Egypt. J. Plant Prot. Res. Inst. (2021), 4 (1): 10 –16

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Preliminary tests for alternative low cost protein sources for the artificial larval diet of Mediterranean fruit flies *Ceratitis capitata* (Diptera: Tephritidae)

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ARTICLE INFO

Article History

Received: 10 / 1 /2021

Accepted: 24 / 3 /2021

Keywords

Fruit fly mass rearing , Mediterranean fruit flies , artificial larval diet ,protein for laboratory rearing , *Ceratitis capitate* and laboratory rearing.

Abstract:

To reduce costs of artificial larval rearing diet of the Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) (Diptera: tephretidae) that used for different purposes, two low costs and locally available replacements for the dried sterile yeast that used as a source of protein, date seeds and poultry feed were used in a preliminary tests , comparing the efficiency of these alternatives (with the traditional yeast based diet) have been executed using quality control tests (Pupal weight, pupal size, emergence, deformity, sex ratio, flight and starvation). Results of the comparison revealed that artificial rearing of medfly on the traditional yeast based diet were very close to diets based on date seeds and poultry feed.

Introduction

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: tephretidae), assumed to be the worst pest in agriculture, causing enormous threats to both production and international trade of fresh horticultural commodities (Klassen *et al.*, 1994 and Hendrichs, 1996), also, causes poverty and malnutrition in developing countries of tropical areas, where climatic conditions are suitable for fruit- and vegetable-based agro-industries (Allwood and Leblanc, 1996 and Allwood, 2000). Laboratory rearing of insects are useful for many purposes such as bioassays, physiological research, rearing of biological control agents, testing of postharvest treatments, SIT programs, etc. (Singh and Ashby, 1985). Low cost but high quality sterile insects is main guarantee for a successful sterile insect technique programs. Proteins (yeast) products are the main nutritional ingredient used in mass-rearing diet for adults and larvae of fruit flies (Schroeder *et al.*, 1972; Cangussu and Zucoloto, 1992, 1997; Placido-Silva *et al.*, 1997; Aluja *et al.*, 2001 and Rohlfs and Hoffmeister 2005).

Deficiency of any of nutritional ingredients will lead to reduction of fecundity and eclosion rates in fruit flies (Kaur and Srivastava, 1991). Drew and Yuval (2000) demonstrated that proteins are crucial for production of pheromones, secretions of male accessory gland, and spermatogenesis. In the nature, flies obtain protein or its precursors by feeding on protein-rich fruit (Such as figs), bird feces, or colonies of bacteria found on leaf surfaces or on decomposing fruit (Hendrichs and Hendrichs, 1990 and Warburg and Yuval, 1997).

Protein-rich nutrition has great potential in promoting male sexual performance but might reduce the ability of males to endure starvation. Chan *et al.* (1990) studied different protein sources in the medfly larval diet such as: casein, corn hydrolyzate, soy protein, soy hydrolyzate, yeast hydrolyzate, lacto albumin hydrolyzate and wheat gluten as replacements for torula yeast. Also, Schroeder *et al.* (1972) studied other protein sources such as, torula yeast type 200, cottonseed protein, soybean protein, Jorgenson yeast combined with whey protein and torula yeast type B and found that only diets formulated with protein from yeast products were adequate nutritionally and physically. Al- Farsi *et al.* (2007) analyzed date seed chemically and reported that it contains protein (2.3–6.4%), fat (5.0–13.2), moisture (3.1–7.1%), phenolics (3102– 4430 mg gallic acid equivalents/ 100 g), antioxidants (580–929 lm trolox equivalents/g) and 22.5–80.2% dietary fiber. Date seeds are potential sources of dietary fiber supplements (McKee and Latner, 2000). Also, other studies of the chemical composition and nutritional quality revealed that date seeds contained crude protein 6.25, 10.4 % crude fat, 22.0 % crude fiber, 1.1 % ash and 60.0 % carbohydrates on dry weight basis (Chatfield and Adams, 1940; Dowson and Aten, 1962 and El-Shurafa *et al.*, 1982).

The aim of the present research paper is to study a preliminary test for alternative low cost protein sources for the artificial larval diet of *C. capitata*.

Materials and methods

1. Fly strain: Mediterranean fruit fly lab strain (Horticultural crops insects Department Laboratories - Plant Pprotection Research Institute).

The adult flies feed normally upon water, sugar and hydrolyzed protein (4:1). Date seeds toasted Grinded and sifted and added to the larval rearing diet replacing the yeast. Date seeds/ Poultry feed replacing the yeast by weight. Poultry feed ingredients: Yellow corn, Soybean meal 48%, sprouted Soybean seeds, Wheat bran, Mono calcium phosphate, Limestone powder, Table salt, A mixture of vitamins and mineral salts, L. lysis Hydrochloride, D.L. Methionin, Sodium bicarbonate and invertase enzyme. Poultry feed protein content 16 %, Crude fat 3.55 % and Crude fiber 2.7 (Table 1).

Table (1): Showed the composition of the Standard (Control), 100 % (Date seeds / Poultry feed), 75% (Date seeds / Poultry feed) and 50 % (Date seeds / Poultry feed).

	Standard (Control)	100 % (Date seeds / Poultry feed)	75% (Date seeds / Poultry feed)	50 % (Date seeds / Poultry feed)
Sugar	**82**	**82**	**82**	**82**
Protein	82 Dried sterile yeast	82 Date seeds/ Poultry feed	61.5 Date seeds/ Poultry feed + 20.5 yeast	41 Date seeds/ Poultry feed + 41 yeast
Wheat bran	330	330	330	330
Sodium benzoate	3	3	3	3
Citric acid	3	3	3	3
Water	500	500	500	500

2. Quality control tests: Experiments details:(IAEA, 2014):

2.1. Weight of pupae:

Record average weights of 10 pupae two days before emergence (Five replicates).

2.2. Size pupae:

Measured volumetrically by counting the number of pupae per one ml using graduated cylinder and record the average size per pupa (Five replicates).

2.3. Emergence percentage:

Calculate number of successfully emerged flies for each 100 pupae and estimate the emergence percentage (Five replicates).

2.4. Ratio of Deformed flies:

Calculate number of flies that failed to emerge , or emerge with deformation for each 100 pupae to estimate the Deformity percentage (Five replicates).

2.5. Sex ratio:

For each group of 100 emerged adult flies, The numbers of males and females are counted and recorded (five replicates).

2.6. Flight ability:

100 pupae for each treatment placed into one PVC cylinder coated internally by talcum powder and put inside a plastic transparent bag with metal frame inside to make it swallowed, on emergence, calculate the flies succeeded to fly and escape the cylinder to be the flier individuals (five replicates).

2.7. Survival under stress:

A number of 100 flies are caged separately (transparent cups with top with a fiber mish top cover to supply aeration) without any feeding material or water and calculate how long the fly can persist alive without any nutrition.

Results and discussion

1. Date seeds:

1.1. Pupal weight:

Results in Table (2) revealed that control and 50 % pupae have the superior weight with mean of 11.12 mg /pupa and 11.56 mg /pupa respectively, but they differ significantly from 75 % and 100 % those came in the second order with mean weights of 8.4 mg/pupa and 7.5 mg /pupa respectively.

1.2. Pupal size:

Table (2) show that there was no significant difference between control pupae and 50 % date diet pupae with mean pupal size of 0.0205 ml /pupa and 0.0202 ml/pupa respectively while they differ significantly from 75 % and 100 % date diets pupae those have means of 0.0175 and 0.0165 ml/pupa respectively.

1.3. Emergence percentage:

The four diets have differed significantly from each other table (2), control diet has the superior emergence percentage with mean of 97 % followed by 50 % date seed diet with mean percentage of 95.2, then 75 % diet with mean of 91.2 and finally 100 % date seed diet as the least emergence percentage mean of 86.2.

1.4. Deformity percentage:

For the deformed adults up on emergence, results in table (2) revealed that there were no significant differences among the four diet, control, 50 % , 75 % and 100 % date seed diets with deformed adult flies means of 0.8 %, 0.4 %, 0.4 % and 0.6 % respectively.

1.5. Sex ratio:

Table (2) data showed that Sex ratios of adult flies results from the four diets came with means of, 48♂ : 52♀, 49♂ : 51♀, 55♂ : 45♀ and 52♂ : 48♀ for control, 50 % , 75 % and 100 % date seed diets respectively.

1.6. Flight ability:

Table (2) show that there was no significant difference between flight ability of the adult flies resulted from 50 % and 75 % date diet pupae with mean percent fliers of 70.8 % and 71.2 % respectively while they differ significantly from control and 100 % date diet pupae those have means of 66 % and 65.6 respectively.

1.7. Survival under Stress (Starvation):

The four diets have differed significantly among each other table

(2), 75 % date seed diet has the longest life span (life span without feeding as an indicator to the ability of the fly that survive under stress of food absence), with mean of 118 hour/adult fly, followed by 100 % date seed diet with mean life span of 112.6 hour/fly, then control diet with mean of 107.6 hour/fly and finally 50 % date seed diet as the least life span with mean of 102.7 hour/fly.

Table (2) : Pupal weight, size, emergence, deformity, sex ratio, flight and starvation of medfly reared on standard, 50, 75 and 100 % date seed diets.

Treatment parameter	Control	50 %	75 %	100 %
Pupal weight	11.12 ± 0.053 a1	11.56 ± 0.024 a1	8.4 ± 0.122 b1	7.5 ± 0.049 b1
Pupal size	0.0205 ± 0.0002 a2	0.0202 ± 0.0002 a2	0.0175 ± 0.0003 b2	0.0165 ± 0.0004 b2
Emergence	97 ± 0.316 a3	95.2 ± 0.374 b3	91.2 ± 0.374 c3	86.2 ± 0.374 d3
Deformity	0.8 ± 0.374 a4	0.4 ± 0.245 a4	0.4 ± 0.245 a4	0.6 ± 0.245 a4
Sex ratio	48♂ : 52♀	49♂ : 51♀	55♂ : 45♀	52♂ : 48♀
Flight	66 ± 0.316 b5	70.8 ± 0.374 a5	71.2 ± 0.374 a5	65.6 ± 0.4 b5
Starvation	107.6 ± 0.510 c6	102.7 ± 0.422 d6	118 ± 0.286 a6	112.6 ± 0.504 b6

2. Poultry feed :

2.1. Pupal weight:

Results in Table (3) revealed that control has the superior weight with mean of 11.12 mg /pupa and differ significantly from the rest of the diets, followed by both 50 % and 100 % Poultry feed diet those have mean pupal weight of 9.24and 9.0 mg /pupa respectively, but they differ significantly from 75 % which came with least mean weight of 8.52 mg/ y.

2.2. Pupal size:

Table (3) show that control pupae has the superior size with mean of 0.205 ml / pupa and differ significantly from the rest diets, followed by 50 % diet that has a mean of 0.193 ml/pupa , also, they differ significantly from the rest diets, finally, 75 % and 100 % there was no significant difference between control pupae and 50 % date diet pupae with mean pupal size of 0.0205 ml /pupa and 0.0202 ml/pupa respectively while they differ significantly from 75 % and 100 % Poultry feed diet pupae have no significant differencs between them and have a mean pupal size of 0.0185 and 0.0189 ml/pupa respectively.

2.3. Emergence percentage:

Data in Table (3) show that control pupae has the largest percentage of emergence with mean of 96.45 % and differ significantly from the rest diets, followed by 50 % and 100 % diets with a mean emergence percentage of 95.05 % and 95.25 % ,with no significant difference between them, while 75 % came with the least percentage (mean of 90.9 %).

2.4. Deformity percentage:

Results in Table (3) revealed that there were no significant differences among the four diet, control, 50 % , 75 % and 100 % Poultry feed diets with deformed adult flies means of 0.6 %, 0.8 %, 0.5 % and 0.6 % respectively.

2.5. Sex ratio :

Table (3) data showed that Sex ratios of adult flies results from the four diets came with means of, 48♂ : 52♀, 52♂ : 48♀, 44♂ : 56♀ and 51♂ : 49♀for control, 50 % , 75 % and 100 % Poultry feed diets respectively.

2.6. Flight ability:

Table (3) show that there were a significant difference among the flight ability of the adult flies resulted from the four diets, 100 % diet flies came as the first rank with mean of 74.6 %, followed by 75 % poultry feed diet fliers with mean of 72.15 % then 50 % diet with mean of 68.25 % and finally, control diet with mean flier percentage of 66.4 %.

2.7. Survival under Stress (Starvation):

The four diets have differed significantly among each other Table (3), 50 % poultry feed diet has the longest life span, with mean of 112.2 hour/adult fly, followed by controldiet diet with mean life span of 107.6 hour/fly, then 75 % diet with mean of 93.2 hour/fly and finally 100 % poultry feed diet as the shortest life span with mean of 85.12 hour/fly.

Table (3) : Pupal weight, size, emergence, deformity, sex ratio, flight and starvation of medfly reared on standard, 50, 75 and 100 % poultry feed diets.

Treatment parameter	Control	50 %	75 %	100 %
Pupal weight	11.12 ± 0.583 a1	9.24 ± 0.510 b1	8.52 ± 0.128 c1	9.0 ± 0.054 b1
Pupal size	0.205 ± 0.000 a2	0.193 ± 0.001 b2	0.0185 ± 0.001 c2	0.0189 ± 0.001 bc2
Emergence	96.45 ± 0.278 a3	95.05 ± 0.320 b3	90.9 ± 0.332 c3	95.25 ± 0.194 b3
Deformity	0.6 ± 0.245 a4	0.8 ± 0.374 a4	0.5 ± 0.289 a4	0.6 ± 0.4 a4
Sex ratio	48♂ : 52♀	52♂ : 48♀	44♂ : 56♀	51♂ : 49♀
Flight	66.4 ± 0.510 d5	68.25 ± 0.403 c5	72.15 ± 0.384 b5	74.6 ± 0.510 a5
Starvation	107.6 ± 0.510 b6	112.2 ± 0.374 a6	93.2 ± 0.564 c6	85.12 ± 0.393 d6

Results of quality control tests (Pupal weight, pupal size, emergence, deformity, sex ratio, flight and starvation - for both of the Mediterranean and Peach fruit flies) revealed that traditional wheat bran based artificial larval diet was very close to the new diets based on date seeds and poultry feed (Used as an alternative sources for proteins) in spite of the significant differences that showed through the statistical analysis of the results but these differences will compensated after successive generations reared upon the same new diets (Kamikado *et al*., 1987; Souza *et al*., 1988 and Economopoulos, 1992) whose demonstrated that adaptation of the fruit flies to the laboratory artificial diet for colonization need several generations.

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