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Laboratory studies on the nutritional content effect of fruits on the competitive ability for *Bactrocera zonata* and *Ceratitis capitata*

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Abstract
The study showed that the nutritional content of fruits affects the growth of adult flying insects and their larval stages, and given the importance of fruit flies, represented by both the (*Bactrocera zonata* and *Ceratitis capitata*).Infecting fruits during the physiological maturity stage of fruits, especially with the presence of mixing in horticultural crops, especially in rural Egypt, this may lead to the settlement of flies inside old horticultural farms.Therefore, the study focused on the fruit content of proteins, fats, and carbohydrates necessary for the growth of larvae of the peach and fruit flies in each of the fruits under study, which are (Guava - Mango - Peaches).The results also showed that the competitive dominance of *Bactrocera zonata* larvae over *Ceratitis capitata* larvae, with both flies preferring Guava fruits with the presence of both Peach and Mango fruits. It was also shown that peach fly larvae prefer the fat and carbohydrate content of the fruit content, while the fruit fly larvae prefer the protein content. There was also a strong significant correlation between fruit content and the growth of larvae at their three ages inside the fruit, while the relationship was direct between the larvae of the two flies. It has also been shown that the relationship between the larvae of the two flies and protein was a positive, direct relationship. Increasing the protein component in the fruits leads to an increase in the growth of the larvae in their three stages of life. While the inverse relationship was negative between the growth of the larvae of the two flies, thus the growth of the *Bactrocera zonata* larvae prevails over the growth of the *Ceratitis capitata* larvae because the *Ceratitiscapitata* larvae need about twice the nutritional requirement of the *Bactrocera zonata* larvae in terms of proteins. While the biochemical analysis of the pupal stage of the two insects indicated to that there were no significantly differences between them in protein and lipids.

Keywords: Competition between Peach fruit fly larvae and Mediterranean fruit fly larvae - biochemical analysis of the larval and pupal stages - protein, fats and carbohydrates - fruits (Guava - Mango - Peaches)

Introduction
Current study contains a general summery about recent studies and the future researches ways on the topic of resources of allocation and compensation during the vital processes in many insects, with concentration on the important role of resources during these processes, study also show the role of compensatory mechanisms on treating nutritional shortage which deported from the earlier stages of the developmental processes. Also, current study aim to firstly review the resources allocation in the development processes “open and closed” then turn on to the other topic which show the modeling resources customization and its trade-offs. Also, current study contains review the recent methodological development processes such as methods of response surfaces and many experiments beside the nutritional geometry. Current study also contains the important role of compensatory behavior and physiology (David Nestel *et. al.* 2016) [9]. Lay eggs in the rotten fruits, larvaes feed on the yeast which grows on the rotten fruits which represents an essential source of protein. Because the yeast population. As fruits decomposition increases, ripe fruits were founded contains less protein content of rotting fruits. Also we assumed that *Z. indianusD. simulans* larvas which different in their nutritional protein requirements. Response to survival, development period, and the number of ovaries from the composition of the larval diet system with *Z. indianus* better performance across a wider range of proteins contents. (Cristiane Matavelli *et al.* 2015) [6]. Carbohydrates and proteins consider the most major macronutrients which have serious effects on many organisms fitness such as the serious pest *Drosophila melanogaster*. Study led to know us how these successive macronutrients shape the important components of fitness in *D. melanogaster* through using of the nutritional geometry whereas most of nutritional geometric analyses which carried out on these species which have been conducted with using semi-synthetic diets that were not defined chemically well. Current study aimed to combined both of use of nutritional chemically and geometry defined diets to comparing the patterns of larvals and adults which expressed of it across about 34 diets completely different in both of proteins to carbohydrates (P:C) ratio and in proteins plus carbohydrates (P+C) concentrations. (Taehwan Jang and Kwang Pum 2018) [19]. To summaries’, specific hunger for both sucrose and proteins in larvae of fruit flies were noticed and documented, which consider a model system for work steps on the mechanisms system (work) of the nutrient hunger and regulation. Insect larvas were valued protein ratio in their diets very highly system whereas a highly ratio of them settled on a patch even when it needs sucrose which led to low growth rate. While current study concentration on the big essential macronutrients and the future researches may be evaluate insects' larvals ability to regulate the other important nutrients and the certain mechanisms which led to such ability. In two laboratory experiments which carried out with artificial diets, insect's larvae were selectively deprived of both of sucrose and protein then fed with a system diet which provides them with the missing nutrients. Whereas allowed to these larvals they move freely between two adjacent food patches, with notice that insects larvas

prefer to stay on one certain yeast which containing on patch and more of sucrose led to providing patch, (Sebastian Schwarz *et. al.* (2014) [16]. Insect's caterpillars face serious nutritional challenges when feeding on different plants. This is beside the harmful secondary metabolites and different proteins due to water limit, plant tissues may be rich in carbohydrates which may be affective seriously on the optimal larval performance. (Branislav Babic *et. al.* 2008). In insects feeding affects primarily on adult size during the nymphal or larval stages but measures of insect adult size such as the body weight also completely to changes with adults feed. Study founded that manipulation of larval diet system had serious affects on all measures of insect adult sizes, Gonçalo M. Poçaset *et. al.* (2022) [10]. Effects of the composition and concentration of amino acids (aa) and sucrose in larval diets on several indicators of development of the *C. capitat*a. Proteins and fats level beside different sucrose contents in the diet system have low effects on most development process. However the sucrose content affected significantly on the ability of insects larvas to accumulate fats and proteins reserve. Adults which had different sucrose diet system did not different significantly in their fat and protein contents. Results obtained also that deletion of glycine was the most serious effects followed by deletion of all non-essential AA and serine. High concentrations in the diet system led to harmful effects on the growth process. Fat contents in the pupating larvaes and also protein levels were affected depends on analysis of lipids frequency distribution suggesting that midges appear to regulate content the level of lipids in emerging adults within certain range limits regardless the diet system history of larval or lipids content, David Nestel *et. al*. (2004) [8] and Sudhakar Krittika *et. al.* (2019) [18]. The diet system of larvals has a short-term effectiveness on resistance and desiccation of the starvation of *C. cosyra*. Whereas the diet system of the adults does not compensate for starvation resistance which results from a larval diet system. On the other side during the period that *C. cosyra* insects were fed on adult diets found that their starvation and desiccation resistance were declined irrespective of the diet system which offered to them. Water contents play an important role in the ability of *C. cosyra* insect to tolerance the water loss. Christopher W. Weldonet. *et.al*. (2019) [5].

The importance of the research and its objectives

Monitoring the phenomenon of competition between fruit flies and an explanation for the dominance of the peach fly over the fruit fly with the presence of the two flies in the same seasonand the sexual maturity of the two flies.

Materials and methods

Experiments were carried out on flies *B. zonata* and *C.cabitata* which were reared in Plant Protection Research Institute, Giza, Egypt during summer season 2023. Adult flies were kept in control environment (Temperature 30 ± 2 C°, 70± 5% R.H. and 17:7 L.D photo phase) in cages with sizes (80cm, 50cm, 40cm). The successive flies were fed on sugar and enzymatic protein hydrolysate with ratio 3:1 respectively; further more supplied with a water source. Flies were bred so that both flies reached sexual maturity at the same time to ensure that eggs were laid in the samples of fruits in the same treatment. (Two mature fruits of Peaches, Guava and one fruit of Mango) were exposed to an equal number of in sect's foe two fruit flies under the study. At rate of five replicates in equal cages. Each cage contained fifty pairs of adult's insects in the case of laying eggs and four cages were contained an equal number of both flies. Each cage contained twenty-five pairs of both mature flies, along with the necessary nutrition (sugar, protein and water). (Two mature fruits of Guava, two fruits Peach and one Mango fruit were exposed to flies for forty-eight hoursaccording to P.S. Messenger and N.E. Flitters (1959). The treated fruits were incubated for seven days, then the number of individuals of the adult's stage of the insect emerging form the treated fruits were counted and the appropriate type of fruits were identified to conduct an analysis of the proteins, fats and carbohydrates for each threes treated fruits. (As shown in the Fig1&2)

Preparation the samples for biochemical analysis

A. Preparation the samples for biochemical analysis
Successive samples were prepared homogenized in distilled water then centrifuged on 6000 rpm for 10 min at 5°C using (BECKMAN GS-6R Centrifuge). After centrifugation the supernatant fluid was divided into small aliquots with size (0.5 ml) and then stored at –20 °C until analysis of the main components. Three replicates were carried out for each biochemical determination.

B. Preparation of protein reagent: Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml of 95% ethanol. 100 ml 85% (w/v) phosphoric acid were added. The resulting soln was diluted to a final volume of 1 liter.

C. Protein assay: 50 µl of pupal homogenate were pipetted into a test tube containing 50 µl phosphate buffer (pH 6.6), then 5 ml of protein reagent were added to the test tube and the contents were vortexes. The absorbance at 595 nm was measured after 2.0 min. against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml of protein reagent. The content of protein was estimated as mg/g. body weight.

Determination of total proteins content: We determined total protein by method of Bradford (1976) [2] by using a standard of Bovine serum albumin.

A. Preparation of plant tissue homogenate samples

1. Sample Preparation: Weight a small amount (typically 0.1-0.5 gram) of the plant tissue and grind it into a fine powder. Transfer the powder to a test tube or flask.
2. Hydrolysis: Add 3-5 mL of 2 M hydrochloric acid (HCl) to the test tube or flask containing the plant tissue powder. Heat the mixture in a boiling water bath for 2-3 hours to hydrolyze the carbohydrates into simple sugars. After hydrolysis, allow the mixture to cool to room temperature.
3. Neutralization: Neutralize the hydrolysate by adding sodium hydroxide (NaOH) solution drop-wise until the pH of the solution is between 6 and 7. The neutralization is important to prevent the acid from interfering with the subsequent colorimetric reaction.

B. Preparation of anthron reagent

Anthron reagent was prepared by adding 28 ml of H2O to 72.0 ml concentrated H2SO4 (98%) and 50 mg of anthron with vigorous shaking.

C. Preparation of standard curve of glucose

From the stock solution, 250 mg of glucose in 100 ml of distilled water, serial concentrations of glucose-containing 50, 100, 150, 200, and 250 µg glucose per ml water were prepared in five cleaned tubes. To each tube, 05 ml of anthron reagent was placed, and all tubes were placed in a boiling water bath for 10 min. Tubes were left to cool for 15 min. at room temperature, then O.D. readings were made at 620 nm.

Determination of the total carbohydrates percentage

The total carbohydrates were determined by method which described by Morris (1948) [15], Dubois *et al*., (1956) [14] using anthron reagent.

Statistical Analysis

Killing rates were corrected by using Abbott's formula and statistical processing was done using ANOVA, and the least significant difference was measured by measuring the correlation between fruits content and the dominance of the *Bactrocerazonata* over the *Ceratitis capitata.*

Results and discussion

The fruits were incubated for 7 days, then the numbers of individuals of the adult stage of the insect emerging from the fruits were counted, and the appropriate type of fruit was identified to conduct an analysis of the protein, fats, and carbohydrates of each of the fruits. Data showed that there was a significant difference between the infection of the fruits under study, amounting to about LSD = 13.96, and that Guava fruits were the most infected. It was also shown that the type of Guava fruit was the preferred type for both insects. It was also shown that the peach fly was dominant over the fruit fly in the event that the eggs of both insects were laid in the same fruits, through entomological counting of the flies emerging from replicates of the mixed experiment as shown in (Fig 1).

After placing the studied fruits in the cage and exposing them to fully ripe insects for 24 hours, the pricks in the fruits were counted and it was found that guava fruits were the favorite of the two flies because of their nutritional content compared to peaches and mango fruits, as shown in Fig (2).

By incubating the pricked fruits in experimental cages on the Guava fruits, it was found that both flies were in Guava fruits that were subjected to pricking separately (each cage contains 50 pairs of one of the two flies in the state of sexual maturity), they were approximately equal in number of insects resulting from laying eggs in Guava fruits, with a ratio of approximately 1:1 for males and females. While fruits exposed to mixed fly cages (25 pairs of both flies) were used to lay eggs, *B. zonata* larvae dominated on *C. capita* by approximately 80% to 90% of the adults. While the *C. capita* did not complete

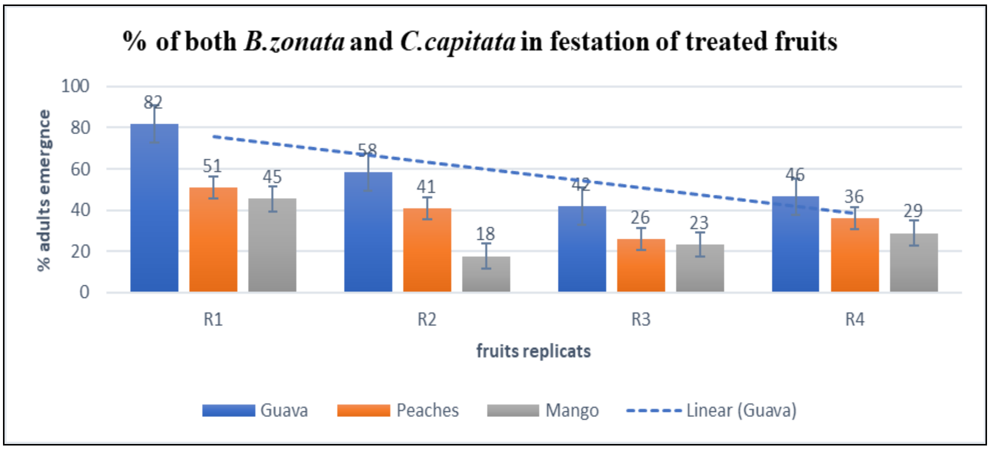

Fig 1: Percentages of both peach adults fly and fruit adults fly infestations of Guava, Peaches and Mango fruits under laboratory conditions during season (2023).

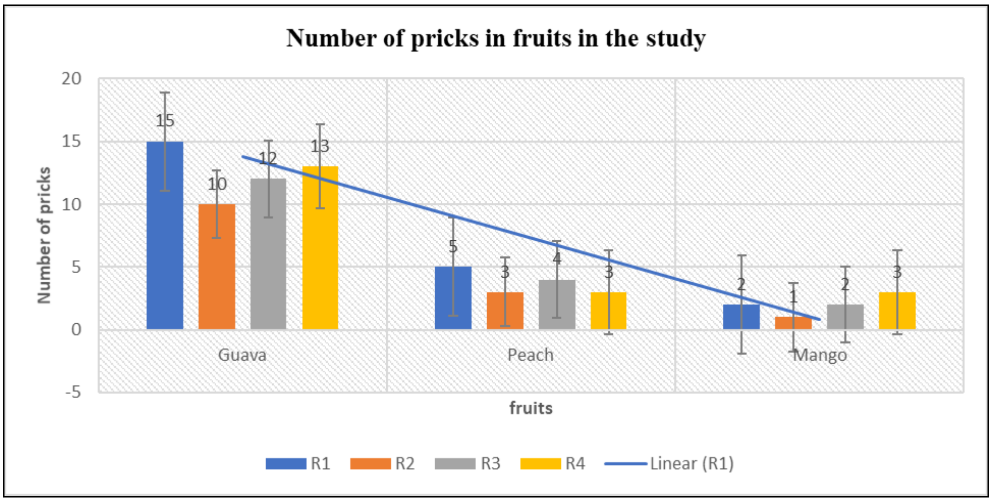

Fig 2: Number of pricks in guava, peach and mango fruits infected with fruit flies under study.

Its exit rate at the same rate as *B. zonata*. Through statistical analysis, it was revealed that there was a significant difference and that LSD = 14.18. as shown in (Fig 3).

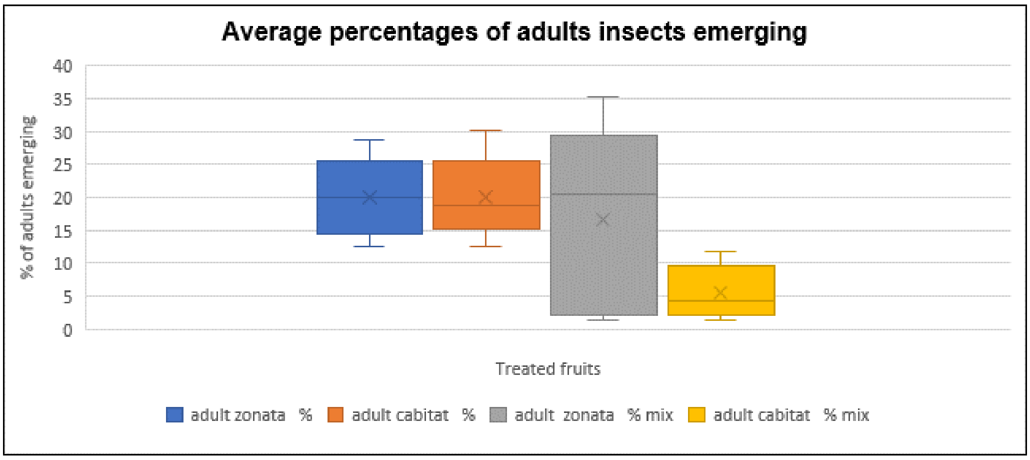

Fig 3: Percentages of mixed treatments of Guava fruits exposed to both *B. zonata* and *C. capita*higher flies.

Successive Tephritid contain three larval traits were significantly affected by the successive fruit species and effect of the successive fruits on the treatment larvals were different between different successive Tephritids. Also, the successive polyphagous species such as; *Bactrocera zonata*, *Ceratitis catoirii*, *C. rosa*, and *C. capitata* were able to surviving on a wide range of fruits than the oligophagous species such as *Zeugodacus cucurbitae*, *Dacus demmerezi*, and *Neoceratitis cyanescens*. Results indicated also to that the pupals weights were positive correlation significantly with the larvals survival period, and on the other side was negative correlation significantly with the developmental period for the successive polyphagous species. Abir Hafsi *et. al.* (2016) [1]. indicated to that nutrients of the larval stage period has correlated significantly effects with both of the growth of the fruit flies, body weight and fecundity. In current study authors investigated the effects of the diet composition on the life history traits of The oriental fruits fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). There was a strong effect of diet on daily fertility, as flies on the fruit-based diet had the highest daily fertility, while they had the lowest daily fertility on the sugar-rich diet. Daily fecundity of fruit flies on standard, high-sugar diets gradually increased from day 1 to day 15, while it decreased on high-protein, fruit-based diets (Tephritidae). Than The Anh. *et. al.* (2022) [20]. Results indicated also to that serious case of the insect adaptation with a new larval environment consists of fruits waste and authors also were concluded that successive fruits flies when feeding on the orange bagasse as the only diet system of larvals development, morphological, nutritional, chemical, and sexual behavioral adjectives different from those of flies which fed on the artificial diet or other fruit flies which originates from the wild fruits. Carlos Pascacio-Villafán *et. al.* (2023) [4] who referred to a consequence of modifications of the digestive enzymatic process and nutritionally poor diet system can be noticed as first increases in the proteolytic activity and in the abundance of transcripts associated to serine-proteases. Marco Bonelli *et. al.* (2020) [12] referred to that the lipids content aggregates during the larvals dispersal phase and also during the first 20 h of the pre-pupals stage period. Also, these levels go down a bit through the pupals stage and also during the first half of the pharate adults stage period and the trend is declining radically toward the last hours of metamorphosis. And also indicated to that carbohydrates and glycogen were highly percentage (content) when used from the moment the larvals leave the diet system until the mid pupals stages period, then the glycogen secrets increases toward to the mid pharate adult and the content were decreases again until reach to the low levels at the moment of the adults stage. Also protein contents were decreased during the pre pupals stages period recovering toward the pharate adult's stage. David Nestel (2003) [7] and Marisa A. Rodrigues (2015) [13].

It has been shown that Guava fruits have the highest percentage of protein and fat and a good percentage of carbohydrates, which makes them preferable for laying eggs and growing larvae inside the fruits. It was also shown that the larvae of *Bactrocerazonata* flies were dominant in growing inside fruits over the larvae of *Ceratitis capitata*, and by analyzing the fruit content of Guava fruits.

Statistical analysis of the nutritional content of the infested and control fruits revealed a significant difference between the fruits infested with each of the two flies separately and between the infested fruits with both flies and the control, with the least significant difference of about (LSD = 28.8), as it was shown that the fruit content and the nutritional requirement of the larvae there was a difference between them. This leads to the dominance of *Bactrocerazonata* larvae over *Ceratitis capitata* larvae. The fruit fly needs a higher protein content and higher fat content than the *Bactrocerazonata*. While the *Bactrocerazonata* needs less carbohydrates and fats, as the amount of fats in the larvae's feed was the main factor in the formation of the ovaries of the adult insect, as they begin to form during the parthenogenetic stage. Thus, this explains the sexual maturity of the fruit flies. After 3 days, they could be fertilized, while the peach flies need about 15 days for ovarian formation and sexual maturity.

By analyzing the nutritional content of infected Guava fruits, a gram of weight (mg/100gm body weight) of the control contained protein, lipid, and carbohydrates in proportions of 2.48 - 22.23 - 37.54%, respectively. While the need for larvae in fruits infested with solitary fruit flies, the grams of weight contained 5.82 - 15.62 - 83.30%, respectively, for solitary fruit flies, while in fruits infested with the peach fly, they contained 1.59 - 14.95 - 47.98%, respectively. Thus, it became clear that the nutritional content of protein and carbohydrates for growing fruit fly larvae was higher by about 27.32% - 57.6%, respectively. From the growth of peach fly larvae. Perhaps this may explain that the physiological maturity of the fruit fly occurs about 3 days after the insect emerges, while the physiological maturity of the peach fly occurs about 15 days after the emergence of the adult insectas shown in (Fig 4).

Biochemical analysis of the pupae and larvae of both flies showed that there was a significant difference for the larvae and pupae of the two flies, with the least significant difference about (LSD = 46.81). As *Ceratitis capitata* larvae contain nearly twice the protein content of *Bactrocerazonata* larvae, about 49.3%. While the carbohydrate content was 50.1%, as for fats, the content for fruit flies was lower by about 6.4%. As for the

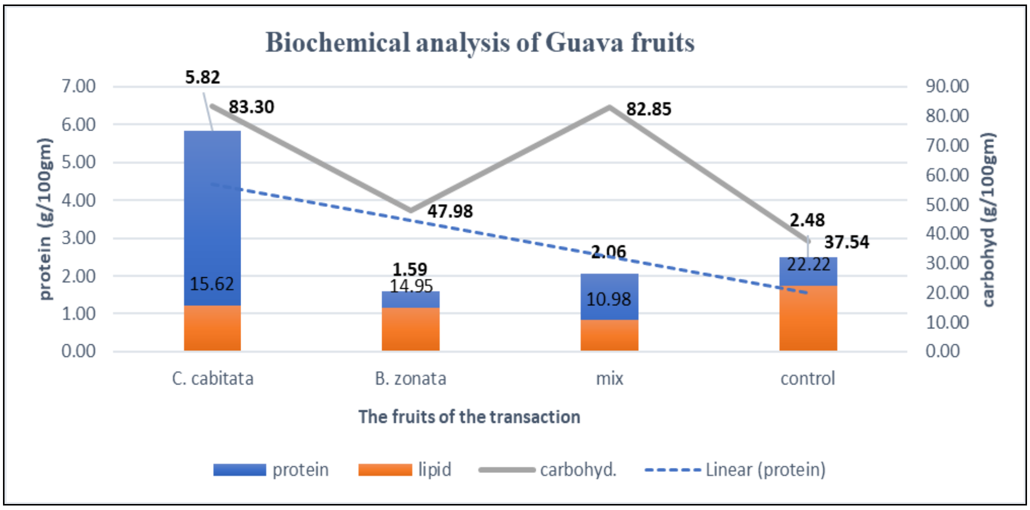

Fig 4: Nutritional content analysis of fruits infected with fruit flies.

Analysis of the nutritional content of Guava fruits infected with fruit flies
virgins, they were similar in content, approximately 84.03% - 83.47% - 89.44% of protein, fats and carbohydrates, respectively.
Perhaps this may be due to nutritional changes in the larvae to form pupae, which affects the sexual maturity of the *Bactrocerazonata* insect after emergence and the dominance of the *Ceratitis capitata* larvae in the fruit content as shown in (Fig 5).

Conclusions

It has been shown that the nutritional content of fruits affects the growth of larvae at their three ages before the jumping stage to form pupae, which led to the dominance of peach fly larvae over fruit flies due to their low nutritional needs for protein and carbohydrates.
While it requires a greater content of fat, which is a low-need element for the fruit fly, this may explain the early sexual maturity of the fruit fly, which may not exceed 3-4 days at optimal temperatures, and the late sexual maturity of the peach fly, which may reach 15-17 days. This may lead to the need to conduct studies on the development of the sexual maturity of the two insects to find out why the sexual maturity of the peach fly is later than that of the fruit fly**.**The study showed that in the presence of both the *Ceratitis capitata*and the *Bactrocerazonata*, attention is paid to combating the *Bactrocerazonata*because of its dominance, and integrated control (IPM) can be applied by eliminating the males because their life cycle is not complete, as the females need about 15 days to complete sexual maturity.

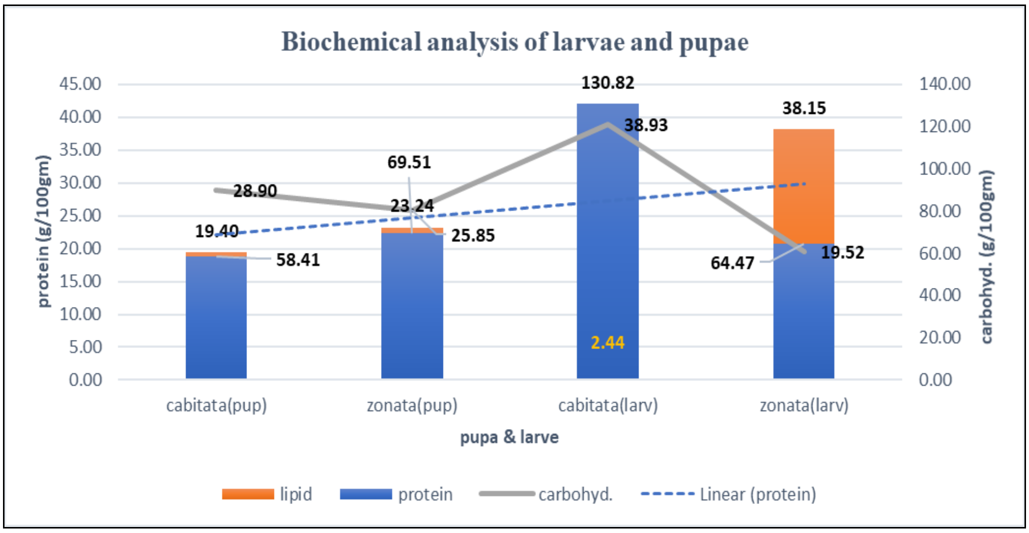

Fig 5: Biochemical analysis of the larval and pupal content of fruit flies and its effect on the fruit content of Guavafruitson the survival and growth of larvae and pupae

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