MEDITERRANEAN FRUIT FLY PUPAL WEIGHT AS A PRINCIPAL INDICATOR FOR MASS-REARED FLIES' QUALITY USED IN STERILE INSECT TECHNIQUE (SIT) PROGRAM

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ABSTRACT: *To estimate any correlation between pupal weight and different biological parameters used as quality control tests to estimate the fly quality for mass-reared Mediterranean Fruit Fly Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) in SIT programs, and consequently quality control tests needed could be minimized thus reducing labor and costs, obtained results revealed that pupal size, adult deformity percentage, adult flight ability and starvation ability tests are related to pupal weigh with a significant correlation thus it could be minimized to one of them while pupation depth, emergence percentage, and sex ratio came without a significant correlation with pupal weight so, they must be executed within the quality control tests of the Mediterranean Fruit Fly SIT programs.*

Keywords: Mediterranean fruit fly, Ceratitis capitata (Wiedemann), Diptera: Tephritidae, quality control, mass rearing, SIT programs, control.

INTRODUCTION:

Nephritis fruit flies are believed to be the most dangerous impact to fruit production all over the world [1, 2, 3]. The mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: *Tephritidae*) is the most destructive pest attacking fruits and vegetable in the world, causing an extensive loss in yield [4]. It is highly polyphagous and attacks more than 350 species of host plants belonging to about 67 families [5]. One of the greater aspects is the fact that even in fruit fly-controlled countries, the European market's rejection increase day after day. They are easily translocated because of global trade and passenger trafficking. The risk of fruit flies as key and potential quarantine pests is becoming increasingly realized [6]. Control routines applied against it include awareness of the great damage caused by fruit flies led to a demand for the development of control strategies against the fruit flies. Most of the developing countries income is highly dependent on agriculture, so effective control methods to suppress or eradicate this pest are of utmost importance. This includes spray, use of food attractants and other semi-chemicals for catching and killing both sexes [7] baiting and male annihilation techniques, biological control (entomopathogens, parasitoids, and predators), agricultural control means such as orchard sanitation, fruit bagging, and early harvesting [1-3, 8]. The application of SIT programs in control represents a road mark for the traditional pest control strategy. Effective execution of SIT programs is based on the opportunity of mating the sterile mass-reared males to wild females and thus reproduction failure results. This success can be achieved when the over-flooding ratio is maintained in the field (mass-reared sterile males: wild males' flies). Mass-rearing and releasing a given number of sterile fruit flies into the field is only part of the success of the SIT program, since managers need to ensure that, once, sterile males compete effectively with wild males and mate with wild females in the field and transfer their sperm successfully. An important basis for the success of the SIT program is the presence of effective procedures for monitoring and providing periodic feedback on the quality and competitiveness of sterile fruit flies. These procedures could result in significant gains both in efficiency and effectiveness. On the other hand, neglecting insect quality could lead to substantial program cost increases and failure of the pest control program. A successful series of experiments have been developed by many scientists all over the world to compare tephritid fruit flies that are produced based on different rearing protocols. These attempts resulted in the production of many fruit fly quality control manuals [9-12]. In the production facility, serious conflicts could arise, quantity versus quality i.e. the need for a high number of mass-reared irradiated males (to maintain over flooding ratio) against high-quality mass-reared irradiated males (those can compete with the wild males). Examples of such conflicts include the attempts to increase mass-rearing production but this may lead to a reduction of the size and quality of the sterile flies, also, managers may be not interested in the quality and may prefer to replace an older strain with another strain that is less laboratory-adapted and more difficult to rear, but more competitive in the field. So, it strongly recommended constructing a separate product Quality Control unit for SIT program [13[. Each program that incorporates the SIT component requires an efficient monitoring system to assess over-flooding ratios, the spatial distribution of the released sterile males, etc. Elsewhere [14, 15], the researchers mentioned that the SIT has been proven very effective for the suppression, or eradication of populations of the Mediterranean fruit fly. Nevertheless, releasing only sterile males increase the biological efficiency of the program application but dramatically increases the costs [16]. Others [17], discussed using classical Mendelian genetics which has two disadvantages. First, 50 % reduction in productivity of the mass-reared colony. Secondly, sex-linked translocations are not stable and break down the Genetic Sexing Strain during successive generations under mass-rearing. A filter-rearing system (FRS) was developed to avoid this breakdown [18]. Herein, a try to indicate that weight is a valuable indicator of the overall viability of pupae, and show that the larger flies will have a higher ability to fly, live longer, and have a higher propensity to mate than smaller ones [19]. Therefore, using weight to compare the overall quality of pupae from different facilities must be done with caution, i.e. pupal weight gives a robust measure of pupal quality and correlates well with other quality parameters, and can be a predictor for tests performed late.

MATERIAL & METHOD

Medfly strain

Mediterranean fruit fly lab strain (laboratories of biology) that reared upon an artificial larval diet composed of Sugar (as a source for carbohydrate) 8.45 %, Dried sterile yeast (as a source of protein) 8.45 %, Wheat bran (as a bulking

agent) 33 %, Sodium benzoate 0.3 %, Citric acid 0.3 %, and Water 50 %. The adult flies feed normally upon water, sugar and hydrolyzed protein (4:1).

Experiments:

Experiments sequences:

To correlate pupal weight with other biological parameters, a group of tests executed sequentially, begin with a determination of the pupation depth of medfly popped larvae then isolate pupae of different depths and weigh them, then measure their sizes at the same time recording pupal duration. Emerged flies were checked out to record the emergence ratio and deformity ratio, upon fly emergence, the sex ratio was calculated, and finally, the flight ability and starvation tests were executed and according to the statistically analyzed results, the correlation is estimated.

EXPERIMENTS DETAILS

(FAO, 2014, 2019[20] and Parker *et al.,* 2021)[21]**:**

1. Pupation depth

Using a wooden box that each of the four sides is composed of 10 strips (1 cm x 15 cm) to give a 10 cm depth, this box is filled with fine sand as a pupation media, putting the trays that containing the larval artificial media 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 sides 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. Pupal weight

For each group of pupae resulting from the Pupation depth record the average weight for every 10 pupae 2 days after pupation. Record the weight for each group and save for the next test.

3. Pupal size

For each pupal weight category, count the number of pupae per one ml in a 10 ml graduated cylinder and record the average size per pupa, record and save the pupae.

4. Emergence ratio

For each pupal weight category, calculate the number of successfully emerged flies for every 100 pupae to calculate the percentage of emergence.

5. Deformity ratio

For each pupal weight category, calculate the number of flies that failed to emerge successfully, or emerge with deformation every 100 pupae to calculate the percentage of Deformity.

6. Sex ratio

For each pupal weight category, calculate the number of males and females for every 100 pupae and calculate the sex ratio.

7. Flight ability

For each pupal weight category, using the 20 cm long x 18 cm diameter plastic cylinder covered from inside with talcum powder put the cylinder inside a petry dish with 100 pupae. Calculated the number of flies that succeeded to fly outside the cylinder.

8. Starvation

For each pupal weight category, leave flies individually in a separate cup without any food material, recording date and time for emergence and death to calculate how long the fly can persist alive without any food.

Statistical analysis

Results were subjected to statistical analysis using IBM SPSS statistics version 23 one-way ANOVA test and correlation coefficient test.

RESULTS

1. Pupation depth

Obtained results revealed that medfly larvae penetrate the sand pupation medium not deeper than 4 cm. percent of pupae found within the first cm of the sand 27.73 % of the total pupae as the second rank, while it is 52.21 % within the second cm depth as the superior rank, and 17.19 % within 3 cm in the third rand and the deepest pupation level (4 cm) came with the least pupation percentage 2.86 % with a significant difference among the four levels. There was no correlation between pupation depth and percent of pupae collected from each level

Table (1): Percent of medfly pupae collected from different pupation depths.

	Pupation depth	% pupae
1	1cm	27.73 ± 0.013 b
2	2	52.21 ± 0.033 a
3	3	17.19 ± 0.024 c
4	4	2.86 ± 0.027 d
Correlation is not significant at the 0.05 level (2-tailed). rp = 0.680		

2. Pupal weight

Calculating the mean pupal weight for pupae collected from each depth level revealed that there was a gradual decrease in pupal weight as the depth increased, the mean pupal weight for the first pupation depth (1 cm) was 8.02 mg, 7.56 mg for the second level, 6.58 mg for the third level and finally, 5.18 mg for the last level (4 cm depth), with a significant difference among the four levels. The correlation test revealed that there was a significant correlation at the 0.05 level (2-tailed). rp = 0.976.

Table (2): Pupal weight of medfly collected from different pupation depths.

	Pupal weight	Pupation depth
1	8.02 ± 0.0006 a	1
2	7.56 ± 0.0008 b	2
3	6.58 ± 0.0011 c	3
4	5.18 ± 0.0012 d	4
Correlation is significant at the 0.05 level (2-tailed). r_p = 0.976		

3. Pupal size

Measurements of the pupal size revealed that gradual decrease in pupal size as the pupal weight decreased, the mean pupal size for the first pupation weight (8.02 mg) was 0.018.4 ml, 0.0175 ml for the second weight (7.56 mg), 0.0164 ml for the third weight (6.58 mg) and finally, 0.0154 ml for the last eight (5.18mg), with significant differences among the four levels, also correlation test revealed that there is no significant correlation at the 0.05 level.

Table (3): Pupal size of medfly collected from different pupation depths.

	Pupal weight	Pupal Size
1	8.02 ± 0.0006 a	0.018.4 ±0.0002 A
2	7.56 ± 0.0008 b	0.0175 ±0.00017 B
3	6.58 ± 0.0011 c	0.0164 ±0.00025 C
4	5.18 ± 0.0012 d	0.0154 ±0.00014 D
Correlation is significant at the 0.05 level (2-tailed). rp = 0.741		

4. Emergence percentage

The same evidence appeared in emergence percentage tests, the means of the emerged flies gradually decreased as the pupal weight increased, the mean emergence percentage for the first pupal weight was 96.8%, 88.78 % for the second weight, 83.12 % for the third weight and finally, 79.6 % for the last weight, with a significant difference among the four levels, also correlation test revealed that no significant correlation at the 0.05 level.

Table (4): Percent emergence of medfly adults collected from different pupation depths.

	Pupal weight	% emergence
1	8.02 ± 0.0006 a	96.8 ± 0.195 A
2	7.56 ± 0.0008 b	88.78 ± 0.213 B
3	6.58 ± 0.0011 c	83.12 ± 0.15 C
4	5.18 ± 0.0012 d	79.6 ± 0.122 D
Correlation is not significant at the 0.05 level (2-tailed). rp = 0.924		

5. Deformity percentage

calculating the percentage of the deformed flies, revealed that mean of the deformed flies increased as the pupal weight decreased, the mean of the deformed flies for the first pupal weight was 1.4 %, 1.8 % for the second weight, 2.2 % for the third weight and finally, 3.4 % of the pupae for the last weight was deformed, with significant differences among the four levels, with a significant correlation at the 0.01 level (2-tailed). rp = 0.990.

Table (5): Percent deformed pupae of medfly collected from different pupation depths.

	Pupal weight	% deformed Pupae
1	8.02 ± 0.0006 a	1.4 ± 0.245 A
2	7.56 ± 0.0008 b	1.8 ± 0.374 A
3	6.58 ± 0.0011 c	2.2 ± 0.020 A
4	5.18 ± 0.0012 d	3.4 ± 0.0245 B
Correlation is significant at the 0.01 level (2-tailed). rp = 0.990		

6. Sex ratio

Results revealed that there was no relation between pupal weight and male-to-female ratio and there was no correlation between them. For superior pupal weight (8.02 mg) the sex ratio was 53.8 ♂: 43.2 ♀, 40.2 ♂: 48.8 ♀ for 7.56 mg pupae, 44.8 ♂: 39 ♀ for 6.58 mg pupae and for 5.18 mg pupae sex ratio was 42.2 ♂: 38 ♀ these results with significant differences among these ratios. Also, the correlation test revealed that there is no significant correlation at 0.05.

Table (6): Sex ratios of medfly collected as pupae from different pupation depths

	Pupal weight	males	females
1	8.02 ± 0.0006 a	53.8 ± 0.374 A	43.2 ± 0.663 II
2	7.56 ± 0.0008 b	40.2 ± 0.374 D	48.8 ± 0.663 I
3	6.58 ± 0.0011 c	44.8 ± 0.374 B	39 ± 0.837 III
4	5.18 ± 0.0012 d	42.2 ± 0.663 C	38 ± 0.707 III
		Correlation is not significant at the 0.05 level (2-tailed). rp = 0.514	Correlation is not significant at the 0.05 level (2-tailed). rp = 0.742

7. Flight ability

Results of the flight ability tests revealed that there is also a gradual decrease in the percentage of flies that have the ability to pass the flight ability test as the pupal weight decreased, the mean flier percentage for the first pupation weight (8.02 mg) was 85.54 %, 82.32 % for the second weight (7.56 mg), 79.34 % for the third weight (6.58 mg) and finally, 76.32 % for the least pupal weight (5.18mg), with significant differences among the four weights, also correlation test revealed that there is a significant correlation at the 0.05 with rp = 0.973.

Table (7): Percent fliers of medfly collected as pupae from different pupation depths

	Pupal weight	% fliers
1	8.02 ± 0.0006 a	85.54 ± 0.0812 A
2	7.56 ± 0.0008 b	82.32 ± 0.1158 B
3	6.58 ± 0.0011 c	79.34 ± 0.114 C
4	5.18 ± 0.0012 d	76.32 ± 0.128 D
Correlation is significant at the 0.05 level (2-tailed). rp = 0.973		

8. Starvation

The starvation test revealed that the superior-weight pupae have the greatest persistence without any food or water and there is a direct proportionality between the two parameters. For the first pupal weight, the resulting flies can persist starved for a mean of 75.42 hours before death, the second pupal weight has a mean starvation period of 71.19 hours, the third pupal weight gets 67.53 hours' starvation period and finally, the least pupal weight has 63.09 hours' starvation period. The correlation test revealed that there is a significant correlation at the 0.05 level with rp = 0.978.

Table (8): Longevity (Per hours) of survival adults of medfly collected from different pupation depths.

	Pupal weight	Starvation period
1	8.02 ± 0.0006 a	75.42 ± 0.034 A
2	7.56 ± 0.0008 b	71.19 ± 0.029 B
3	6.58 ± 0.0011 c	67.53 ± 0.047 C
4	5.18 ± 0.0012 d	63.09 ± 0.036 D
Correlation is significant at the 0.05 level (2-tailed). rp = 0.978		

CONCLUSION

This study aims to estimate the correlation relationships between pupal weight and different biological parameters that are used as quality control tests to estimate the fly quality for mass-reared medfly in SIT programs, and consequently, quality control tests needed could be minimized thus reducing labor and costs. obtained results revealed that pupal size, adult deformity percentage, adult flight ability, and starvation ability tests are related to pupal weight with a significant correlation with rp of 0.741,0.990,0.973 and 0.973 respectively, thus it could be minimized to one of them and reduce labor and costs for other tests. While pupation depth, emergence percentage and sex ratio came without a significant correlation with pupal weight so, they must be executed within the quality control tests of the Mediterranean Fruit Fly SIT programs.

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