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Recycling of Date By-Product for Mass Rearing of Peach Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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HIGHLIGHTS

- Using date seed powder as Alternative for yeast in rearing of peach fruit fly, *Bactrocera zonata.*
- Date seed provide insects quality parameters corresponding to FAO, IAEA/USDA standards.
- Date seed costs less than yeast.

Abstract: The world is currently facing a severe economic crisis and the rational consumption and reuse of products, including waste are necessary. Agriculture wastes have importance containing beneficial and economic value matters, which unutilized may lead to environmental pollution. Date seed is one of these viable wastes with high nutritional value. Mass rearing of peach fruit flies is important to produce parasitoids for use in biological control programs or sterile insect techniques. In this study powder of date seeds were evaluated as an alternative diet for mass rearing of peach fruit fly *Bactrocera zonata*. The nutrient composition of the tested diet that contained date seed powder was rich in crude proteins (13.1%), crude carbohydrates (40.41), crude fats (4.6), crude fibers (8.4), and ashes (0.29). The results indicated that the date seed powder produced insects with quality parameters equivalent to FAO, IAEA/USDA standards, without differences with those reared on yeast. The histology of the reproductive system of *B. zonata* reveals that the female ovariole is of meristic-polytrophic type with successive stages of egg chambers. The male testis has a single long follicle with successive stages of spermatogenesis. Thus, date seed powder is a potential alternative diet for use in the mass rearing of the peach fruit fly.

Keywords: Alternative diet; biological control; date seed; peach fruit flies.

INTRODUCTION

The peach fruit fly, Bactrocera zonata (Saunders) (Diptera: Tephritidae), is a polyphagous pest of different crops, vegetables, and fruits [1]. In Africa, *B. zonata* has been found in some commercially significant fruits [2,3].

For effective integrated management programs to control the peach fruit fly, sustainable diets for the mass rearing of that insects should be developed to assess its potential parasitoids, preferred hosts, and genetically modified strains [4]. To date, diets based on yeast have been used to rear *B. zonata*. The yeast has a direct consequence on the fecundity and fertility of the adult fruit flies, since it provides the essential growth factors for fruit flies, including peptides, minerals, vitamins, and amino acids [5].

Due to its economic and dietary benefits, the date palm (*Phoenix dactyliferous* L.) is one of the most widely grown trees and its fruit is an important agricultural product in Egypt, which is grown in a variety of locations, including Siwa, Marsa Maruth, and Alwadi Al gadid [6].

A surplus of issues, including environmental degradation, eutrophication, greenhouse gas emissions, and consequences on human health, are brought on by improper handling of organic waste. Recycling these organic wastes, which contain valuable plant material and organic matter, is a promising step to take advantage of their nutritional value and incorporate indirect environmental benefits. These wastes may also be used as effective chemical fertilizer replacements. Recycling agricultural wastes has significant economic benefits since it lowers costs compared with trash transportation, high import costs, and novel chemical fertilizers. Recycling and using organic wastes may contribute to reduce contaminants such as heavy metals and persistent agricultural wastes, which have serious environmental challenges [7].

Agriculture wastes, includes date seed ranging from 10% to 15% of the dates mass [8]. In 2018 more than 8 million tons of date fruit were produced, indicating that date seed represents a significant quantity of trash [9]. The potential production of date seed is approximately 800,000 tons, that has been partially used as soil fertilizer or animal feed [10].

Date seeds have about 8.8 - 9.6% moisture, 3.88 - 5.62% protein, 15.84 -18.01% crude fiber, 10.7 -8.5% ether extract, and 58.80 – 56.79% carbohydrates [11].

The purpose of this study was to evaluate if date seed powder may be used as an alternative diet for mass rearing of the peach fruit fly, contributing to reduce of the use of expensive diets using yeast.

MATERIAL AND METHODS

1. *Bactrocera zonata* rearing under laboratory conditions

The peach fruit flies were obtained from the Laboratory of Plant Protection Research Institute, Dokki, Giza, Egypt, reared at 25±3 ºC, 60±5% R.H. and 12:12 h (L:D) photoperiod. Adult were kept in cages (110 cm length × 8 cm width × 85 cm height) and fed on sugar and protein hydrolysate (3:1) [12]. Small plastic orange-shaped perforated and filled with 2 cm of water was put inside the rearing cage as egg receptacle.

2. Artificial diet

The ratios of the various components required to prepare diets are displayed in Table 1. All diet components were weighed before being combined using a plastic rod to create a homogeneous diet. Briefly, the tested diet had the same amount of date seed power substituting yeast.

Table 1. Composition of larval diets tested for *Bactrocera zonata.*

Ingredients	Control Diet	Tested diet
Water	500 mL	500 mL
Sodium benzoate	3 g	3 g
Sugar	84.5 g	84.5 g
Citric acid	3 g	3 g
Wheat grain	330 g	330 g
Date seed powder	-	84.5 g
Yeast	84.5 g	-

3. Treatment

After preparation of the diets, 200 g were placed into plastic rearing pot. A small piece of black paper was moistened with water and placed in the median region of each pot of the diet. Then 100 eggs (one day old) were scattered onto the wet black paper. The pots were covered with muslin and incubated at 25±3 °C and 60±5% R.H and the number of hatched larvae counted daily. Five replicates with 100 eggs each were used to evaluate the quality control standards for fruit flies in the diets according [13].

Then the trays were transferred to plastic boxes (80 cm length × 8 cm width× 70 cm height) containing 1 cm of sand layer in the bottom for the larvae molt to pupae. The sand was sieved and the number of pupae counted. Three replicates of 50 newly-formed pupae were weighed after one day (one-day-old pupae). Five sets of 100 pupae were transferred to adult rearing cages with sugar and protein hydrolysate (3:1) to obtain the number of adult emergence.

4. Adult fliers and rate of fliers:

One hundred pupae in five replicates from each of the two different tested diets were placed at the bottom of black Plexiglas tubes (8.9 cm in diameter x 10 cm x 126 cm high); with the walls covered with127 unscented talcum powder [13], to determine the proportion of adult fliers. The surviving flies and pupae were counted after the adult flies left the tubes.

5. Biochemical analysis

Adults emerged from diets were homogenized separately in distilled water (1 g of insect mL^{-1}) using a chilled glass–Teflon tissue homogenizer. The samples were centrifuged at 8000 x g at 4 °C for 15 minutes, and the supernatant stored at -20 °C following use to determine the total protein content according [14], total carbohydrates [15], lipids [16], glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) [17].

6. Light microscope

To evaluate the reproductive parameters, 25 day old adult males and females (20 insects for each diet and sex) were anaesthetized with ether and dissected in saline solution (0.1 M NaCl, 0.1 M KCl). Then, the reproductive tracts were transferred to aqueous Bouin's fixative solution for 24 h, washed and dehydrated in a graded ethanol series (70%, 80%, 90%, and 99%), cleared in xylene, and embedded in paraffin wax. Sections 6 µm thickness were obtained with rotatory microtome, stained with hematoxylin and eosin and analyzed using an Olympus BX51 microscope.

7. Analysis of the macronutrients from diets

The macronutrients from the control and tested diets were evaluated in the Haram-Giza Center for Bread Technology. Percentage of crude protein, lipids, fibers, water, and ash were determined according to [18]. Total carbohydrate (%) was estimated according to Shumaila & Mahpar [19].

8. Statistics

A t-Student test was used to compare the variations in quality metrics and nutritional levels for flies grown on the investigated diets. The same test was used to compare the nutritional components of the diets. The statistical analyses were performed using IBM SPSS Statistics Version 22 at 5% of significant level.

RESULTS

The values for the evaluated quality control parameters of peach fruit fly were similar between both diets tested. The percentage of egg hatchability, pupae, adult emergence, flying ability and flying rate were 97.7, 96.7, 95, 99, and 99.7 for insects reared on control diet, respectively and 97.33, 96, 94.3, 98.7, and 99.7 with diet containing date seeds power, respectively (Figure 1).

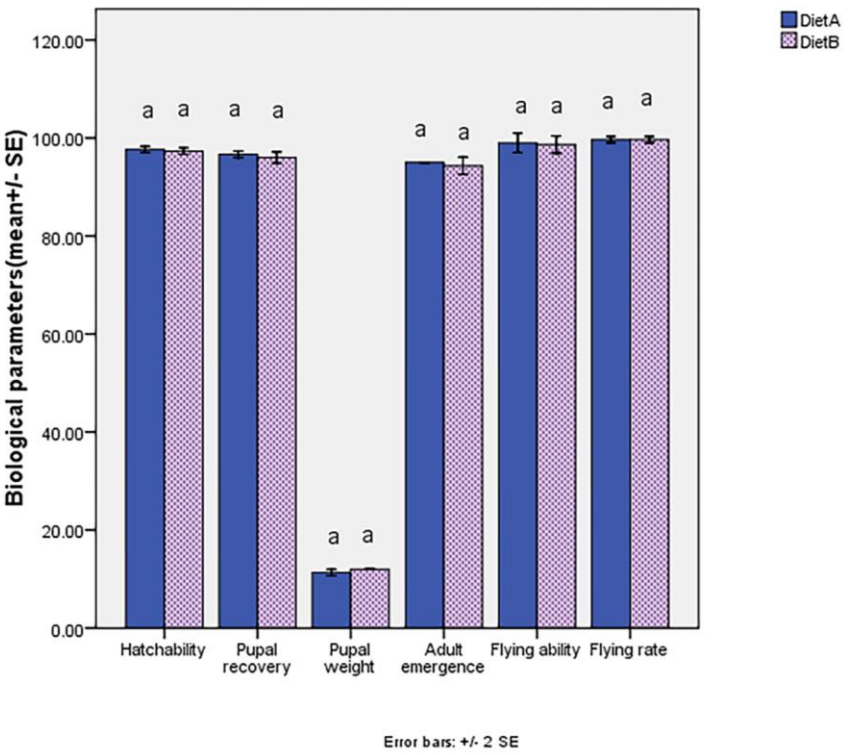

Figure 1. Quality control parameters (mean ± SE) for *Bactrocera zonata* (Diptera: Tephritidae) reared on the two larval diets. Diet A – control. Diet B – treated with date seeds powder. Bars with same letter are similar by t-Student test at 5% significance level.

The percentage of total protein, carbohydrate and lipids in the body of male and female flies were higher in diet with date seeds powder than in the control (Figures 2 and 3), whereas the GOT and GPT body content for male and females flies were similar between the diets (Figures 2 and 3).

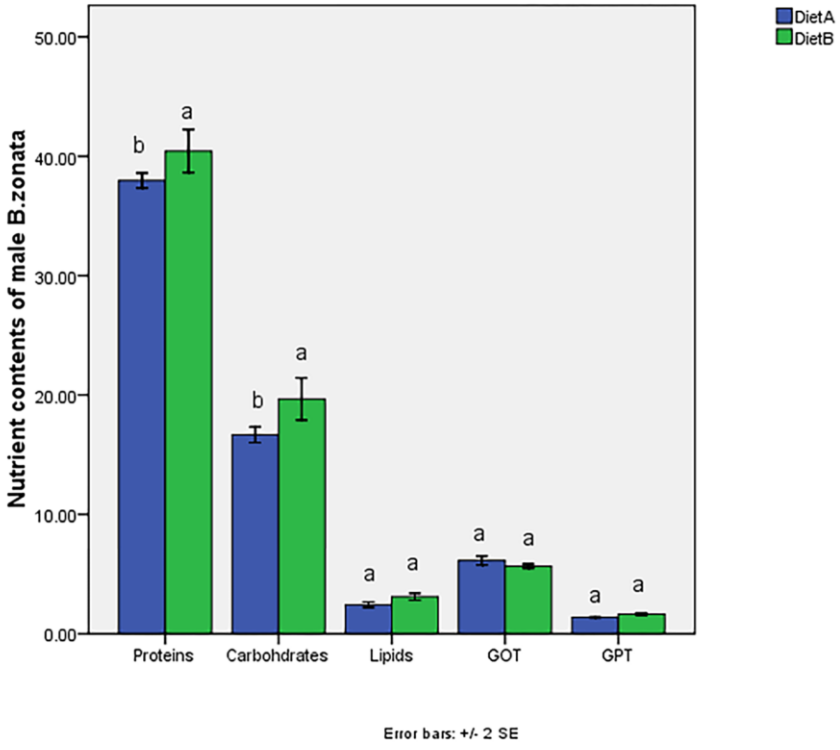

Figure 2. Nutrient content (mean ± SE) in the male body of *Bactrocera zonata* (Diptera: Tephritidae) adult reared on different larval diets. Diet A – control. Diet B – treated with date seeds powder. Bars with different letters differ by t-Student test at 5% significance level. GOT - glutamic oxaloacetic transaminase, GPT - glutamic pyruvic transaminase.

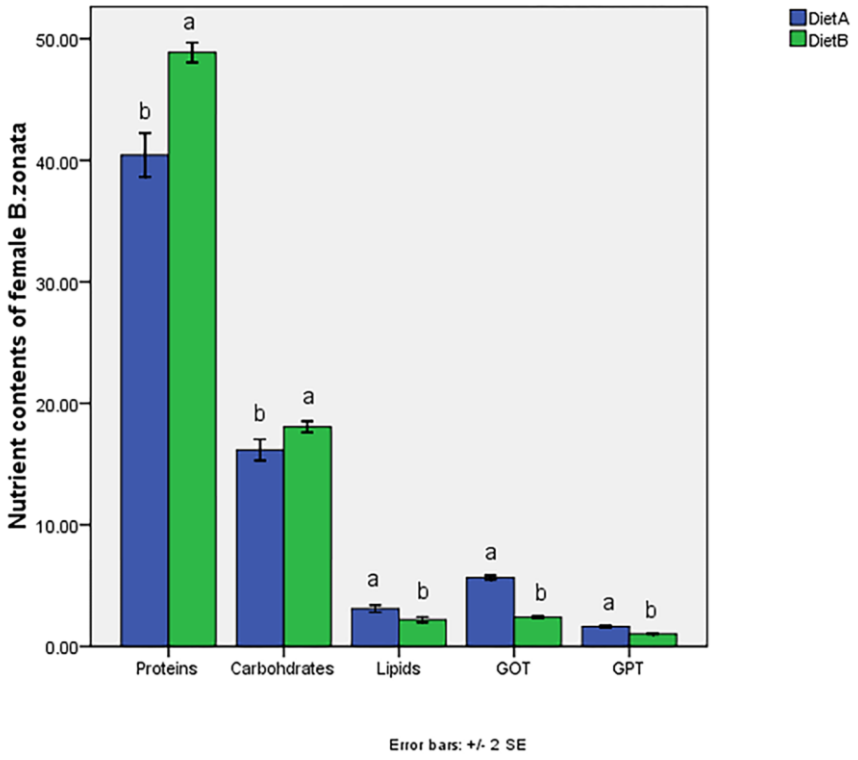

Figure 3. Nutrient content (mean ± SE) in the female body of *Bactrocera zonata* (Diptera: Tephritidae) adult reared on different larval diets. Diet A – control. Diet B – treated with date seeds powder. Bars with different letters differ by t-Student test at 5% significance level. GOT - glutamic oxaloacetic transaminase, GPT - glutamic pyruvic transaminase.

In both diets, female reproductive tract mature at 21-22 days after adult emergence, but male reproductive system matures at the end of the first week of adult lifespan. The ovary of peach fruit fly consists of meroistic-polytrophic ovarioles, with egg follicles in different stage of development (Figure 4). Each egg follicle has one oocytic chamber and a nurse chamber, both surrounded by follicular epithelium (Figure 4).

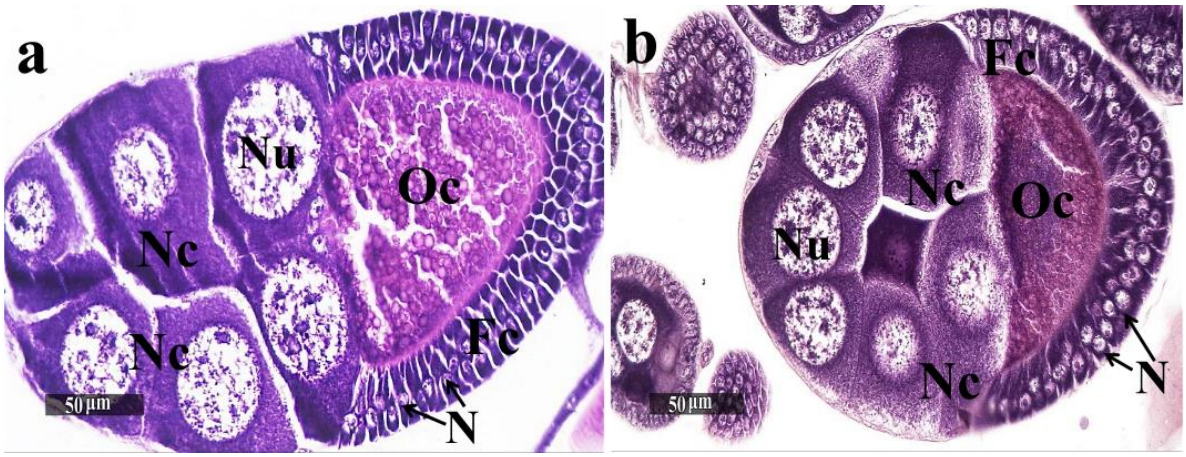

Figure 4. a. Light micrograph of female ovary of *Bactrocera zonata* adults resulted from larvae feed on Diet A-control showing mature egg chamber of female ovary. **b.** Light micrograph of female ovary of *Bactrocera zonata* adults resulted from larvae feed on Diet B-treated with date seeds powder showing mature egg chamber of female ovary where (Oc) oocyte, (Nc) nurse cell, (Nu) nurse cell nucleus, (Fc) follicular cell epithelium, and (N) follicular cell nucleus.

The male testis showed follicles with spermatogonia and spermatocytes at different stage of development (Figure 5). Each spermatocyte undergoes the meiotic division to produce spermatids (Figure 5). The spermatids in the cysts differentiated into spermatozoa (the zone of transformation; Figure 5). The whole testis is surrounded by a peritoneal sheath (Figure 5).

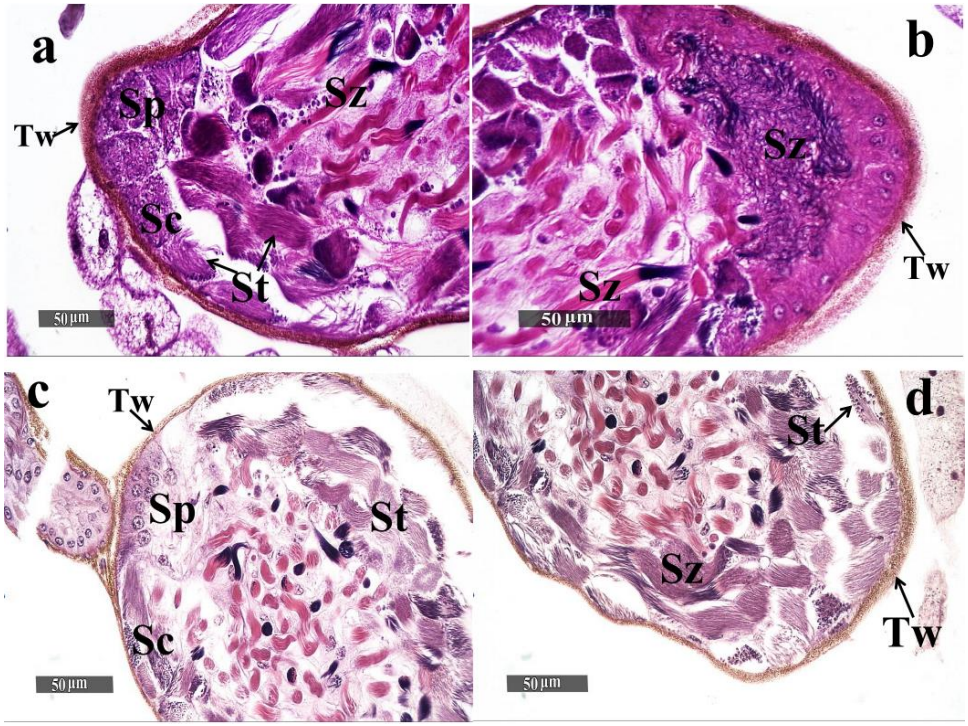

Figure 5. a, b. Light micrographs of male testis of *Bactrocera zonata* adults resulted from larvae feed on Diet A-control showing male testis. **c, d.** Light micrographs of male testis of *Bactrocera zonata* adults resulted from larvae feed on Diet B-treated with date seeds powder showing male testis where (Sp) spermatogonia, (Sc) spermatocytes, (St) spermatid, (Sz) spermatozoa, and (Tw) testis cell wall.

The analyses of nutrients varied between diets (Figure 6). Control diet showed higher protein content (22%) than diet with date seeds powder (13.1%; t = 26.7, df = 4, $p < 0.05$; Figure 6). Carbohydrates content was higher in diet with date seeds powder (40.41%) than control ones (30.35%; t = -17.18, df =4, $p < 0.05$). Ash, fats, fibers, and moisture were similar in both diets (Figure 6).

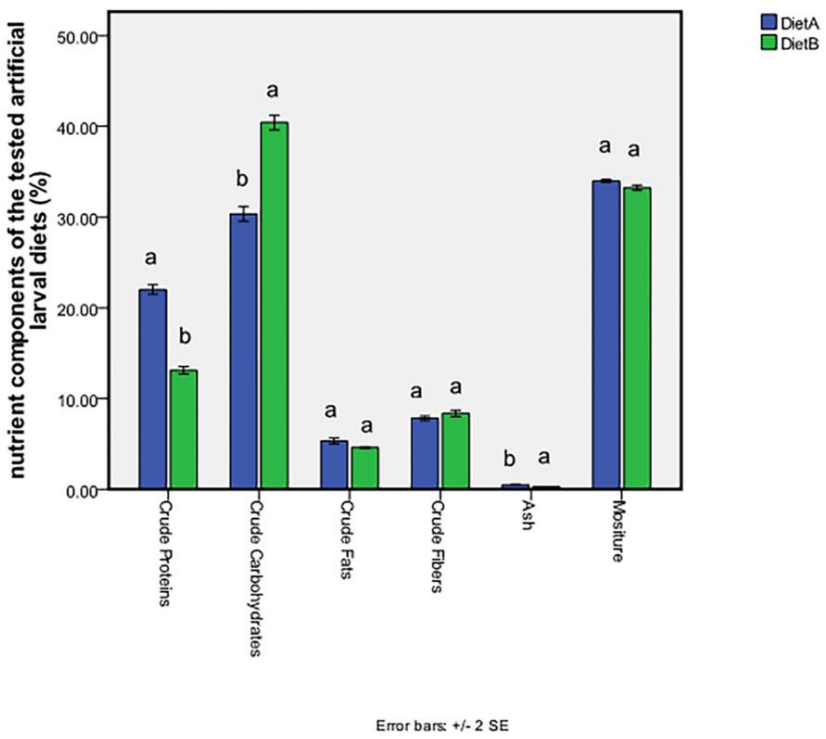

Figure 6. Assessment the different nutrient components of the different tested artificial larval diet (mean ± SE). Diet A – control. Diet B – treated with date seeds powder. Bars with different letters differ by t-Student test at 5% significance level.

DISCUSSION

The quality control parameters of *B. zonata*, including egg hatchability, pupae, adult emergence, flying ability, and flying rate, reveal no differences between insects reared with the different diets. These findings are in agreement with those found by Abdel-Hafeez and coauthors [20] that have pointed out absence of difference in emergence percentage and flight ability in *Ceratitis capitata* (Diptera: Tephritidae) larvae reared on conventional yeast artificial diet and that with date seeds powder. Also these results are in harmony with Maset and coauthors [21] that determined the optimal artificial larval diet and the respective larval density for *C. capitata* on diets based on corn flour, sugarcane bagasse, and lyophilized carrot powder, considering biological parameters of quality control and economic viability. According to FAO/IAEA [13] our findings shown that date seed powder diet produces pupae >7.5 mg in weight and 93% of adult emergence.

The analyses of insect body to verify how the feeding of peach fruit larvae is affected by the nutrient content of the tested diets reveal higher protein, carbohydrate and lipid in flies emerged from diet with date seeds powder than that with yeast. The obtained data agree with those from Rendon and coauthors [22] that examined how body nutrient contents of an invasive fly, *Drosophila suzukii* were affected by dietary protein and carbohydrate showing that flies that received rich-protein diet have lower carbohydrate, protein and, lipid body content compared to flies fed on rich-carbohydrate and poor-protein in their diets.

The ovariole of *B. zonata* female likely all other Diptera, is of meristic polytrophic type [23]. Shehata and coauthors [24] found that the ovaries of a newly-emerged *B. zonata* male are underdeveloped and they reached maturity on the 21st or 22nd day of adult lifespan. *Bactrocera zonata* males reared on both diets reach maturity at the end of the first week of adult male lifespan, which has been characterized by the presence of all spermatogenesis stages in the testis follicle [25]. Together, these findings indicate that diet with date seed powder is suitable for reproduction of the peach fruit fly.

Our findings shown that the percentage of carbohydrates is higher than protein in diet based on date seed powder. These two components in larval diets of fruit flies are responsible basically on the body size, development and survival of adults [26]. Behmer [27] hypothesized that whereas some phytophagous insect larvae prefer high-protein diets, others prefer diets high in carbohydrates. As demonstrated by Hafsi and coauthors. [28], polyphagous tephritids have different dietary needs from oligophagous tephritids and the performance of polyphagous species is invasive fly, *D. suzukii* associated with diets rich in lipids, carbohydrates, and fibers but not proteins. Date seeds powder diet here evaluated contain 13.1% of proteins, similar to the suitable amount for larval rearing artificial diet of oriental fruit flies [29]. High carbohydrate content in larval artificial diets with wheat germ produce peach fruit flies with good quality parameters [30].

The low crude fats content in the both diet tested here has no effect on the quality of peach fruit flies between the tested artificial diets. Diets based on wheat germ with ca. 3% fats are suitable for rearing of peach fruit fly with quality parameters equivalent to FAO/ IAEA/USDA standards [30].

The fiber content of diet with date seeds powder is similar to that with yeast about 10%. The fiber content is essential in insect mass rearing for permitting a most favorable milieu for larval development [29]. The suitable crude fiber content has been claimed to range from 5 to 26% [31], which occurs in the diets here evaluated.

CONCLUSIONS

Date seed powder used in an artificial diet for mass rearing of peach fruit fly *B. zonata* as alternative for that with yeast provide insects with good quality parameters, including biochemical components and reproduction, but with lower costs than diets rich in yeast. Overall, our findings provide evidence that date seed powder may be used in the mass rearing of peach fruit fly that are used for sterile insect technique or to produce parasitoid for use in biological control programs.

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Conflicts of Interest: “The authors declare no conflict of interest.”

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