dynamics and effects on larval development. *Natur wissenschaften*,2014:101:61-68.
- Chaiphongpachara T, Tubsamut P. Geometric morphometry of pupae to identify four medically important flies (Order: Diptera) in Thailand. *Biodiversitas Journal of Biological Diversity*,2019:20(6).
- Dujardin JP. Modern Morphometrics of Arthropods: A Phenotypic Approach to Species Recognition and Population Structure. In: *Genetics and Evolution of Infectious Diseases*. Elsevier: 2024:385-425.
- Posnien N, Hopfen C, Hilbrant M, Ramos-Womack M, Murat S, Schönauer A, et al. Evolution of eye morphology and rhodopsin expression in the *Drosophila melanogaster* species subgroup. *PLoS one*,2012:7(5):e37346.
- Bitner-Mathé BC, Klaczko LB. Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata* wings. *Heredity*,1999:83(6):688-696.
- Spratford CM, Kumar JP. Dissection and immunostaining of imaginal discs from *Drosophila melanogaster*. *Journal of visualized experiments: JoVE*,2014:(91):51792.