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Investigation of the correlations among biological parameters of mass reared peach
fruit fly *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract:

The peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most important species of family Tephritidae. It is a polyphagous species attacking more than 50 species of fruit and vegetables and It causes serious economic losses, either by direct damage to fruits or indirectly by warranting quarantine and phytosanitary measures. To perform required studies on biology, attractants, various biological control agents, sterile insect technique (SIT), and postharvest treatment also, to obtain an accurate result that guarantee success of the applied control methods, it is required a regular supply of good quality insects. This study is aimed to minimize quality control test used to evaluate the quality of laboratory mass produced peach fruit fly *B. zonata*. through finding the correlations among these biological parameters and choose some of these tests as a representative for the rest traditional tests, thus minimize time, costs and labors. Results revealed that six parameter out of the eight studied parameters (survival ability, number, size weight, emergence percentage and flight ability [of pupae per each pupation depth]) have the same trend, while only two parameters follow random trend (percent pupal deformity and sex ratio). results revealed that pupae collected 2 cm under sand surface have the best quality since have the largest size, highest weight, survive longer, have highest emergence percentage, highest percent of fliers and highest percent of pupae (with means of 0.0346 ml, 11.98 mg, 67.8 hrs. 98.67 %, 97.33 % and 39.71 % respectively) , followed by pupae from 3 cm depth then first cm and finally the forth cm depth. Also, correlation tests results revealed that there was a strong direct correlation among the five parameters (with correlation coefficient r ranging from 0.997 to 0.877). Pupal weight correlates well with other quality parameters and can be a predictor for tests performed late, its weight gives a robust measure of fly quality. So, use it to compare overall quality of pupae from different facilities is highly recommended. thus, minimized labor, time and costs consumed by other tests.

Introduction

Fruit flies are believed to be the most serious threat to horticultural products all over the world (Allwood and Drew, 1997; Barnes, 2004 and Ekesi and Billah, 2007). As, they are

highly polyphagous, attacking more than 350 species of host plants belonging to about 67 families (Aluja and Mangan, 2008). One of them the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most important species of fruit

flies infested more than 50 species of fruit and vegetables and It causes direct damage to fruits or indirectly by warranting quarantine and phytosanitary measures (OEPP/EPPO, 2005). One Of the greater aspects is the fact that fruit flies represent a great risk as key and potential quarantine pests since they are easily translocated due to the global trade and passenger trafficking. This leading to even in fruit fly controlled countries, European markets rejection increase day after day. (Ole-MoiYoi and Lux, 2004). Myers *et al.* (1998) defined pest eradication as the getting rid of all individuals of a pest species from a distinct geographic area without any possibility of reinvasion. Eradication procedures include pesticide spraying (Full or partial), use of attractants (sexual, olfactory or food) for catch and kill (Steiner *et al.*, 1961), in addition to, baiting and male annihilation techniques, biological control (pathogens, parasitoids and predators), and agricultural control means (Orchard sanitation, fruit bagging, and early harvesting) (Klassen, 1989; Allwood, 1997; Barnes, 2004; Mau *et al.*, 2007 and Ekesi, and Billah, 2007). More than hundred years has elapsed since Runner (1916) demonstrated that X-rays induced sterility in cigarette beetles, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) . Lindquist (1955) mentioned that the control of the screwworm *Cochliomyia hominivorax* Coquerel, through a technique of sterilizing the males by somehow. These sterilized males released in large numbers into the field to mate with wild females, thus compete with wild males, copulate with wild females leading to population decline. Released individuals for sterile insect technique (SIT) must be in good condition. based on classical Mendelian genetics, Busch-Petersen and Kafu 1989 demonstrated that X-rays induced sterility has

disadvantage that 50% reduction in the productivity of the mass-reared colony. many innovations have been made to improve production efficiency (Robinson, 2002), male competitiveness (Shelly, 2001), and thereby increase induced sterility in wild populations (Rendon *et al.*, 2004). sterile males only releasing will increase the biological efficiency of SIT process but increase the costs dramatically (Hendrichs *et al.*, 1995) . Hendrichs, 2000 and Chakroun *et al.*, 2017 studied doses and method of insect sterilization. And in 2002, Hendrichs *et al.* insured that the SIT has been proven to be very effective for the suppression, or eradication of populations of the Mediterranean fruit fly. A successful series of experiments have been developed by many scientists all over the world to compare tephritid fruit flies that produced based on different rearing protocols. These attempts resulted in the production of many fruit fly quality control manuals (e.g. Orozco *et al.*, 1983; Boller and Chamber, 1977 and Brazzel *et al.*, 1986). Calkins *et al.* (1996), strongly recommended to construct a separate product quality control unit for SIT program. each program that incorporates the SIT component requires an efficient monitoring system to assess over-flooding ratios, spatial distribution of the released sterile males, etc. (Vreysen *et al.*, 2005). To execute required studies on biology, response to attractants, efficacy of the various biological control agents, SIT, and postharvest treatment also, obtain an accurate result that guarantee success of the applied control methods, it is required a regular supply of good quality insects. quality control parameters must be established and closely monitored in mass rearing procedures (Ekesi and Mohamed, 2011).

This study is aimed minimized labor, time and costs consumed by quality control tests through finding the correlations among biological parameters used to evaluate the quality of laboratory mass produced peach fruit fly and consequently, choose one or two tests as a representatives to each correlated quality control tests. Thus, minimized labor, time and costs.

Materials and methods

1. Peach fruit fly *Bactrocera zonata* strain:

Peach fruit fly lab strain (horticultural yields department laboratories - plant protection research institute) that reared upon an artificial larval diet composed of Sugar (as a source for carbohydrate) 8 %, dried sterile yeast (as a source for protein) 10.5 %, wheat bran (as a bulking agent) 26.7 %, sodium benzoate 0.53 %, citric acid 0.53 % and water 53.4 %. The adult flies feed normally upon water, sugar and hydrolyzed protein (4:1).

2. Experiments:

2.1. Experiments sequences:

To correlate biological parameters, a group of tests executed sequentially, begin with determination of pupation depth of medfly popped larvae then isolate pupae of different depths and weight them, then measure their sizes. Emerged flies checked out to recorded emergence ratio and deformity ratio, upon fly emergence, sex ratio calculated finally, Flight ability and Survival under Stress tests executed and according to the statistically analyzed results, the correlation is estimated.

2.2. Experiments details:(IAEA 2014):

2.2.1. Pupation depth:

Using a wooden box that each of the four sides is composed of 6 strips (1 cm x 15 cm) to give a 6 cm depth, this box is filled with a fine sand as a pupation media, putting the trays that containing the larval artificial media (In which eggs were seeded) on the top of

the sand till larvae reach pupation and pop out to pupate inside the sand in different depths, remove the first (top) strip of the four side and collect the sand, sieve and collect the pupae found. Repeat with the rest of the strips and collect the pupae of each depth sequentially, classify pupae into categories according to weight, record and save for the next test

2.2.2. Pupal weight:

For each group of pupae resulted from the pupation depth record the average weight for 5 lots of 100 pupae two days before emergence (since age at sampling is critical because pupae lose water and, therefore, weight). Record the weight for each group and save for the next test.

2.2.3. Pupal size:

For each pupal weight category , count (Five lots) the number of pupae per one ml using graduated cylinder and record the average size per pupa, record and save the pupae.

2.2.4. Emergence ratio:

For each pupal weight category, calculate number of successfully emerged flies for each 100 pupae (Using five lots) to calculate the percentage of emergence.

2.2.5. Deformity ratio:

For each pupal weight category, calculate number of flies that failed to emerge , or emerge with deformation for each 100 pupae (Using five lots) to calculate the percentage of deformity.

2.2.6. Sex ratio:

For each pupal weight category, a group of 100 pupae separated in a petri dish, Once it has been determined that no further emergence will occur, The numbers of emerged males and females are counted and recorded.

2.2.7. Flight ability:

PVC tubes are used to investigate flight ability of the newly emerged flies (Outside diameter 8.9 cm with 3 mm thick walls; painted black so that light enters only at the top; 10 cm high).

Avoid using an abrasive cleaner. Petri dish lids, 90-100 mm in diameter, should be painted black or the bottom overlaid with black paper. Strip of porous paper, 1 cm wide, and formed into a ring 6 cm in diameter. Two days before emergence, For each pupal weight category, 100 pupae are placed within the ring of paper, in the bottom of the Petri dish. the inside of the tube is lightly coated with unscented talcum powder to prevent the flies walking out. Tubes are tapped on a firm surface to remove excess talc, and the talc should be wiped off the bottom. The PVC tube with talc is placed in the darkened Petri dish lid. Five replicates (Five tubes with 100 pupae each) are set up for each lot to be tested.

2.2.8. Survival under stress:

For each pupal weight category, five lots each of 100 flies are transferred separately to cup (Covered top with a fiber mish to supply aeration) using an aspirator, or preferably a suction pump, Leave flies without any feeding material or water, recording date and time for emergence and death to calculate how long the fly can persist alive without any food.

3. Statistical analysis:

Table (1): Percent of peach fruit fly *Bactrocera zonata* pupae collected from different pupation depths.

No.	Pupation depth	% pupae
1	1cm	14.2 ± 0.0447 c
2	2	39.71 ± 0.0049 a
3	3	33.24 ± 0.0035 b
4	4	11.88± 0.0038 d

1.2.Pupal weight :

Relating pupal weight to depth of pupation, calculating the mean pupal weight for pupae collected from each depth level revealed that the mean pupal weight for the first pupation depth (1 cm) was 11.75 mg, 11.98 mg for the second level,

Table (2) : Pupal weight of peach fruit fly *Bactrocera zonata* collected from different pupation depths.

No.	Pupation depth	Pupal weight
1	1	11.75 ± 0.0017 c
2	2	11.98 ± 0.0027 a
3	3	11.90 ± 0.0170 b
4	4	11.65± 0.0064 d

Results subjected to statistical analysis using IBM SPSS statistics version 23 one-way ANOVA test and correlation coefficient test (IBM Corp, 2020).

Results and discussion:

1. Estimating biological parameters of the mass reared flies:

1.1. Pupation depth:

Results revealed that peach fruit fly *Bactrocera zonata* larvae penetrate the sand pupation medium not deeper than 5 cm, but a few pupae descended to 5 cm, so they are eliminated from calculations. Percent of pupae found within the first cm of the sand 14.2 % of the total pupae, while it is 39.71 % within the second cm depth, and 33.24 % within 3 cm in the third rand and the deepest pupation level (4 cm) came with the least pupation percentage of 11.88 % . There was a significant difference among the four levels. 2 cm depth pupae came as the superior percentage of pupal number followed by the 3 cm depth pupae then the first cm depth pupae while larvae those pupate within the 5th cm depth was few pupae, so all records of the 5th cm pupation depth (If found) are eliminated (Table 1) .

11.90 mg for the third level and finally, 11.65 mg for the last level (4 cm depth), with a significant differences among the four levels 2cm pupae was the weightiest followed by 3 cm then one cm while pupae within 4 cm deep was the lighter (Table 2).

1.3. Pupal size:

Measurements of the pupal size revealed that, the mean pupal size for the first pupation depth (0.0197 ml), 0.0236 ml for the second depth, 0.0227 ml for the third cm depth and finally, 0.0205 ml for the last pupation depth (4 cm), also revealed that there were

significant differences among the four size groups, [pupae of the second cm pupation depth came as the largest pupae followed by 3 cm depth and then the pupae in the first cm depth and the 4cm depth of the pupation medium contain the smaller pupae (Table 3).

Table (3): Pupal size of peach fruit fly *Bactrocera zonata* collected from different pupation depths.

No.	Pupation depth	Pupal Size
1	1cm	0.0197 ±0.0012 D
2	2	0.0236 ±0.0019 A
3	3	0.0227 ±0.0007 B
4	4	0.205±0.0007 C

1.4. Emergence percentage:

Regarding to emergence percentage tests, the mean emergence percentage for the first pupal weight was 93.67 %, 98.67 % , 96.67 % and 92.67 % for the successive depth

categories with a significant differences among the four levels, where 2 cm depth came with the highest percentage of emergence, followed by 3 cm, then one cm and finally the fourth cm depth (Table 4).

Table (4) : Percent emergence of peach fruit fly *Bactrocera zonata* adult collected from different pupation depths.

No.	Pupation depth	% emergence
1	1	93.67 ± 0.0016 C
2	2	98.67 ± 0.002 A
3	3	96.67 ± 0.0017 B
4	4	92.67 ± 0.0018 D

1.5. Deformity percentage:

Calculating the percentage of the deformed flies, revealed that the mean of the deformed flies for the first pupal cm pupation depth was 2.3 %, 0.9

% for the second cm, 1.6 % for the third cm and finally, 3.5 % of the pupae for the last pupation depth was deformed, with a significant differences among the four levels (Table 5) .

Table (5): Percent deformed pupae of peach fruit fly *Bactrocera zonata* collected from different pupation depths.

No.	Pupation depth	% deformed Pupae
1	1	2.3 ± 0.0224 B
2	2	0.9 ± 0.0079 D
3	3	1.6 ± 0.0114 C
4	4	3.5 ± 0.0170 A

1.6. Sex ratio:

Results revealed that there was no relation between pupation depth and male to female ratio. For first pupation cm, the sex ratio was 50.21 ♂ : 48.79 ♀, 49.24 ♂ : 50.76 ♀ for 2 cm pupae, 49.3

♂ : 50.7 ♀ for 3 cm pupae and for 4 cm pupae sex ratio was 49.64 ♂ : 50.36 ♀ these results with a significant differences among these ratios (Table 6) .

Table (6) : Sex ratios of peach fruit fly *Bactrocera zonata* collected as pupae from different pupation depths.

	Pupation depth	Males	Females
1	1	50.21 ± 0.332 A	48.79 ± 0.0332 III
2	2	49.24 ± 0.068 C	50.76 ± 0.067 I
3	3	49.30 ± 0.447 C	50.70 ± 0.044 I
4	4	49.64 ± 0.075 B	50.36 ± 0.075 II

1.7. Flight ability:

Results of the flight ability tests revealed that the highest percentage of flies those have the ability to pass flight ability test (97.33 %) was resulted from 2 cm pupation depth, followed by 3 cm

depth with 96.33 % , then 95.67 % for flies resulted from one cm depth and those resulted from 4 cm pupation depth have the least percentage of fliers with significant differences among the four categories (Table 7).

Table (7) : Percent fliers of peach fruit fly *Bactrocera zonata* collected as pupae from different pupation depths.

No.	Pupation depth	% fliers
1	1	95.67 ± 0.0035 C
2	2	97.33 ± 0.0070 A
3	3	96.33 ± 0.0017 B
4	4	93.67± 0.0018 D

1.8. Survival under stress:

Survival test revealed that flies differ significantly in there with standing survival without food or water. Pupae of 2 cm pupation depth have the greatest persistence with a mean of 67.8 hrs. 3 cm pupae came in the second

level with a mean of 64.7 hrs. For the first pupation cm, the resulted flies can persist starved for mean of 61.4 hrs. as the third rank. While the least survival persistence was recorded for the flies resulted from 4 cm pupation depth with mean age of 58.7 hrs. (Table 8).

Table (8) : Longevity (Per hours) of survival adults of peach fruit fly *Bactrocera zonata* collected from different pupation depths.

No.	Pupation depth	Adult longevity
1	1	61.4 ± 0.0101 C
2	2	67.8 ± 0.0152 A
3	3	64.7 ± 0.0174 B
4	4	58.7 ± 0.0171 D

2. Estimating the correlations among the biological parameters:

Correlation tests revealed that there were a strong direct correlation among the five parameters [Survival, no of pupae, weight of pupae, emergence percentage and number of flies those have flight ability (of pupae per each pupation depth)] with r range values of 0.877 : 0.997, and the correlations are significant at the 0.01 level (2-tailed).

While these parameters (Survival, no of pupae, weight of pupae, emergence percentage and number of flies those have flight ability [of pupae per each pupation depth]) have a strong reversed correlation with the no of the deformed flies resulted from different pupation depth with r range values of – 0.920 : - 0.994) also, these correlations are significant at the 0.01 level (2-tailed) (Table 9).

Table (9): Correlation relationships among different biological parameters of laboratory reared peach fruit fly.

	Pupal size	Pupal No. per Depth	Weight per Depth	Age	Emergence percentage	Percent fliers	Percent deformity	Male Ratio	Female Ratio
Depth	.292	-.126	-.328	-.300-	-.234-	-.585**	.500*	-.459-*	.642**
Pupal size		.527*	.432	.455*	.487*	.282	-.345-	-.583-**	.603**
Pupal No. per Depth			.967**	.979**	.991**	.877**	-.920-**	-.773-**	.656**
Weight per Depth				.984**	.979**	.949**	-.969-**	-.634-**	.481*
Age					.997**	.940**	-.967-**	-.664-**	.524*
Emergence percentage						.918**	-.952-**	-.710-**	.579**
Percent fliers							-.994-**	-.406-	.220
Percent deformity								.493*	-.317-
Male Ratio									-.967-**

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Results revealed that six parameter out of the eight studied parameters [Survival ability, number, size weight, emergence percentage and flight ability (Of pupae per each pupation depth)] have the same trend, while only two parameters follow random trend (Percent pupal deformity and sex ratio). Also, pupae collected 2 cm under sand surface have the best quality since have the largest size, highest weight, survive longer, have highest emergence percentage, highest percent of fliers and highest percent of pupae, followed by pupae from 3 cm depth then first cm and finally the forth cm depth. Correlation tests results revealed that there was a strong direct correlation among the five parameters [Survival, number, weight, emergence percentage and number of flies those have flight ability (For pupae collected from each pupation depth)]. Obtained results revealed also that, pupal size, adult deformity percentage, adult flight ability and starvation ability tests are related to pupal weight. pupal weight correlates well with other quality

parameters and can be a predictor for tests performed late and gives a robust measure of fly quality. So, use it to compare overall quality of pupae from different facilities is highly recommended, thus minimized labor, time and costs consumed by other tests.

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