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Original Research

Evaluation of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) Parameters for Establishing A Mass Rearing on Ceratitis Capitata (Wied.) (Diptera: Tephritidae)

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the major fruit crop pests in Morocco and the world. The braconid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) is a larval-pupal endoparasitoid that can be used as a native biological control agent against this pest. In this study, several rearing parameters were controlled to optimize a profitable rearing strategy. density and quality of host larvae, 2) exposure time to parasitoids, and 3) sex ratio of parasitoids. To investigate the effect of each parameter, we concluded that the third instar of C. capitata in a proportion of 2:1 larva: female, exposed for 24h to parasitoids with a proportion of 1:1 and 2:1 female: male. These parameters demonstrate good performance, influenced by parasitism at approximately 80%, high flight ability at 88%, low pupal mortality, and high female progeny production. A diet of pure honey and water for parasitoid adults gives a high longevity of almost 70 days. Other parameters did not differ much throughout the trials. The results in our study can be used to provide a suitable protocol and environment for mass rearing of *D. longicaudata* as a biological control against *C. capitata.*

Keywords: Biological control, *Ceratitis capitata, Diachasmimorpha longicaudata,* mass-rearing.

INTRODUCTION

Phytophagous insects are a major agricultural problem. The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most undesirable pests in Morocco and world; the use of chemicals insecticides and fumigants contribute to fight against it, but because of the wide range of hosts on which grows (>350 plant species); makes their elimination difficult. For this reason, biological control seems to be an effective solution. The success of a classical biological control program relies in large part on efficient rearing of large numbers of healthy beneficial insects for shipment and release. *Diachasmimorpha longicaudata* (Ashmead) is the most important parasitoid used to fight this enemy (Cancino & Montoya, 2008); (Cruz et al., 2018); (Viscarret et al., 2006); (Montoya et al., 2000). *D. longicaudata* is a larval-prepupal endoparasitoid of several tephritid genera that include C. capitata. For this reason, it's used for augmentative mass-rearing of parasitoid. While the general rearing procedure for this parasitoid is well-understood, several technical challenges remain to optimize production. Biological parameters of *D. longicaudata* have been studied on *Anastrepha* (Meirelles et al., 22013) ;(ancino et al., 2002a) ;(Montoya, Liedo, Benrey, Cancino, et al., 2000), and *Ceratitis capitata* (Wiedemann) (Cruz et al., 2018; Meirelles et al., 2013; Montoya, Liedo, Benrey, Barrera, et al., 2000; Viscarret et al., 2006).

The success of *D. longicaudata* as a control agent depends on its parasitism, the quality and quantity of parasitoid females produced. Thus, the main objective of the mass production of this parasitoid is high female production, since the higher the number of females produced, the more efficient and cheaper will be their production, and the more accessible will be their use in biological control programs (Oliveira et al., 2014).

The main objective of this study is to help for better understanding of the relation between parasitism, host density, exposure time, parasitoid sex-ratio…, and parasitoid emergence may also help improve mass rearing of *D. longicaudata*. Our study was designed to evaluate different rearing parameters of *D. longicaudata*: parasitoid sex-ratio, optimal number of hosts offered to parasitoid, effect of exposition duration of host larvae to parasitoids, optimal instar used in the rearing program and flight ability as a quality control of the parasitoid.

METHODS

1. Insect rearing

The insects used in this study were maintained in the insect rearing facility of the Omnium Agricole du Souss Auxiliary Production Site, Chtouka, Morocco. The *Ceratitis capitata* colony was established in 2015 from wild fruit flies collected from infested argan fruits from the Hassan II Institute of Agronomy and Veterinary Sciences, Agadir, Morocco (IAV's orchards) and since been reared continuously at the facility's laboratory where they were maintained under controlled conditions (temperature of 25±1ºC, relative humidity of 65±10%, and photoperiod of 12 hours (Elaini et al., 2019). To provide the larvae used in the rearing processes, *C. capitata* eggs were collected and distributed to plastic containers (24×11×5cm), in which the larvae were fed on an artificial diet (Elaini et al., 2020). Seven days later the larvae reached the third instar and became ready to be used.

Diachasmimorpha longicaudata colony was begun from flies imported from Spain and has since been reared continuously *on Ceratitis capitata* larvae and a diet based on water and pure honey *ad libitum* in a piece of cotton placed on a Petri dish. *D. longicaudata* wasps were held in cages constructed from pieces of transparent plastic (30×30×30cm), with mixture on three sides providing ventilation, and a sleeve in one side to facilities insects handling; ended with a hole that allowed insects to be added or removed from the cage, and to provide nutrition. The colony was reared in the same facility and under the same conditions as *C. capitata* colony.

2. Experimental procedure

Longevity

Longevity was determined under laboratory conditions (rearing conditions), samples were exposed to four different groups of nutriment: with honey-without water, with water-without honey, with water-with honey, and without honey-without water. Three trails were made for each type of condition: isolated male, isolated female and male-female together. 10 parasitoids newly emerged were introduced into a box (24×11×5cm) covered with a mesh with one type of nutriment each time. The number of parasitoid and the sex-ratio was recorded daily until all parasitoids had died (Appiah et al., 2013). Ten replicates were made for each group of nutriment. The same test was elaborated to the males and the females. For the third trial (males and females together) the number inside the box was 10 parasitoids for each sex.

Optimal host instars.

In case of our host, *C. capitata*, three instars were used. Seven days old, 20 males and 20 females of *D. longicaudata* per replicate were placed in a plastic cage (30×30×30cm) with honey and water. In a parasitism unit consisted of a Petri dish bottom (9cm×0,7cm); 20 first instar larvae (2-4 days) with larval diet were placed and covered with a mesh. This unit was exposed to parasitoids for five hours. After parasitism, the contents of the Petri dish (larvae and their artificial diet) were transferred to a plastic box (24×11×5cm), and added with dry bran to minimize humidity, and maintained at 26°C for 14 days to pupae and adult emergence (López et al., 2009),(Oliveira et al., 2014). After emergence, the number of parasitoid females and males, number of fruit flies, and number of dead pupae were quantified. The sex ratio was calculated using the formula: number of females/ (number of males + number of females). The parasitism rate was calculated based on the formula: number of parasitoids/ (number of parasitoids emerged + number of fruit flies emerged). The same test was done for the second instar (5-6 days old), and the third instar (7-8 days old). Ten replicates were carried out for each instar test with a control for each test in which 20 non parasitized larvae were controlled into the emergence.

Optimal exposition duration of host larvae to parasitoids.

Eight different host exposure times were evaluated in this test: 2h, 4h, 6h, 8h, 10h, 20h, 24h, 48h and a control consisted of *C. capitata* larvae not exposed to parasitoid. The assay was set up with seven days old host larvae of *C. capitata* and seven-day-old *D. longicaudata* adults. The protocol of exposition was the same as demonstrated in the bioassay 2, except the number which was 10 male and 10 females of parasitoid and 10 larvae per each replicate in a density of 1:1 *Ceratitis capitata* larvae/ *Diachasmimopha longicaudata* female. The test was repeated ten times with a control for each test in which 10 non parasitized larvae were controlled into the emergence. Number of parasitoid females and males, fruit flies emerged were recorded, sex ratio and parasitism rate were calculated, and number of dead pupae was quantified (Cancino et al., 2002a).

Optimal density of host larvae offered to parasitoids.

Seven days old, 10 males and 10 females of *D. longicaudata* per replicate were placed in a plastic cage (30×30×30cm), Six densities of *C. capitata* larvae (seven days old) were offered to parasitoid in different proportions in the following order: ½:1, 1:1, 2:1, 3:1, 4:1, 5:1(host larvae: parasitoid female). These larvae were exposed to the parasitism unit for five hours. Ten replicates were carried out for each density test with a control test in which 10 non parasitized larvae were controlled into the emergence (Cruz et al., 2018). After emergence, the number of parasitoid females and males, number of fruit flies, and number of dead pupae were quantified. The sex ratio and the parasitism rate were calculated.

Optimal sex-ratio of parasitoid.

Seven days old, males and females of *D. longicaudata* were placed in a plastic cage (30×30×30cm) in four proportions: 1:2, 1:1, 2:1, and 3:1 female: male. Different densities of *C. capitata* larvae (seven days old) were offered to parasitoids in the parasitism unit to respect the proportion of 1:1 larva: female. Ten replicates were carried out with a control test in which 10 non parasitized larvae were controlled into the emergence. After emergence, the number of parasitoid females and males, number of fruit flies, and number of dead pupae were quantified. The sex ratio and the parasitism rate were calculated (Oliveira et al., 2014).

Flight ability.

Flight ability identified as the percentage of parasitoids that could fly. 100 parasitized pupae were placed inside of a 7,5cm diameter x 7cm height black cylinder. The inside walls of the cylinder were coated with neutral powder so the parasitoids could not leave the cylinder by walking (Cancino et al., 2002b). This cylinder was placed in a plastic cage (30x30x30cm), with a source of light and to a 12:12 L:D period. Inside the cage at the top a trap plate was installed to catch all parasitoid flights from cylinder and to make sure that the fliers wouldn't go back to the cylinder. 3 replicates were carried out with ten cages each. The number of non emerged pupae, dead parasitoids inside the cylinder (no fliers), fruit flies and empty pupae was recorded. The percent fliers were estimated as follows: number of adult parasitoids outside the cylinder / (number of empty pupae– number of fruit flies' adults) x 100.

3. Statistical Analysis:

All statistical analyses were performed using Minitab 16 software. The comparisons of the frequencies of the calculated biological parameters were carried out with ANOVA test. The existence of significant differences between the means was confirmed with the Turkey test where 95% certainty and p <0.05 considered statistically significant.

RESULTS AND DISCUSSION

Longevity:

The mean longevity of males and females of both test (isolated or combined), under four different food types shows no statistically significant difference among days (F=1,37; df=3; P=0,249). Survival of the parasitoids exposed to these conditions is shown in Fig. 2. For types of food there was a significant difference in parasitoid survival for both sexes (F=26,22; df=3; P=0,000). Peak survival value was observed in the test where parasitoids had access to honey+water or only honey, in the other hand survival was the same when parasitoids feed only water without any food and it was much lower than those feeding honey or honey+water (Fig. 1)

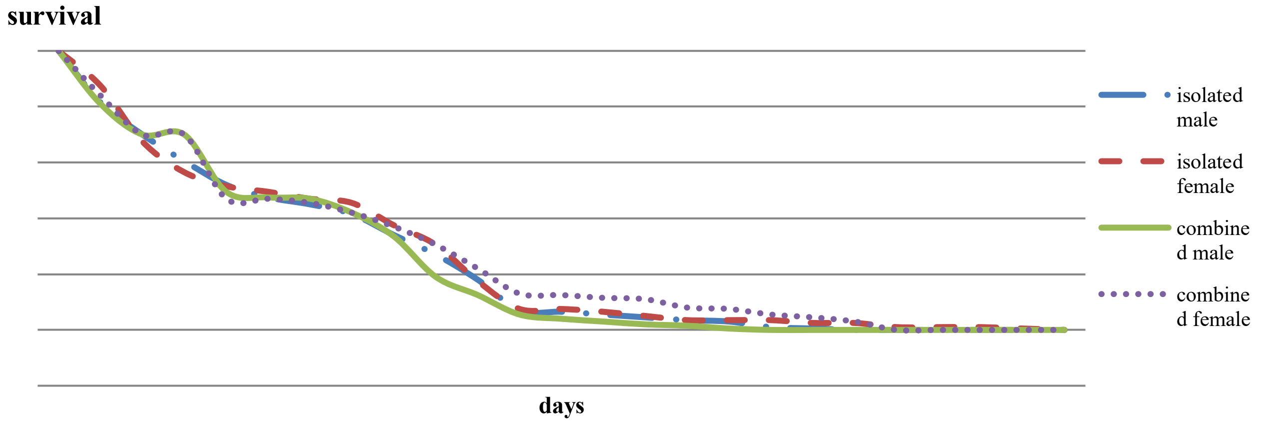

Figure 1. Survival of combined and isolated males and females of *D. longicaudata* under the same conditions.

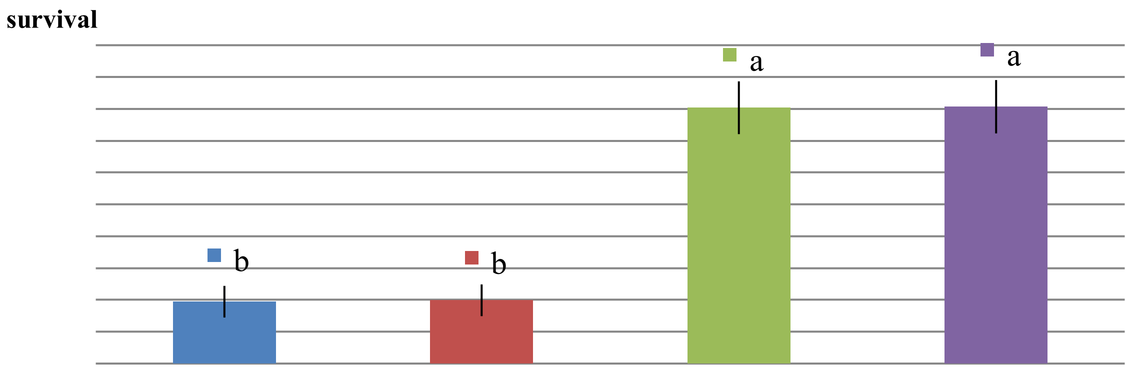

Figure 2. Survival of *D. longicaudata* feed with different diets. Bars marked with the same letters do not differ significantly from each other.

Optimal instar:

There was a significant interaction between the numbers of emerged parasitoids and the instars in relation to the parasitism rate (F = 48,6; df = 3; P = 0,000), and the mortality rate (F = 34,11; df = 3; P = 0,000). It's clear that second and third-instar larvae were preferred over the first instar, with a small increase in the third instar. However, it did not differ across instars in the sex ratio (F = 0,04; df = 2; P = 0.963). It is important to note that first-instar larvae also had parasitized but with mortality rate very high (38.5±5.67). There was not a significant difference between the numbers of emerged male and female in relation to the second and the third instar, with the highest proportion of females from third-instar larvae. The size of parasitoid emerging was affected by the instar of host larvae used; the second instar gives smaller parasitoids in comparison with the third.

Table 1: Parasitism rate, mortality rate, sex ratio and number of offspring of *D. longicaudata* reared in first, second, and third instar of *C. capitata* larvae. Values with the same letters do not differ from each other.

	treatment		
	First instar	Second instar	Third instar
parasitism %	19,13±6,90 b	81,93±7,96 a	83,06±6,30 a
mortality %	38,5±5,67 a	4,5±0,49 b	3±1,10 b
sex ratio	0,5045±0,12 a	0,5319±0,02 a	0,5278±0,01 a
Number of females	1,2±0,55 b	8,1±0,84 a	10±0,99 a
Number of males	1,1±0,45 b	7,6±0,99 a	9,4±1,12 a

Exposure time

Exposure time significantly affected *D. longicaudata* oviposition and emergence. The proportion of parasitism rate increased with exposure time from (17,08±2,74) at 2hr to (71,78±4,87) at 24hr (F=27,2, df = 7, p < 0,001). While higher exposure duration (>24h) negatively affected the parasitism rate and the total parasitoid production by result. These data show that the higher parasitism rate reached with 24h of exposure duration with more or less duration beside 24h gives significantly fewer parasitoids (Fig. 1).

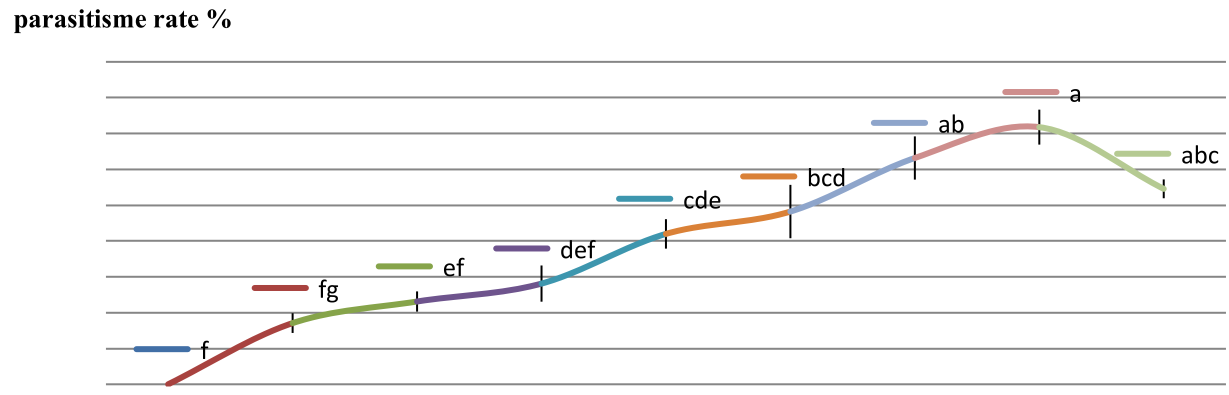

Figure 3. Parasitism rate of *D. longicaudata* reared in third-instar larvae *C. capitata* subjected to different exposure times.

Exposure time did not affect significantly the mortality rate in host larvae with all duration, it's averaged from (11±5,04) at 2 hr to (5±2,23) at 48 hr. (F = 0,7, df = 8, p = 0,694), with no significant differences between the sex ratio across all duration. There was a significant interaction between the numbers of emerged parasitoids and the exposure duration in relation to the parasitism rate that increased with the exposure duration to reach the highest value in the 24h of exposure (71.78±4.87) ;(P<0.005; F=26.87). The percentage of parasitism was significantly reduced based on the increase in exposure time over 24h.

Optimal density:

The number of larvae of *C.capitata* offered to the parasitoid *D. longicaudata* significantly affected the parasitism rate (F = 62,12; df = 6; P = 0.000), the maximum number of parasitized larvae was observed for the proportions ½:1, 1:1 and 2:1 larvae: female and decreased as the number of larvae offered increased, it affects also mortality rate (F = 17,41; df = 6; P = 0.000), the proportion ½:1 larvae: female chows the highest value (36±5,81) while no statistically significant differences were observed between other proportions, for sex ratio (F =51,37; df = 6; P = 0.000) lowest rates were observed when 5 larvae were offered per female (0,327±0,041), and offspring (F = 50,29; df = 6; P = 0.000) the number of offspring rose as the number of larvae offered for parasitism increased (fig 2).

When all parameters were compared, it was concluded that the proportion 2:1 larva: female seems to be the best proportion; despite of the higher value of offspring registered when female offered five larvae, however the rise of number affected negatively the parasitism rate and the sex ratio.

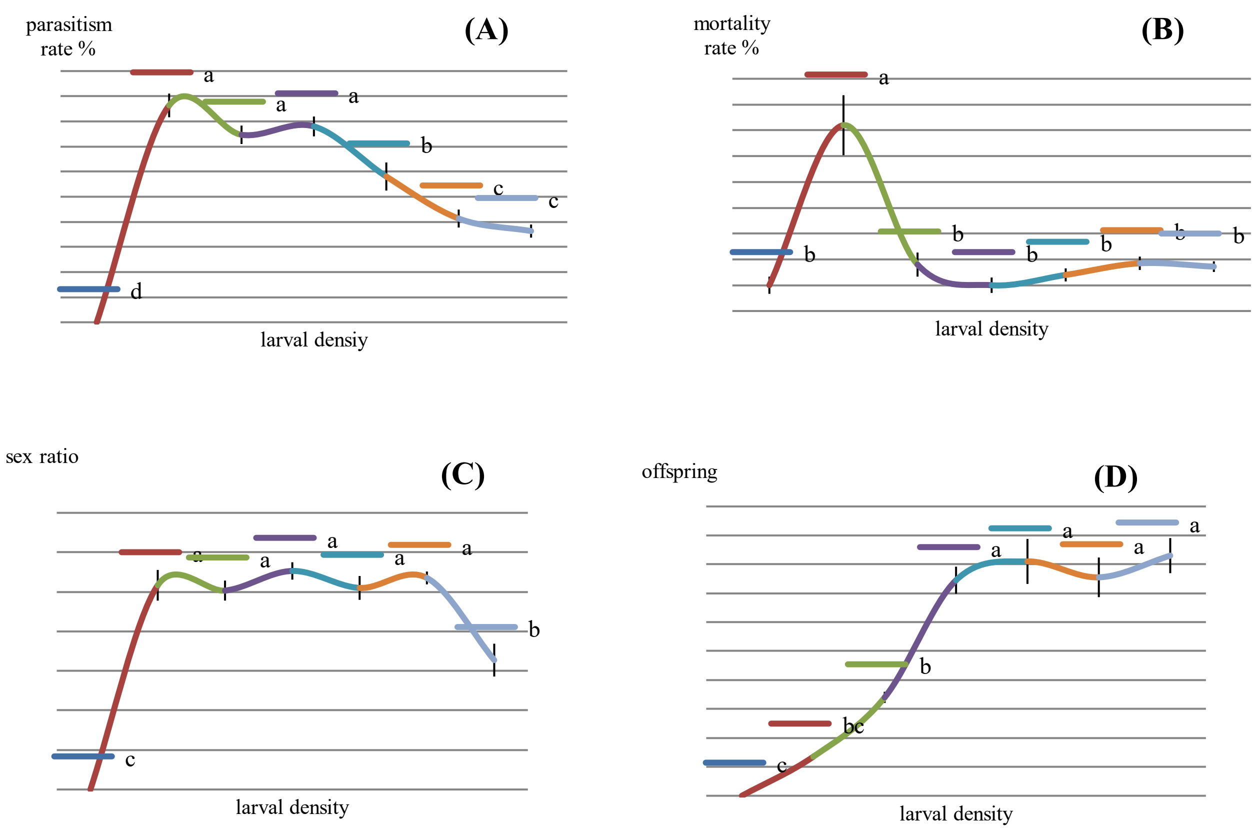

Figure 4. Parasitism rate (A), Mortality rate (B), sex ratio (C) and number of offspring (D) of D. longicaudata reared in third-instar larvae of C. capitata subjected to different larval densities.

Sex ratio:

There was not a significant interaction between the sex ratio of parasitoid in the cage and parasitism rate (F=1,7; df = 3; P = 0,183) when all proportions were used in the cage or mortality rate (F=0,6; df = 4; P = 0,664), that gives the same pupal mortality when 1/2, 1, 2 and 3 female/male were combined together in the cage.

Table 2: Parasitism rate, mortality rate, sex ratio and number of offspring of *D. longicaudata* reared in third instar of *C. capitata* larvae with different proportion of female/male in the cage.

	Treatment (proportions female: male)			
	1/2	**1/1**	**2/1**	**3/1**
Parasitism % (Mean± SE)	76,71±8,23 a	78,99±10,26 a	74,35±7,01 a	56,28±5,49 a
Mortality % (Mean± SE)	7±3,34 a	7,5±2,26 a	3,5±2,47 a	7,33±5,49 a
Sex ratio (Mean± SE)	0,37±0,04 b	0,51±0,01 a	0,55±0,01 a	0,45±0,03 ab
Number of offspring (Mean± SE)	7,1±0,75 b	14,5±1,89 a	14,3±1,38 a	15,7±1,46 a

The number of offspring (F = 27,25; df = 4; P =0,000) and sex ratio (F = 61,2; df = 4; P = 0.000) were statistically significant among sex ratio proportions used. For the number of offspring, the proportions 1:1, 2:1 and 3:1 showed no difference between each other, but they were significantly differed from the proportion ½:1 female/male which gives the lowest value (7,1±0,7519). For sex ratio the highest value was observed in the proportion 1:1 and 2:1 and reduced when three females were combined with one male, the lowest mortality rate was observed in comparison to the other treatments in the test where number of males were more than females (Table 2).

Flight ability:

There was not a significant difference among parasitism rates (F=0,57; df=2; P=0,574), mortality rate (F=2,25; df=2; P=0,125), and flight ability (F=0,31; df=2; P=0,737) between all tests and replicates been done. A high percentage of flighers was observed (88,70±1,67), parasitism rate also shows a high percentage.

DISCUSSION

In our study we did not observe any effect of mating on female or male longevity, where all trials present the same longevity. There was not any difference reported between male and female in longevity in contrary of what (Appiah et al., 2013), reported that the female lives a little longer than males. In contrast to gender, food had a significant effect on parasitoid survival for all groups.

The second and the third instar of *C.capitata* are the best to use as host for parasitism, with little preference for the third in reason of the quality of offspring; big and large parasitoids that may have higher levels of fertility and fecundity and even survival and resistance of natural conditions (López et al., 2009); (Meirelles et al., 2013). The first instar on the other hand presents a low parasitism rate, high mortality and low progeny number (Table 1). However, differences in host size or quality may or may not necessarily influence reproductive parameters.

Duration of host exposure to *D. longicaudata* affects parasitization rate and emergence of parasitoid progeny, (Vargas et al., 2002) reported that 6h of exposure gives good results, (Cancino & Montoya, 2008) reported that 2 to 3h gives the best results for *D.longicaudata* reared on *Anastrepha ludens*, and (La-Spina et al., 2018) confirmed that 4h exposure time gives highest parasitization and parasitoid production with *Psyttalia lounsburyi*. Our study showed that a 24-hours host exposure time gives the highest production of parasitoids and parasitism rate. The sex ratio of wasp offspring was stable and did not vary significantly over different exposure times (Fig.3).

Higher parasitoid production was observed in the progeny in ratios of 1:1 and 2:1 (larvae/females). Ratios above 4:1 reduced the sex ratio, and ratios below 1:1 caused high pupal mortality rates. The highest parasitism rate was obtained in the ratio of host larvae/females (1:1 and 2:1) (Fig.4). Our results were compatible as (Cruz et al., 2018) were reported. Results observed for the ratio ½:1 can be explained by super parasitism; when we reduced the number of host larvae, the females tended to increase oviposition activity due to the low number of host larvae by females, which resulted in higher parasitism and mortality rates.

Our study shows that the sex ratio of parasitoid started influence the progeny; when the number of females was more or equal to number of males the progeny was higher, in contrary more male produce less offspring. Ratios 1:1 and 2:1 produce the highest progeny sex ratio, above or under these ratios' offspring ratio reduced (table.2).

Flight ability is one of the most important parameters in mass-rearing and quality control of *D. longicaudata* (Cancino et al., 2002b), because it's an indicator of distribution capacity in the field. Various studies have found that the confinement of insects to small cages with food and no adverse conditions may noticeably reduce their flight capacity (Cancino et al., 2002b) ;(Cancino & Montoya, 2008). Our results show a high flight capacity of parasitoid although the processes of rearing in a small cage, our results were similar than what (Cancino et al., 2002b) reported for reared parasitoid and lower than wild parasitoids.

Developmental time of *D. longicaudata* at 25°C reported in our study is between 16 and 18 days for both sexes all over the tests made in this study. It's important to report that there is a male dominated sex ratio on the first and second day of emergence in all trials of the study, it may be a strategy increases the likelihood of males copulating newly emerged females.

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