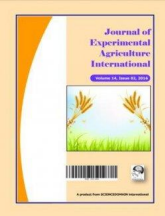

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Food Attractants Used in the Fruit Fly Monitoring (Diptera: Tephritidae) in a Commercial Orchard of *Psidium guajava*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fruit flies are considered an important fruit pest for world fruit production because they cause significant losses to the production and limit fruit free transport due to quarantine restrictions imposed by importing countries. Fruit flies population dynamics knowledge of a particular region is an important factor in the management of this pest. Traps are devices created to attract and capture flies by sexual attraction or food attraction, both put inside the trap. It reduces fruit flies population in

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the orchard, decreasing the pressure and chances of an infestation. This research aimed to evaluate the efficiency of food baits in the fruit flies capture on guava plant culture, in order to establish an adequate program for this pest in the municipality of Nova Floresta – PB. Monitoring of the adult fruit-fly was performed with aid of plastic traps of the type PET bottle 330 mL of Bio Anastrepha® 5.0%, 440 mL of sugarcane molasses diluted to 10.0% and 520 mL of 30.0% fruit juice (guava). The traps were installed in the central part of the trees, approximately 1.50 m above ground. Every 15 days adult fruit flies were caught, the occasion that food baits were replaced. From these data were evaluated: efficiency of food baits, MAD index (flies/trap/day) and food baits cost-benefit analysis. The best food attractant at the lowest cost was Bio Anastrepha® at 5.0%, with a catch margin of 63.83%, followed by fruit juice at 30.0% and sugarcane molasses at 10.0%, with 34.04% and 2.13%, respectively.

Keywords: Anastrepha spp.; Ceratitis capitata; food bait; integrated pest management.

1. INTRODUCTION

Fruit world production is around 609 million tons, currently, the largest world's producers are China and India which together produce 43.6% of the world total and they have their productions intended mainly to internal market [1]. Brazil ranks third in the world ranking and is responsible for 5.7% of the harvested volume, with a production of 41.5 million tons. The Brazilian fruit culture has been, over the years, preparing to compete more actively in the international market aiming to increase its stake in the country economy [2]. About fruits production distribution, consumed destination of fruits on internal market share is higher than in external market, either way, processed or in natural, the exception is orange production, mainly exported in the form of concentrated juice toward the USA. Fruit flies are the designated term to a group of plagues from the Tephritidae family whose economic damages have been recognized worldwide. They are insects that cause direct damage to the final product, fruits, they are classified as fruit tree key-pests, as such, they reach the economic damage level at low population densities, requiring special care during the fruiting period, especially in orchards driven to external markets in the face of quarantine requirements imposed by importing countries of fresh fruits [3].

The Tephritidae family is cosmopolitan, have limited distribution due to host availability and extreme weather conditions. Species of economic importance belong to five genera: *Ceratitis, Anastrepha, Bactrocera, Dacus,* and *Rhagoletis* [4].

This pest also hinders the export of fruits due to phytosanitary barriers imposed by many countries, since it is a quarantine pest due to its large adapting capability to various environments, which helps its dissemination to many regions where there were no records of insect attacks before [5].

Monitoring using traps is essential to verify population level of the Tephritids, and to detect the presence of exotic or quarantine species in the region [6].

Traps are devices created to attract and capture fruit flies, attractants are put inside the trap in order to bring flies by sexual attraction or food attraction. It reduces fruit flies population potentially present in the orchard, decreasing the pressure and chances of an infestation [7]. However, the use of food attractions as a vegetable hydrolyzed protein in Brazilian fruit growing is still restricted, and can be attributed to these factors as their availability in the market and its cost [8]. In Brazil, molasses of sugar cane and fruit juices are attractants used in many fruit growing areas at the expense of hydrolyzed protein, as both for monitoring and for the preparation of toxic baits [9].

Fruit juices have been widely used because of their low cost and easy market acquisition, however, when comparing food attractants, hydrolyzed proteins have shown more durable attractively in the field [10]. Traps containing such attractive are used for the purposes of population detecting and monitoring in the field, of course, also for capture and removal of the largest possible number of individuals [11]. Due to the great importance of Tephritids, it is necessary to stimulate the adoption of management practices for this pest, through monitoring with traps and food attractants that can contribute to the producers for insecticides rational use and environmental preservation. Pest monitoring must provide information that

adequately represents species population fluctuation, though the food attractants usage that is effective, reliable and inexpensive [12].

Given the above, the aim of this study was to evaluate the food attractant efficiency for catching fruit flies in the culture of guava, in order to establish an appropriate program of this pest in the municipality of Nova Floresta – PB.

2. MATERIALS AND METHODS

2.1 Experimental Area

Nova Floresta is located in the Western Microregion Curimataú, and in the Metropolitan region of Barra de Santa Rosa, in the State of Paraíba, borders with Cuité and Picuí in Paraíba and with Jaçanã in the State of Rio Grande do Norte. Region climate is classified as AS', hot and dry with rainfall concentrated in the months of March to July [13]. Total rainfall recorded during the experiment was 580.16 mm.

The experimental area selected for this study was the Farm Monte Videl, geographical coordinates S: 06°27'38.79" and W: 36°12'36.99", total area is 12 hectares, where is located the orchard to do guava exploration *Psidium guajava* L. (Myrtaceae) with a total area of 2.5 hectares.

The plants of the mentioned orchard have a spacing of 6.0 × 4.0 meters with a height of plants around 2.5 up 3.0 meters, being the predominant guava variety, Paluma. The property also has a diversity of fruit species such as orange, graviola, lemon, acerola, cashew, and mango. Population survey of fruit flies on guava plants was carried out from August 2014 to July 2015.

2.2 Adults Collection Through Traps

Monitoring of adult fruit flies was carried out with aid of traps made of PET plastic bottle containing different food attractants, such as: hydrolyzed corn protein (Bio Anastrepha®) 5.0% (400 mL water + 30 mL protein); cane sugar molasses (400 mL Water + 40 mL molasses) to 10.0%; and guava fruit juice (400 mL water + 40 g sugar + 120 mL juice) at 30.0%. These traps were installed in the trees central part, approximately 1.50 m above ground. The bottles were inspected biweekly, at this time the captured fly specimens were collected, and the food attractants were replaced. These specimens were washed with water on a sieve and then well packed in plastic containers with hydrated alcohol 70.0% and they were properly labeled, and lately sent to the Invertebrate Zoology Laboratory of Agricultural Sciences Center of the Federal University of Paraíba - Areia/PB in order to perform the material screening, where males and females of the genre *Anastrepha* and *Ceratitis* were separated and preserved in alcohol at 70.0% for an ulterior identification of species.

2.3 Fruit Flies Identification of Species

Samples of fruit flies were separated by sex and only females were identified through the acolytes present in the ovipositor, since males did not present the diagnostic characters for a specific identification [14], identification keys were utilized [15].

In order to proceed with the identification, aculeus of females have been turned inside out, detached from the eversible membrane and assembled in glycerin between slide and coverslip and examined under a magnifying glass and optical microscope. Aculeus examination was performed under an optical microscope under a magnification of 20 to 40×.

2.4 Population Fluctuation of Fruit Flies

Surveys of the *Anastrepha* and *Ceratitis* species for population fluctuations study were carried out through the species present in the traps. Population fluctuation was based on the total number of adult females of *Anastrepha* and *Ceratitis* per month, the analysis was done in relation to pluviometrical precipitation and temperature with the attractants and MAD index. Climatic data were obtained from the PROCLIMA website.

2.5 MAD Index

MAD index represents the number of flies caught per trap/day (MAD). The MAD index was calculated by the formula (number of caught flies /number of installed traps/number of collect days) [16]. Its tolerance index is a function of requirements degree from market or fruit destination. If a destination is the United States of America, the MAD must be less than 1, in Brazil, this index drops to 0.5. For to calculate the MAD index, the following formula was used:

$$MAD = N/A \times D$$

At where:

MAD = flies/ trap/ day;
N = total number of flies caught;
A = number of traps evaluated;
D = interval days between collections.

2.6 Statistical Analysis

The experimental design was a randomized block with three treatments and 10 replications, each block consisted of a plant containing each of three PET traps having food attractants, a total of 30 PET bottles. Average number of adults/month, number of males and females and MAD index were data subjected to analysis of variance and the means values were compared by Tukey test at 5.0% probability. The data were analyzed using the software Assistat, version 7.7 beta [17].

3. RESULTS AND DISCUSSION

3.1 Food Attractants Efficiency

The Bio Anastrepha® at 5.0% was efficient in the flies capture for genera, *Anastrepha* and the *Ceratitis capitata* (Wiedemann) species in guava commercial orchard in the municipality of Nova Floresta - PB (Table 1). For genus *Anastrepha* capture of fruit flies, there was a difference between attractants Bio Anastrepha® and fruit juice compared to molasses, this last one has caught fewer flies from cited genus. Results found by [18] corroborate this study, where the commercial product Tephritid (corn hydrolyzed) presented higher capture efficiency compared to other attractants. For *Ceratitis* genus fruit flies capture, 5.0% Bio Anastrepha® was statistically superior to the other treatments (Table 1). Data found by [9] evidenced that Bio Anastrepha® alone have captured approximately 16.3 and 20.4 times more adults of *C. capitata* than sugar cane molasses. Found that the liquid attractants Bio Anastrepha® and CeraTrap® accounted for more than 60% of the total number of *C. capitata* captured during the six exposure periods [8].

Analyzing the capture of fruit flies (males and females) in the genera *Anastrepha* and *Ceratitis*, (Table 2) it is observed that for *Anastrepha* males fruit juice 30.0% was the food attractant which the best performance in the fruit flies capture, mean of 3.41 individuals/trap, but did not differ statistically from the average number of individuals caught in the traps containing the attractant 5.0% Bio Anastrepha®. Similar results were found for females capture of this genus; however, the attractant Bio Anastrepha® at 5.0% presented a superior result (5.75) when compared to fruit juice 30.0% (2.66 individuals by trap) (Table 2).

About genus *Ceratitis* males capture, there was no significant difference between the tested attractants, but there was significance for females only for Bio Anastrepha® 5.0% (4.58 individuals/trap) compared to the other attractants tested (Table 2). Attractants based in hydrolyzed protein and Bio Anastrepha® have effectively attracted females and males of *C. capitata* and *Anastrepha* spp. in orange orchard Pêra in the municipality of Nova Europa in São Paulo [19].

It is also possible to observe in Table 2 that the number of captured females with the attractant Bio Anastrepha® at 5.0% was superior to all other attractants, this possibly happened because it was a specific attractant for *Anastrepha*, also extending to capture females of species *C. capitata*. Food attractant based on protein presented higher fruit flies capture rate. Probably because it is an essential nutrient in the ovarian maturation and egg production in adult females, besides being important in the growth immature stage, nutritive material for survival and

Table 1. Adults average number/ month of *Anastrepha* spp. and *Ceratitis capitata* caught in PET through different types of attractants in a commercial orchard in the municipality of Nova Floresta - PB from August/2014 to July/2015

Attractants	Fruit flies		Total
	Anastrepha spp.	*Ceratitis capitata*	
Bio Anastrepha®5.0%	8.41 a	5.08 a	12.49 a
Molasses at 10.0%	0.75 b	0.50 b	1.25 b
Fruit juice 30.0%	6.07 a	0.16 b	6.23 ab

Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at 5.0% probability

Table 2. Adults mean number (males and females) of *Anastrepha* spp. and *Ceratitis capitata* captured in PET traps type through different attractants in a commercial orchard in Nova Floresta - PB from August/ 2014 to July/2015

Attractants	*Anastrepha* spp.		*Ceratitis capitata*	
	Males	Females	Males	Females
Bio Anastrepha® 5.0%	2.66 ab	5.75 a	0.50 a	4.58 a
Molasses at 10.0%	0.25 b	0.50 b	0.33 a	0.16 b
Fruit juice 30.0%	3.41 a	2.66 ab	0.00 a	0.16 b

Averages followed by the same letter in a column are not significantly different by Tukey test at 5.0% probability

storage for the pupal stage, and utilization in adulthood [20,21].

Table 3 shows *Anastrepha* females caught numbers per food attractant, where it was verified that traps containing Bio Anastrepha® 5.0% has caught the largest quantity of species and hence the largest number of individuals, totaling 60 fruit flies and 5 species of *Anastrepha*: *Anastrepha sororcula* (Zucchi), *Anastrepha dissimilis* (Stone), *Anastrepha obliqua* (Macquart), *Anastrepha hadropickeli* (Canal, Uramoto & Zucchi) and *Anastrepha fraterculus* (Wiedemann), representing 63.83% of captured fruit flies.For food traps using molasses at 10.0%, only was observed two captured species (*A. sororcula* and *A. dissimilis*), it represented 2.13% of the total number of captured individuals. About food traps containing fruit juice at 30.0%, three species of fruit flies were captured (*A. sororcula, A. dissimilis* and *A. obliqua*) with 32 captured fruit flies and 34.04% individuals total. Three from the tested attractants, Bio Anastrepha® 5.0% attracted a larger number of fruit flies samples, except fruit juice for the *A. obliqua* species. It was observed that the juice used as attractant have captured more individuals than the hydrolyzed protein at 5.0% and molasses at 10.0% [22]. Traps containing Bio Anastrepha® 5.0% was the attractant that has captured the largest number of fruit flies (62.11%) compared to Torula® (37.89%) [6].

For species caught in the traps, *A. sororcula* was the species that presented the greatest percentage among the other specimens caught (77.66%), while *A. dissimilis* reached 10.64%, *A. obliqua* 9.58%, *A. hadropickeli* and *A. fraterculus*, both 1.06% from the total of captured species during the sampling period.

3.2 MAD Index by Attractant

It was observed in Table 4 the averages of the MAD index for *Anastrepha*, which statistically there were no differences between attractant Bio Anastrepha® at 5.0% and fruit juice at 30.0%, in the other hand the MAD index was superior when 10.0% molasses was used. For the genus *Ceratitis*, it was verified a significant average on the MAD index in relation to the other food attractions tested when Bio Anastrepha® 5.0% was used. When MAD index presents its value equal to 0.5 or greater than 0.5 indicates the level in orchard infestation then chemical control actions should be initiated [23]. That way, it was observed that for both genus, *Anastrepha* and *Ceratitis* the MAD index remained below the control levels.

3.3 Population Fluctuation

At the species level, it was observed (Fig. 1), captured fruit flies population fluctuation in different food attractants, where the letter "A", "B" and "C" represent species caught in the traps containing the Bio Anastrepha® 5.0%, sugar cane molasses 10.0% and fruit juice 30.0%, respectively. Graph "A" (Fig. 1), it was recorded population peak of *C. capitata* in December 2014, followed by *A. sororcula* which presented population peak in June 2015. Hydrolyzed protein solutions are the most used attractant to capture both female and male individuals of fruit flies [24] however, they attract more females [25,26] than males. In the graph "B", population peaks were observed for two species of *Anastrepha* (*A. dissimilis* and *A. obliqua*), besides the capture registered of *C. capitata* for sugar cane molasses attractant at 10.0%. Also, in Fig. 1, in graph "C" was verified a population peak for *A. sororcula* in July 2015 when food attractant fruit juice at 30.0% was used.

During the experiment, by the Bio Anastrepha® population curve - MAD (Fig. 2) can be observed a period of dominance ranging from Nov/14 to Jan/15 where population peak of *Ceratitis capitata* species was observed in the month of December/14, reaching a MAD of

Table 3. Number of *Anastrepha* females captured in the PET traps through different attractants, in commercial orchard in the municipality of Nova Floresta – PB from August/2014 to July/2015

Attractants	*A. sororcula*	*A. dissimilis*	*A. obliqua*	*A. hadropickeli*	*A. fraterculus*	Total	%
Bio Anastrepha®5.0%	50	05	04	01	01	60	63.83
Molasses at 10.0%	01	01	-	-	-	02	2.13
Fruit juice 30.0%	22	04	05	-	-	32	34.04
Total	73	10	09	01	01	94	100
%	77.66	10.64	9.58	1.06	1.06	-	100

Table 4. MAD index of fruit flies captured in PET traps through different attractants in commercial orchard in the municipality of Nova Floresta from August/2014 to July/2015

Attractants	*Anastrepha* spp.	*Ceratitis capitata*
Bio Anastrepha®5.0%	0.0248 a	0.0158 a
Molasses at 10.0%	0.0016 b	0.0006 b
Fruit juice 30.0%	0.0199 a	0.0005 b

Averages followed by the same letter in a column are not significantly different by Tukey test at 5.0% probability

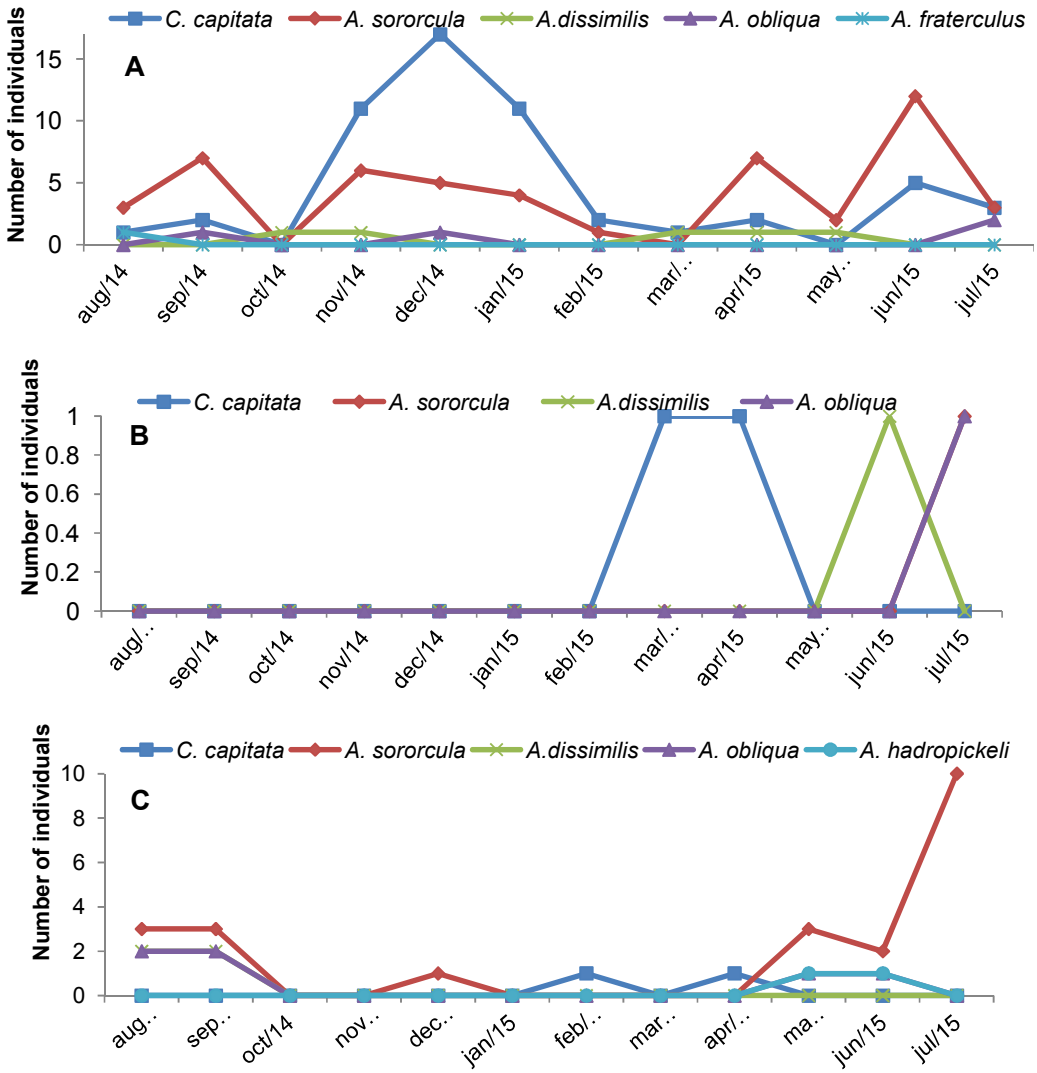

Fig. 1. Population fluctuation of fruit flies caught in the PET traps through A: Hydrolyzed protein at 5.0%; B: Molasses at 10.0% and C: Fruit juice at 30.0% in a commercial orchard in Nova Floresta - PB from August/2014 to June/2015

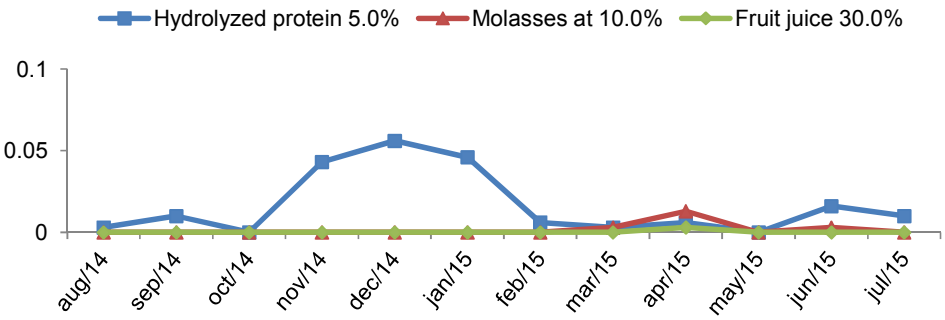

Fig. 2. Population fluctuation of fruit flies/trap/day of *Ceratitis capitata* (females + males) captured in PET traps by different attractants in the commercial orchard in the municipality of Nova Floresta - PB from August/2014 to July/2015

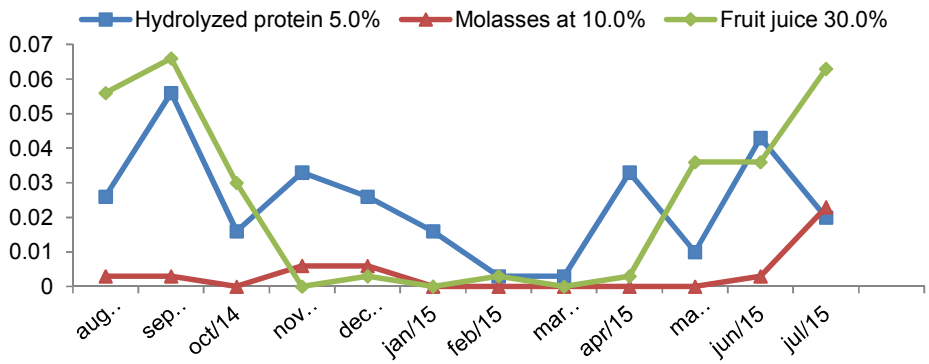

Fig. 3. Population fluctuation of flies/trap/day (MAD) of *Anastrepha* spp. (female + males) captured in PET traps through different attractants in a commercial orchard in the municipality of Nova Floresta - PB from August/2014 to July/2015

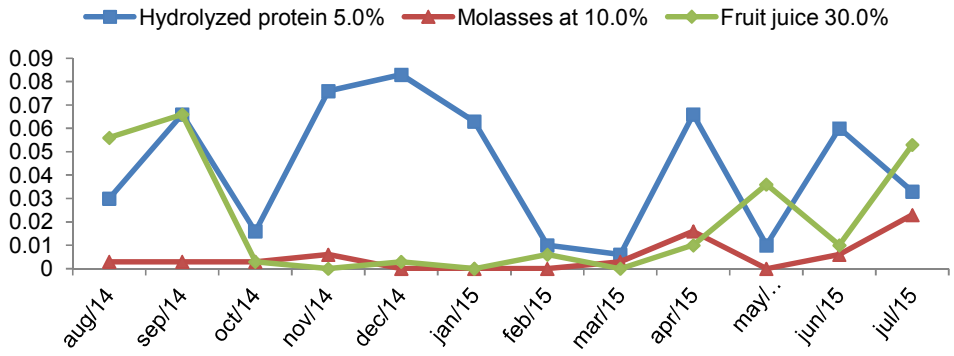

Fig. 4. Population fluctuation of flies/trap/day (MAD) of *Ceratitis capitata* and *Anastrepha* spp. (male + female), captured in PET traps through different attractants in a commercial orchard in the municipality of Nova Floresta - PB from August/2014 to July/2015

0.056. Probably this occurrence must be related to populations residing in the guava orchard, descendants of migrant populations of the surrounding fruit trees.

Population curves for (MAD) *Anastrepha* spp., it is verified that fruit flies population peak was obtained with the attractants molasses and Bio Anastrepha®, both in the month of September/14, with MAD 0.066 and 0.056, respectively (Fig. 3). MAD referring to the fruit juice attractant varied from 0.0 to 0.066 during the sampling period, molasses besides reaching one of the population peaks also reached the lowest values of the MAD index ranging from 0.0 to 0.023 for the genus *Anastrepha*. It is possible that the low MAD rates presented for *Anastrepha* in relation to fruit juice attractant at 30.0% and molasses at 10.0% have been affected to the detriment of the shortage of food or perhaps for the specificity of the Bio Anastrepha® attractant.

When population fluctuation (MAD) was evaluated, for the two genera mentioned above, a population peak was observed by traps containing the food attractant Bio Anastrepha® 5.0% of 0.083 in the month of December/2014, it was also verified that MAD remained equal to or greater than 0.006 (Fig. 4) during the whole sampling period. According to [27], studying the population fluctuation and survey of fruit flies in guava in the municipality of Russas in Ceará, the evaluated orchard presented a considerable increase from the month of April, when it was found 6.04 MAD, with population peak occurring in May (15.25 MAD), decreasing to 6.32 MAD in June.

The low MAD values observed in Fig. 1, Fig. 2, and Fig. 3 can probably be explained by the fact that the farm owner uses several other fruit fly control techniques within his property, such as: adhesive glue on PET bottles painted with the

yellow color, use of luminous traps, besides the thinning of fruit damaged by birds or other pests present in the area, for example, the yellow beetle (*Costalimaita ferruginea vulgata*) that according to the farm owner was one of the key plagues at his orchard.

4. CONCLUSIONS

The food attractant Bio Anastrepha® to 5.0% attracts with effectiveness males and females, especially *Anastrepha* spp. and *C. capitata*. The greatest MAD found in population fluctuation curves was from the attractant Bio Anastrepha® 5.0% to the value of 0.083. Molasses at 10.0% was inefficient in attractiveness of adult *Anastrepha* spp. and *C. capitata.* Using Bio Anastrepha® at 5.0% results in the best cost benefit when compared to the other food attractants tested (10.0% molasses and 30.0% fruit juice).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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