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Which artificial larval diet is better for *Ceratitis capitata* (Diptera: Tephritidae) rearing?

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Abstract

Background: There is an increasing demand for biological agents for integrated pest management programs, requiring a constant supply of insects in terms of quantity and quality. In this context, the development of insect-rearing methods and quality control parameters are essential in order to provide best-quality and economic viability products. The medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the most economically important species of fruit flies in several fruit-producing areas of Brazil, being massively reared in many countries for Sterile Insect Technique (SIT) programs. To achieve mass production scale, suitable larval diets are necessary for medfly rearing at laboratory conditions. Therefore, the objectives of this study were to determine the best artificial larval diet and the respective larval density for *C. capitata* on diets based on corn flour (CF), sugarcane bagasse (SB), and lyophilized carrot powder (CP), considering biological parameters of quality control and economic viability.

Results: Based on the obtained results and although all diets produced quality pupae according to FAO/IAEA/USDA standards, the SB artificial diet promoted better results in the number of larvae, larval weight, number of pupae, and pupal weight, in comparison with CF and CP diets, as well as being the most economically viable. The optimal larval densities on CF, SB, and CP diets were, respectively, 0.5, 2.0, and 1.0 mL of eggs/kg of diet.

Conclusions: The diet that was most suitable for insect yield and economic viability was the SB diet, followed by the CF and CP diets, in this order.

Keywords: Medfly, Sterile insect technique, Artificial diet, Quality control, Economic viability

Background

The maintenance of insect colonies at the laboratory is essential to carry out research and applications of different strategies in integrated pest management programs, requiring a continuous supply of insects in terms of quantity and quality (Parra et al., 2002). With the increase in companies and the commercialization of biological agents, the need to monitor the quality of insects reared at laboratory conditions also increases, being essential for the development of insect-rearing methods and quality control parameters in order to provide best-quality and economic viability products to guarantee the success of integrated pest management programs (Parker, 2005; Parra et al., 2002).

Many fruit fly species have been the target of investigations aiming at their mass rearing, which is necessary for the sterile insect technique (SIT) application or for the production of natural enemies (Walder, 2002). Sterile male flies released in the field need to present competitive biological parameters against the wild males, so that they can copulate with the wild females and ensure

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the viability of this self-control technique. The Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a fruit fly species with major economic and quarantine importance in many fruit-producing areas of Brazil, such as the Vale do Rio São Francisco (Habibe et al., 2008; Szyniszewska & Tatem, 2014). The possibility of using the SIT for its control has been explored since the 1960s (Ohinata et al., 1977).

The diet is probably the most important component of mass rearing, once pest management programs depend on the success to produce natural enemies with satisfactory biological and reproductive performance (Parker, 2005). The medfly, reared in a laboratory, maintains the capacity of surviving even with broad nutrition fluctuations (Chang, 2004; Chang et al., 2001; Nash & Chapman, 2014; Nestel & Nemny-Lavy, 2008; Nestel et al., 2004; Zucoloto, 1993). *C. capitata* is a polyphagous and multivoltine species, which facilitates its adaptation to larval diets (Malavasi & Zucchi, 2000), and the development of suitable diets for *C. capitata* larvae rearing on a large scale has allowed more than ten integrated pest management programs (IDIDAS 2019).

After artificial selection, the medfly can even be reared successfully in artificial diets, derived entirely from a non-vegetable ingredient (Zucoloto, 1993). Good nutrition during the larval phase is important for the suitable development of insects, affecting the survival and the size of adults (Cresoni-Pereira & Zucoloto, 2009). Larval diets can influence the mating success of adults and insects reared on poor diets can suffer reduced fitness (Anjos-Duarte et al., 2011; Kaspi et al., 2002; Navarro-Campos et al., 2011; Nestel & Nemny-Lavy, 2008).

Diets based on sugarcane bagasse have been used for a long time to rear *C. capitata*. Studies with diets containing this bulky agent showed that a large number of high-quality pupae can be raised without decreasing the fecundity and fertility of adults following consecutive generations (Vargas et al., 1983). Chang et al. (2000) developed a meridic diet for *C. capitata* with corn cob as a bulky agent. All ingredients of this diet, except one, were chemically characterized. Corn cob, although containing some nutrients, is basically a nutritionally inert ingredient (Chang et al., 2001). Mitchell et al. (1965) developed a larval diet based on lyophilized carrot powder as a bulky agent, which has been used by several laboratories (Peleg & Rhode, 1970; Zucoloto, 1987). The USDA laboratory has already used fresh and dehydrated carrots for rearing this species (Christenson et al., 1956).

Several mass-rearing facilities in the world are capable of producing sterile medflies that are used for population eradication and area-wide suppression.

The Biofactory MOSCAMED Brazil, a medfly mass-rearing facility inaugurated in 2006 in northeastern Brazil, released more than 8 million sterile males in pilot projects at fruit-growing areas of the Vale do São Francisco (MOSCAMED Report 2007), and those amounts were obtained rearing the larvae on a diet with sugarcane bagasse (Walder, 2002). Unfortunately, financial constraints related to logistic and labor costs significantly limited their medfly production in the following years. The availability of less costly diets in the region could have minimized those constraints.

An adequate artificial diet that meets nutritional requirements and quality control is essential for fruit flies mass rearing in the laboratory (Walder et al., 2014). In this context, to reduce production costs, the optimization or development of new diets cannot stop, and studies assessing biological parameters of quality control are necessary before encouraging the application of an SIT program against this pest in Brazil.

Therefore the present study aimed to evaluate and compare modified and novel larval diets, determining the most efficient and economically viable diet for *C. capitata* mass rearing in Brazil.

Methods

Ceratitis capitata rearing under laboratory conditions

The strain of *C. capitata* used to provide eggs for the tests of the present study was obtained from a population established originally with wild pupae from infested mangos in Petrolina, PE, Brazil, and has been maintained under semi-mass rearing conditions at the Center for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP) adapting the procedures described by Caceres (2002) for *Anastrepha fraterculus* rearing at laboratory conditions. The colony was kept in climatized rooms under controlled conditions at 25 ± 2 ℃ and 75 ± 5% RH throughout the life cycle, and 12:12 h (L:D) photoperiod. The total dark condition was used for pupae development. Medflies adults were kept in "sandwich" cages (100 (length) × 6 (width) × 75 (height) cm) from the El Pino biofactory, Guatemala, containing voile cloth on the sides. Their feed consisted of refined sugar (*Caravelas*®*—Usina Colombo S/A*, Ariranha, SP) combined with lyophilized beer yeast extract (*Bionis*®—YE MF *Biorigin*, Lençóis Paulista, SP) (3:1), as described by Walder et al. (2014), and water ad libitum. The ovipositing cages were distributed in a randomized design, and the oviposition occurred on the cage screens. The eggs were collected after 24 h in trays containing water. In order to obtain the laboratory-reared larvae, eggs collected from the mother colony were kept bubbled in a water bath at 24˚C for 48 h before being seeded in the artificial larval diets for the tests described below.

Artificial larval diets for *Ceratitis capitata* rearing

The three distinct artificial larval diets tested are a) novel diet based on corn flour (CF) (initial pH = 4.2–4.5); b) modified diet of Graham and Dudley (1959), Taylor et al. (1991) and Chaudhury and Skoda (2007), based on sugarcane bagasse (SB) (initial pH = 3.5–3.7); c) novel diet based on lyophilized carrot powder (CP) (initial pH = 3.5). The three diets are described in Table 1.

To determine the optimal larval density for each artificial diet described, five different volumes of eggs were inoculated separately: 0.3, 0.5, 0.8, 1.0, and 2.0 mL of eggs/1 kg of larval diet. Each aliquot of eggs was previously bubbled in water and then seeded to evaluate the larval development in each treatment.

The preparation of diets occurred one day before the tests, and 1 kg of each diet (2 cm thick) was placed in polyethylene trays (27.3 × 17.5 cm). Each tray was covered with a polyester screen (with 105 µm holes) to avoid the entrance of *Drosophila* spp. or other contaminating agents. The different volumes of eggs were seeded separately on the three diets. Each tray was stored in a rearing room (24 ± 2 ℃, 75 ± 5% RH, and total dark). Four replications were performed for each volume of seeded eggs tested on each artificial larval diet treatment, one tray per replication. After the larvae reached the final instar larvae (third instar) and started abandoning the respective diet tray, they were collected and placed in plastic pots (500 mL) containing dry vermiculite for pupation.

Evaluation of the effects on biological parameters of *Ceratitis capitata* larvae rearing with different artificial diets

For each volume of seeded eggs on each artificial larval diet treatment, the quality control parameters recommended and described by FAO/IAEA/USDA (2014) for fruit flies were evaluated, such as the total number and the weight of third instar larvae; mean larval period; total number and weight of pupae; average weight and average diameter of pupae; egg-pupal recovery (%); mean pupal period; adult emergence (%); and mean sex ratio (female/female + male). To assess adult emergence and sex ratio, 1,000 pupae for each treatment were placed in Petri dishes, and the respective percentage was calculated 4 days after the emergence.

For the selection of the optimal seeding density for each of the three artificial larval diets, the mean values of quality control parameters recommended by FAO/IAEA/USDA (2014) for medfly were considered. For the sterile insect technique, values above 93% for adult emergence, before the irradiation, and higher than 7.5 mg pupae weight are acceptable for bisexual lineages of this species in facilities that produce sterile males.

In another experiment, after analyzing the biological parameters and determining the optimal larval density for each artificial diet tested, the best volume of eggs determined was chosen to be seeded in each treatment. The artificial diets were inserted in larger trays

Table 1 Composition and cost of 1 kg of the three tested larval diets for *Ceratitis capitata*

Ingredients	CF diet[1]			SB diet[2]			CP diet[3]		
	g or mL/kg diet	% of diet (mass)	Cost (US$/kg)	g or mL/kg diet	% of diet (mass)	Cost (US$/kg)	g or mL/kg diet	% of diet (mass)	Cost (US$/kg)
Corn flour	300 g	30.00	0.69	64.98 g	6.50	0.00	–	–	–
Brewer's yeast	50 g	5.00	0.09	99 g	9.90	0.18	70 g	7.00	0.13
Crystal sugar	30 g	3.00	0.06	119.9 g	11.99	0.24	–	–	–
Sugarcane bagasse	–	–	–	99 g	9.90	0.16	–	–	–
Wheat germ	–	–	–	29.9 g	2.99	0.06	–	–	–
Lyophilized carrot powder	–	–	–	–	–	–	150 g	15.00	0.88
Sodium benzoate	2 g	0.20	0.03	2.99 g	0.30	0.05	2.5 g	0.25	0.04
Nipagin	2 mL	0.02	0.01	–	–	–	2 g	0.20	0.05
Ethyl alcohol	2 g	0.16	0.00	–	–	–	–	–	–
Citric acid	6 g	0.60	0.05	9.9 g	0.99	0.08	8 g	0.80	0.06
Antibiotic	–	–	–	0.299 g	0.03	0.02	–	–	–
Water	1120 mL	61.02	–	573.8 mL	57.40	–	330 mL	76.75	–
Total	1 kg	100	0.63	1 kg	100	0.79	1 kg	100	1.16

[1] CF diet: diet based on corn flour, [2]SB diet: based on sugarcane bagasse, [3]CP diet: based on lyophilized carrot powder

(47.2 × 30.2 cm), with up to 3 kg per tray (until 2 cm thick), and four replications were performed for each treatment. The same quality control parameters mentioned before were evaluated, along with adult flight ability to determine the most efficient artificial diet for medfly larvae rearing. To assess the percentage of fliers, 50 pupae from each treatment were positioned in the bottom of black Plexiglas tubes (8.9 cm diameter × 10 cm high) whose walls were coated with unscented talcum powder (FAO/IAEA/USDA, 2014). After emerged flies had flown from the tubes, the remaining flies and unemerged pupae were counted. Four replicates per treatment were distributed in a randomized design.

Data analyses

For each volume of eggs seeded on each artificial larval diet, the biological parameters "number of larvae," "larval weight," "larval period," "number of pupae," "pupal weight," "pupal diameter," and "pupal period" were analyzed applying the *F* test for variance analysis ($p < 0.05$), and when significative difference was detected, the Tukey test ($\alpha = 0.05$) was used to compare the means. These analyses were performed by the statistical program SAS 9.1 (SAS Institute 2003).

The standard model to analyze proportion data is the binomial regression model, a particular case of a generalized linear model (McCullagh & Nelder, 1989). For this very simple model, we assume that each individual has the same chance of responding to the treatment and they act independently. However, the proportion data (egg-pupal recovery, adult emergence, sex ratio, and flight ability) are overdispersed in the sense that display more variation than the variability specified by the binomial model (Hinde & Demétrio, 1998). In order to account for overdispersion, it was used a quasi-binomial model with the logit link function (Demétrio et al., 2014). The half-normal probability plot with a simulated envelope was used to verify the adequacy of the fitted models, using the "hnp" package (Moral et al., 2017). The F test in this case equivalent to a quasi-likelihood ratio test was applied to test nested models. All analyses were performed in R (R Core Team, 2019).

Furthermore, we calculated the cost to make 1 kg of an artificial diet based on the price of the ingredients purchased in Piracicaba, SP, Brazil, and the amount used for each diet. From the amount of pupae produced per 1 kg of each diet, we determined the amount of diet needed to produce 1 million pupae. Finally, we calculated the production cost of 1 million pupae using each artificial larval diet and a parallel analysis was also performed to demonstrate the percentage share of each ingredient in the total cost of each diet.

Results

Corn flour (CF) diet

Mean (± SE) values from quality control parameters evaluated for *C. capitata* reared on artificial larval diet based on CF with different larval densities are summarized in Table 2 and compared in Fig. 1. The evaluation of the five medfly egg densities on the CF diet found that 2 mL of eggs/kg of diet was superior to the others in the number of pupae, and the concentrations of 0.8 and 1 mL of eggs/kg of diet were also superior to 0.3 mL of eggs/kg of diet. However, pupae from the density of 2 mL of eggs/kg of diet produced lower weight (Table 2). With the CF diet, only the concentrations at 0.3 and 0.5 mL of eggs/kg of diet resulted in pupae weighing at least 7.5 mg and emergence above 93%. The seeding of 0.5 mL of eggs/kg of diet was selected to produce more pupae than the density of 0.3 mL of eggs/kg of diet.

The F test for a linear effect of the volume of eggs showed a value of 15.11 for emergence percentage (p value = 0.0012), which is large when compared to $F_{0.95;1.18} = 4.41$, rejecting the null hypothesis of no volume of eggs effect, allowing for additional variability. Using the half-normal plot, the residuals, based on a quasi-binomial model with volume of eggs linear effect, lie completely within the simulated envelope, indicating the adequacy of the quasi-binomial model (Fig. 1d).

Sugarcane bagasse (SB) diet

On SB diet, mean (± SE) values from quality control parameters evaluated are summarized in Table 2 and compared in Fig. 2. The most pupae were obtained using the density of 2 mL of eggs/kg of diet, and the concentrations of 0.8 and 1.0 mL of eggs/kg of diet also surpassed 0.3 mL of eggs/kg of diet (Table 2). Pupal weight was not significantly different between the five larval densities tested on SB. Because all the densities tested on the SB diet resulted in pupae and emergence over 7.5 mg and 93%, respectively, 2 mL of eggs/kg of diet was selected to obtain higher pupae production.

The F test for a linear effect of the volume of eggs exhibited a value of 1.18 for emergence percentage (p value = 0.2912), in comparison with $F_{0.95;1.18} = 4.41$. Using the half-normal plot, the residuals, based on a quasi-binomial model without volume of eggs effect, lie completely within the simulated envelope, indicating the adequacy of the quasi-binomial model. A plot with the fitted curve and observed percentages versus volume of eggs is presented in Fig. 2d.

Lyophilized carrot powder (CP) diet

On CP diet (Table 2 and Fig. 3), the densities of 1 and 2 mL of eggs/kg of diet resulted in higher pupal

Table 2 Mean (±SE) quality control parameters for *Ceratitis capitata* reared on the three larval diets tested

Quality Control Parameters		Nº of larvae			Larval weight (mg)			Pupal period (days)		
Diets		CF[2]	SB[3]	CP[4]	CF	SB	CP	CF	SB	CP
Density (mL of eggs/kg of diet)	0.3	4389 ± 398 d	1787 ± 198 c	3044 ± 730 c	9.2 ± 0.5 a	11.7 ± 0.3 a	10.2 ± 0.4 a	6.0 ± 0.0 a	7.3 ± 0.3 a	7.5 ± 0.3 a
	0.5	6408 ± 523 c	3573 ± 566 bc	6105 ± 872 bc	8.5 ± 0.2 a	11.1 ± 0.2 ab	9.7 ± 0.2 a	6.0 ± 0.0 a	7.0 ± 0.4 a	7.5 ± 0.3 a
	0.8	9468 ± 559 b	5212 ± 1065 b	8866 ± 1143 bc	7.6 ± 0.6 a	11.1 ± 0.5 ab	9.3 ± 0.5 a	6.0 ± 0.0 a	7.0 ± 0.0 a	7.5 ± 0.3 a
	1.0	11,632 ± 784 b	6307 ± 1659 ab	13,098 ± 1564 b	7.4 ± 0.3 a	10.9 ± 0.4 ab	8.6 ± 0.7 ab	6.0 ± 0.0 a	7.3 ± 0.3 a	6.8 ± 0.3 a
	2.0	20,657 ± 1629 a[1]	13,550 ± 2140 a	20,610 ± 1065 a	5.4 ± 0.3 b	9.6 ± 0.4 b	6.5 ± 0.8 b	5.5 ± 0.3 a	6.5 ± 0.3 a	7.0 ± 0.4 a
ANOVA		$F_{4.19} = 58.58$; C.V. = 1.7%; $P < 10^{-4}$	$F_{4.14} = 17.15$; C.V. = 3.5%; P = 0.0002	$F_{4.16} = 33.93$; C.V. = 19.9%; $P < 10^{-4}$	$F_{4.19} = 13.20$; C.V. = 10.5%; $P < 10^{-4}$	$F_{4.14} = 4.95$; C.V. = 6.5%; P = 0.0095	$F_{4.19} = 6.16$; C.V. = 12.9%; P = 0.0039	$F_{4.19} = 3.00$; C.V. = 4.4%; P = 0.0528	$F_{4.19} = 1.25$; C.V. = 7.8%; P = 0.3324	$F_{4.19} = 1.30$; C.V. = 8.5%; P = 0.3129

Quality Control Parameters		Nº of pupae			Pupal weight (mg)			Pupal diameter (mm)			Pupal period (days)		
Diets		CF	SB	CP	CF	SB	CP	CF	SB	CP	CF	SB	CP
Density (mL of eggs/kg of diet)	0.3	3884 ± 467 c	1748 ± 190 c	2837 ± 796 c	8.1 ± 0.2 a	9.3 ± 0.1 a	8.7 ± 0.2 a	1.7 ± 0.03 a	1.8 ± 0.03 a	1.7 ± 0.03 ab	9.7 ± 0.3 a	9.8 ± 0.5 a	9.5 ± 0.7 a
	0.5	6089 ± 505 bc	3068 ± 470 bc	5726 ± 1034 c	7.5 ± 0.4 a	9.3 ± 0.1 a	8.3 ± 0.1 a	1.6 ± 0.01 ab	1.8 ± 0.05 a	1.8 ± 0.03 a	10.0 ± 0.0 a	10.0 ± 0.4 a	9.3 ± 0.9 a
	0.8	7211 ± 1156 b	5023 ± 1027b	7735 ± 1827 bc	6.3 ± 0.6 ab	9.3 ± 0.3 a	8.3 ± 0.3 a	1.5 ± 0.04 bc	1.8 ± 0.02 a	1.8 ± 0.02 a	10.0 ± 0.0 a	9.8 ± 0.5 a	9.0 ± 0.7 a
	1.0	8599 ± 1237 b	6176 ± 1542b	12,508 ± 1285 b	6.3 ± 0.7 ab	8.9 ± 0.1 a	8.0 ± 0.2 a	1.6 ± 0.03 ab	1.8 ± 0.02 a	1.7 ± 0.03 ab	10.3 ± 0.3 a	10.0 ± 0.6 a	8.5 ± 0.3 a
	2.0	15,551 ± 2103 a	13,105 ± 1874 a	19,747 ± 1069 a	4.9 ± 0.4 b	8.3 ± 0.5 a	6.4 ± 0.4 b	1.4 ± 0.03 c	1.7 ± 0.05 a	1.6 ± 0.04 b	10.7 ± 0.3 a	9.3 ± 0.3 a	8.8 ± 0.5 a
ANOVA		$F_{4.19} = 15.91$; C.V. = 2.8%; $P < 10^{-4}$	$F_{4.14} = 19.27$; C.V. = 3.4%; $P < 10^{-4}$	$F_{4.16} = 29.43$; C.V. = 22.0%; $P < 10^{-4}$	$F_{4.19} = 6.63$; C.V. = 14.8%; P = 0.0028	$F_{4.19} = 2.44$; C.V. = 6.2%; P = 0.0922	$F_{4.19} = 11.48$; C.V. = 6.7%; P = 0.0002	$F_{4.19} = 13.10$; C.V. = 3.7%; $P < 10^{-4}$	$F_{4.19} = 0.96$; C.V. = 4.1%; P = 0.4565	$F_{4.19} = 3.90$; C.V. = 4.1%; P = 0.0229	$F_{4.14} = 2.17$; C.V. = 4.4%; P = 0.1466	$F_{4.19} = 0.46$; C.V. = 9.3%; P = 0.7645	$F_{4.18} = 0.47$; C.V. = 13.1%; P = 0.7600

[1] Means on the same column followed by the same letter do not differ significantly by the Tukey test ($P < 0.05$)

[2] CF diet: diet based on corn flour; [3]SB diet: based on sugarcane bagasse; [4]CP diet: based on lyophilized carrot powder

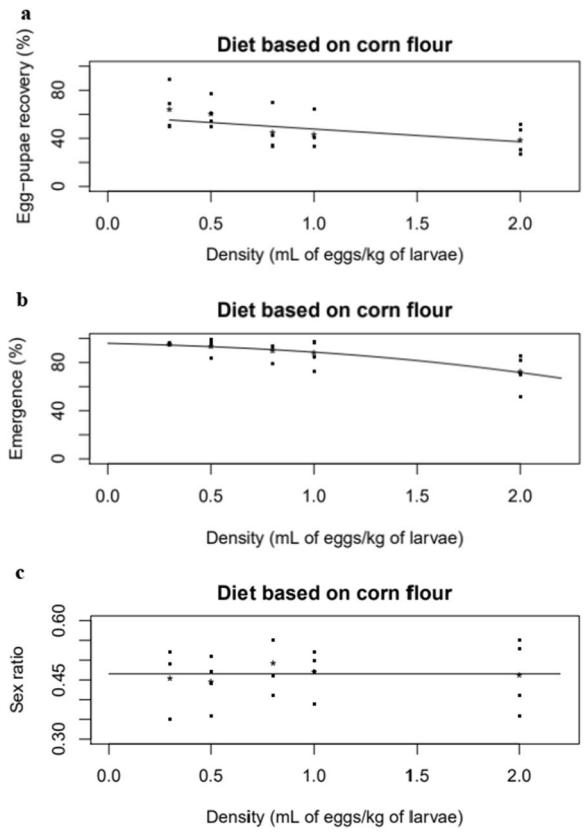

Fig. 1 Larval diet based on corn flour. **a** Egg-pupae recovery: plot with the fitted curve and observed percentages versus density. **b** Emergence: plot with the fitted curve and observed percentages versus density. **c** Sex ratio: plot with the fitted curve and observed proportions versus density

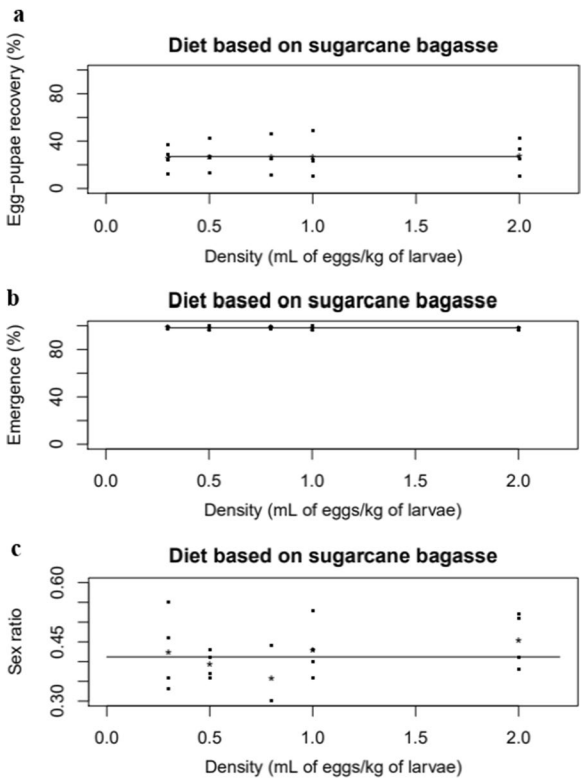

Fig. 2 Larval diet based on sugarcane bagasse. **a** Egg-pupae recovery: plot with the fitted curve and observed percentages versus density. **b** Emergence: plot with the fitted curve and observed percentages versus density. **c** Sex ratio: plot with the fitted curve and observed proportions versus density

productions than the concentrations 0.3 and 0.5 mL of eggs/kg of diet. However, the density of 2 mL of eggs/kg of diet generated lower weight pupae (Table 2). The F test for a linear effect of the volume of eggs showed a value of 16.79 for emergence percentage (p value = 0.0007), which is larger when compared to $F_{0.95;1.18} = 4.41$. Using the half-normal plot, the residuals indicate the adequacy of the quasi-binomial model (Fig. 3d).

In the case of the CP diet, only the density of 2 mL of eggs/kg of diet produced pupae under 7.5 mg and did not reach 93% of emergence. Thus, the concentration that obtained the higher pupae production was chosen (*i.e.*, 1 mL of eggs/kg of diet).

Quality control parameters comparison

After evaluating the artificial larval diets, the optimum volume of eggs seeded for each diet exhibited the following descending order for larval and pupae production: SB > CF > CP (Table 3). The weight of larvae reared on SB diet was greater than those of larvae reared on the other two diets, which did not differ from each other. Just as occurred for larval weight, the SB diet produced heavier pupae than the other tested diets (Table 3).

The F test for diets showed a value of 255.1 (p value < 0.01) for egg-pupal recovery percentage, which is large when compared to $F_{0.95;2.7} = 4.7$. A plot of the fitted percentages with approximate 95% quasi-binomial confidence intervals is shown in Fig. 4c, showing that the diet 1 differs from diets 2 and 3. Analyzing the emergence percentage, the F test for diets showed a smaller observed value of 0.61 (p value = 0.5681), in comparison with $F_{0.95;2.7} = 4.74$, and so we do not reject the null hypothesis of no diet effect, allowing for additional variability. A plot of the fitted percentages (here identical to the overall observed percentages for each diet) with approximate 95% quasi-binomial confidence intervals is shown in Fig. 4d, showing that the diets did not differ significantly.

The F test for diets showed a value of 1.36 for sex ratio (p value = 0.3157), which is smaller in comparison with $F_{0.95;2.7} = 4.74$ (Fig. 4e). Finally, analyzing the flight ability, the F test for diets showed a value of 4.42 (p value = 0.06) against $F_{0.95;2.7} = 4.74$, and a plot of the fitted percentages

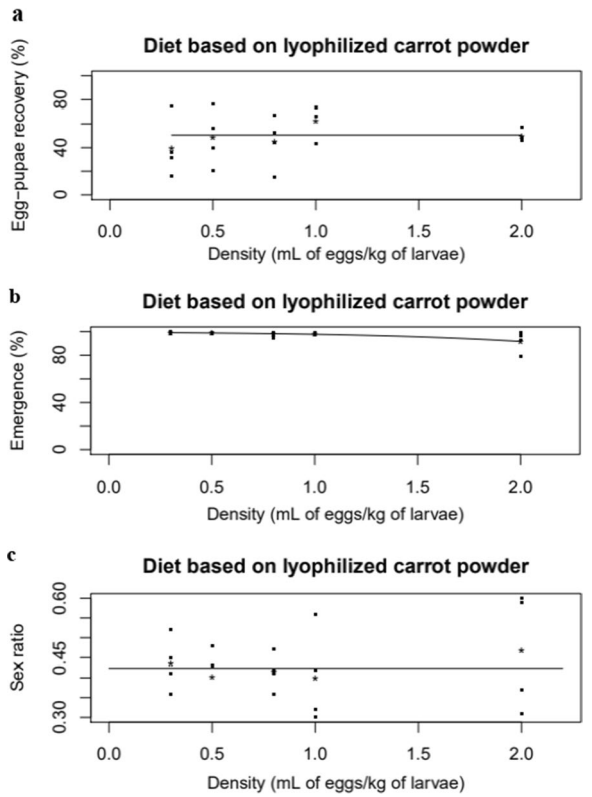

Fig. 3 Larval diet based on lyophilized carrot powder. **a** Egg-pupae recovery: plot with the fitted curve and observed percentages versus density. **b** Emergence: plot with the fitted curve and observed percentages versus density. **c** Sex ratio: plot with the fitted curve and observed proportions versus density

(identical to the overall observed percentages for each diet) with approximate 95% quasi-binomial confidence intervals is shown in Fig. 4f, also showing that the diets did not differ significantly.

It is known that the greater the larval density, the lower the quality of the larvae and pupae produced due to the greater competitiveness. Thus, among all the densities that resulted in pupae with the quality standards recommended by FAO/IAEA/USDA (2014), the one that achieved the highest productivity in each diet was selected.

Discussion

The most efficient artificial larval diet

Regardless of the insect production scale, the maintenance of healthy insects and the establishment of a quality control system in the laboratory are fundamental for the development of basic and applied research in entomology. The production of natural enemies demands systematic rearing practices to ensure satisfactory biological and reproductive performance and high efficiency in field release, and the quality control parameters of the biological agents produced are fundamental factors to guarantee the success of an integrated pest management program (Van Lenteren, 2000). Mass-rearing production in the laboratory without the proper quality control of the insects will affect the viability of the program, directly increasing the cost of management and undermining the credibility of biological control (Bueno, 2009; Van Lenteren, 2000).

In this context, the diet is probably the most important component of insect rearing and constitutes the main cost of a biological program, being essential to the constant process of improving the performance and reducing the costs of the diet to ensure sufficient and adequate quality of fruit flies to achieve success in SIT program (Parker, 2005). Based on the obtained results, the SB artificial diet promoted better results in the number of larvae, larval weight, number of pupae, and pupal weight, in comparison with CF and CP diets.

Table 3 Mean (± SE) quality control parameters for *Ceratitis capitata* reared on the three larval diets tested

Quality control parameters	N° of larvae	Larval weight (mg)	Larval period (days)	Nº of pupae	Pupal weight (mg)	Pupal diameter (mm)	Pupal period (days)	
Larval Diets	CF[2] SB[3] CP[4]	5333 ± 119 b 6533 ± 257 a 4165 ± 241 c[1]	9.0 ± 0.02 b 10.9 ± 0.09 a 8.8 ± 0.02 b	6.0 ± 0.0 7.0 ± 0.0 7.0 ± 0.0	5018 ± 105 b6514 ± 263 a 4135 ± 245 c	7.3 ± 0.1 b 8.8 ± 0.1 a 7.5 ± 0.1 b	1.7 ± 0.02 b 1.8 ± 0.02 a 1.7 ± 0.01 b	9.0 ± 0.0 9.0 ± 0.0 9.0 ± 0.0
ANOVA	$F_{2,9} = 32.12$; C.V. = 6.8%; P = 0.0003	$F_{2,9} = 63.60$; C.V. = 2.7%; $P < 10^{-4}$	Homoscedasticity absence	$F_{2,9} = 33.67$; C.V. = 6.9%; P = 0.0003	$F_{2,9} = 53.16$; C.V. = 2.7%; $P < 10^{-4}$	$F_{2,9} = 18.71$; C.V. = 1.8%; $P = 0.0016$	Homoscedasticity absence	

[1] Means on the same column followed by the same letter do not differ significantly by the Tukey test ($P < 0.05$)

[2] CF diet: diet based on corn flour

[3] SB diet: based on sugarcane bagasse

[4] CP diet: based on lyophilized carrot powder

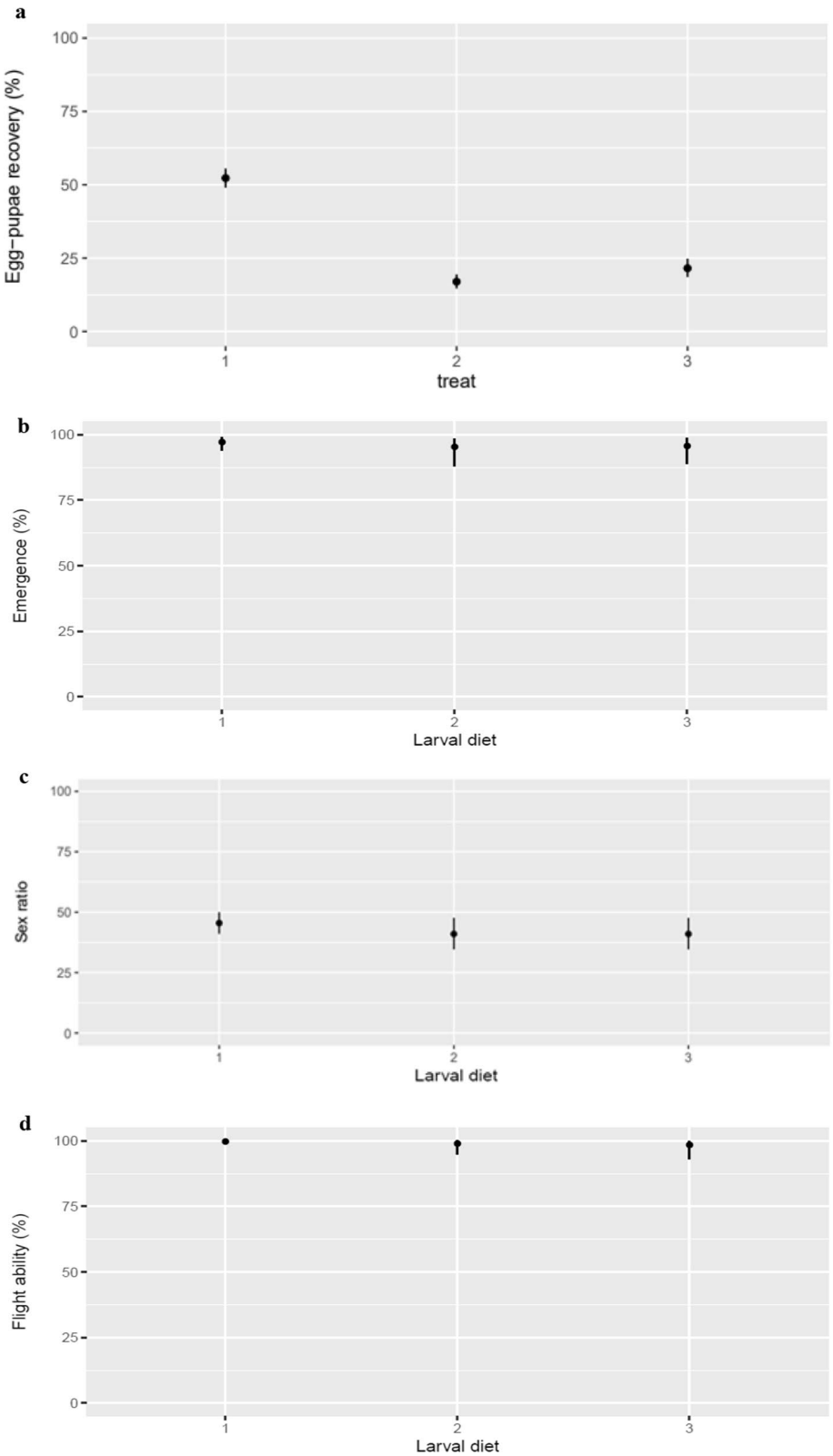

Fig. 4 Plot of fitted proportions with quasi-binomial confidence intervals. Legends for **a**, **b**, **c** and **d** 1: larval diet based on corn flour; 2: larval diet based on sugarcane bagasse; 3: larval diet based on lyophilized carrot powder. **a** Egg-pupae recovery (%). **b** Emergence (%). **c** Sex ratio. **d** Flight ability (%)

Larval diets for *C. capitata* have been developed with both nutritional quality and low production cost (Joachim-Bravo et al., 2006; Nestel and Nemny–Lavy 2008; Silva Neto et al., 2012). Carbohydrate and protein levels in the larval diet can drastically affect the survival, development, and body size of adults (Nash & Chapman, 2014). Nestel et al. (2004) tested three larval diets with different amounts of protein. The diet with the highest amount of protein resulted in the lowest percentage of egg-pupal recovery. As yeast is the main protein source of larval diets, the results of this study indicate the influence of the protein in some of the parameters evaluated. Another ingredient that could be responsible for the difference observed in the larval weight could be the wheat germ. Amira Negm (2020) compared four diets, three containing different amounts of wheat germ and one containing brewer's yeast. The results showed that pupae weight and pupation rate decreased according to the amount of wheat germ. The diet containing brewer's yeast did not differ for such parameters from the diet containing the highest amount of wheat germ. It shows the importance of protein for the cited quality parameters. Moreover, the cane bagasse might have contributed to a better larval mobility inside the diet, resulting in better access of the larvae to the ingredients and a higher final weight of third instar larvae.

Among five larval diets, Pašková (2007) revealed the only diet containing corn flour produced the highest quantity of pupae, as happened with the SB diet in this work. Corroborating the obtained results, Nestel et al. (2004) pointed out that the values of egg-pupal increased as the sugar concentration in the larval diet increased. Although differences found among pupae weight reared separately on the three larval diets evaluated, all of them produced pupae with sufficient quality to achieve a high adult emergence percentage as well as adults with good flight ability according to FAO/IAEA/USDA (2014) standards.

Similar to this work, Nash and Chapman (2014) did not verify differences in the weight of larvae from pupae reared separately on two diets, one containing 50 and the other 70 g of yeast. In another study, Vargas et al. (1983) showed that larval diets with higher amounts of sugar produced heavier pupae, corroborating the results of this research. The diets tested in this study (SB, CF, and CP) contained 11.99, 3.00, and 0.00% sugar, respectively, in their compositions.

Considering now adult emergence parameter, Joachim-Bravo and Zucoloto (1997) showed that the emergence did not vary significantly among insects reared in sugary or non-sugary larval diets. Nash and Chapman (2014) tested larval diets with different amounts of protein and found no difference in emergence. Plácido-Silva et al. (2005) also found no difference in this parameter when larvae were reared on diets with different amounts of protein. Chang et al. (2001) found no difference in emergence and flight ability after testing three diets, and the differences among them in the proportions of corn composts and sugar were similar to those in the diets tested in this work.

The quality parameters observed in fruit flies rearing, in general, are directed to the promotion of high fertility and fertility, short life cycle, good dispersion, and flight ability (Calkins & Parker, 2005; Vreysen and Robinson 2010), but they are not always necessarily a guarantee of successful performance in the field (Rull et al., 2012). Some quality control parameters, such as the number and weight of larvae and pupae, non-flying adults, among others, can provide useful information on the performance of fruit flies in SIT programs (Rull et al., 2012).

The average productivity for the CF, SB, and CP artificial diets was 5,018, 6,514, and 4,135 pupae/kg of diet, respectively. Therefore, it was estimated that to produce 1 million pupae, 199.28 kg, 153.52 kg, and 241.84 kg would be necessary of the CF, SB, and CP larval diets, respectively. As the results of pupae production differed among the three larval diets tested, the SB diet can be considered the most productive one. This larval diet also produced significantly heavier pupae in comparison with other diets.

The economic viability of the diets

Besides the quality control of biological parameters, the development of mass-rearing methods depends on the reduction in the production cost, enabling the pest management programs economically viable. Considering the artificial diets tested, the corn flour represents more than 60% of the total cost of the CF diet, and the lyophilized carrot powder corresponds to more than 75% of the CP diet cost. In the SB diet, the most representative ingredients in terms of cost are beer yeast, crystal sugar, and wheat germ, which together account for more than 70% of the total (Table 1).

According to the results presented in Table 4, SB is the most economically viable diet, requiring US$ 121.47 to produce 1 million pupae and it produces 8,233 pupae per dollar invested, which is very close to values for the CF diet (US$ 124.29 for 1 million pupae and 8,047 pupae per dollar). The knowledge of insect nutrition is important to develop an appropriate diet to increase the medfly population level and to reduce production costs.

Conclusions

The optimal egg seeding densities for *Ceratitis capitata* rearing under laboratory conditions on artificial larval diets based on corn flour, sugarcane bagasse, and

Table 4 Productivity data and costs of the three tested larval diets for *Ceratitis capitata*

Larval diets	US$/kg of diet	Pupae/kg of diet	Pupae/US$1.00 of diet	kg of diet/1 million pupae	US$/1 million pupae
CF[1]	0.63	5018	7965	199.3	125.56
SB[2]	0.79	6514	8246	153.5	121.27
CP[3]	1.16	4135	3565	241.8	280.49

[1] CF: diet based on corn flour

[2] SB: based on sugarcane bagasse

[3] CP: based on lyophilized carrot powder

lyophilized carrot powder are 0.5, 2.0, and 1.0 mL of eggs/kg of diet, respectively; and the most efficient larval diet, in terms of productivity, quality, and costs, was the diet based on sugarcane bagasse, which could be recommended for medfly mass-rearing facilities in Brazil. Despite the findings of this study, more investigations are necessary to evaluate the medfly biological parameters across generations when reared with the larval diet presented.

Abbreviations

SIT: Sterile insect technique; CENA/USP: Centro de Energia Nuclear na Agricultura/Universidade de São Paulo; CF: Larval diet based on corn flour; SB: Larval diet based on sugarcane bagasse; CP: Larval diet based on lyophilized carrot powder.

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Author contributions

BAM, MLZC, LAL, and TAM planned the methodology. BAM, LAL, and MLZC conducted the laboratory work. BAM, CGBD, and TAM made analysis and interpretation of data and drafted the work. VWB wrote, reviewed, and drafted the work. All authors have read and approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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