

Evaluation of liquid artificial larval diets for mass rearing of *Bactrocera cucurbitae* (Coq.)

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As a step towards increase production efficiency and quality over the storage, handling, and waste challenges of bulking agents in traditionally based 'solid' diets, a liquid diet with synthetic sponge cloths has been developed for mass rearing of Melon fly, *Bactrocera cucurbitae* (Coq.) under laboratory condition. Several quantities of Brewer's yeast (8.62, 11.51, 14.38 and 17.25%), and sugar (4.47, 5.96, 7.45 and 8.94%) were tested in liquid larval medium with or without wheat germ oil (WGO). The diets were assessed in terms of pupal yield (%), larval duration (days), pupal weight (gm), percent of adult emergence, active fliers, sex ratio, fecundity, and fertility. Among the different diets tested, larval rearing of *B. cucurbitae* on a liquid diet (LD-I) with 14.2g of brewer's yeast, 7.35g of sugar and wheat germ oil found most suitable. This diet resulted in significantly higher pupal weight with maximum adult emergence (81.00%) and fliers (85.58%). The addition of wheat germ oil to liquid diet increase fertility by 39% and egg hatching by 15%, over the control diet. The diet supplemented with linolenic acid (LD-II) resulted in the 68.85% pupal recovery with 78.33% adult emergence and 81.68% fliers. Although, pupal recovery and weight increased with the increase of brewer's yeast but resulted in the low adult emergence and poor fliers. In the present study, the quality parameters of melon fly reared in LD-I was found close to the recommended pre-irradiation standards of FAO/IAEA/USDA for sterilization of male flies.

Keywords: Brewer's yeast, Fruit-based diet, Liquid larval diet, Melon fly, WGO

India's diverse climate ensures availability of all varieties of fresh fruits and vegetables throughout the year. The country ranks second in fruits and vegetable production in the world. The area under cultivation of fruits and vegetables is 6.40 and 9.57 Mha with annual production of 91.44 and 166.08 MT, respectively. In India, the area under cultivation of cucurbits increased from 0.29 Mha in 2014-15 to 0.37 Mha in 2015-16 with the production of 5.85 MT in 2015-16 compared to 5.0 MT in 2014-15¹. Several factors constrain production; most important is tephritid fruit flies as they cause 90-100% yield loss depending on fruit fly population, locality, and season². It is with reference to various publications and reports; the extent of losses varies between 30 to 100% depending on cucurbit and season³. In addition to the direct losses, its economic impacts result in the loss of export markets as well as the costly requirement of quarantine restrictions and eradication measures⁴.

The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) attacks 61 plant species belonging to 19 different families. Twenty-eight of them are cucurbits and remaining are non-cucurbit hosts belongs to the families, Anacardiaceae, Malvaceae, Moraceae, Capparaceae, Capricaceae, Rutaceae, Rosaceae, Solanaceae, Agavaceae, Sapotaceae, Pandanaceae, Passifloraceae, Lauraceae, Fabaceae, Myrtaceae, Loganiaceae, Vitaceae, Rhamnaceae and geographically distributed throughout the tropics and subtropics of the world, particularly in Southeast Asia⁵. These cucurbits are subjected to damage by melon fly right from the primordial stages of the crop up to harvest⁶. It damages in many ways: i) ovipositoinal injury by the female on fruits and vegetative parts leading to black or brown lesions, ii) internal feeding on inflorescence, roots, leaves and stems, iii) larval feeding damage on ovaries and fruit pulp, and iv) decomposition of fly-damaged fruit tissue by invading saprophytic microorganisms⁷.

Management of tephritids is difficult, they being polyphagous pests with high reproductive potential, wide host range, adaptability to climate and overlapping of generations. Chemical-free approach

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Diets for the mass rearing of tephritid fruit flies have traditionally been based on bulking agents, such as lucerne, bran or carrot, which make up the majority of the larval medium⁸. To overcome storage, handling and waste challenges of such 'solid' diets, liquid diets have been developed which can be placed on reusable inert substrates replacing bulking agents to support the developing larvae⁹. Liquid diets and associated rearing systems are efficient, reliable and economical replacements for the current solid diets. The present study was therefore carried out to evaluate different formulations of liquid larval diet for mass rearing of melon fly and compared with fruit-based diet under controlled laboratory conditions. The efficacy of diets was measured based on pupal recovery (%), larval duration (days), pupal weight (gm), adult emergence (%), active fliers (%), fecundity (eggs/female/day) and egg hatch (%).

expression and fatty acid composition in fruit flies¹³. Another fatty acid such as a linolenic acid is also known to be required for larval growth and wing development in Lepidoptera¹⁴. Hence optimizing the lipid components of culture media is essential for mass rearing of melon fly. In the present study in order to improve fruit fly performance in a liquid diet, four concentrations of brewer's yeast (8.62, 11.51, 14.38 and 17.25%) and four concentrations of sugar (4.47, 5.96, 7.45 and 8.94%) were evaluated with or without 0.15% WGO and linolenic acid.

The studies on the standardization of larval diets for mass rearing of melon fly, *B. cucurbitae* was conducted at Division of Entomology, Indian Agricultural Research Institute, New Delhi during 2016.

Standard Liquid diet composed of brewer's yeast, sugar, antimicrobial agents, citric acid, and water developed by Chang *et al.*¹⁵, was taken as a base for developing effective modified liquid larval medium and it was also tested as the control diet. The different quantity of brewer's yeast (10.65, 14.2, 17.77 and 21.3 g) and sugar (5.5, 7.15, 9.18, 11.02 g) (Table 1) were tested. For testing PUFAs, wheat germ oil and linolenic acid were added. A fruit-based diet composed of pumpkin fruit, yeast extract and antimicrobial agents (methyl paraben and sorbic acid) was also tested. All the diets were compared with the rearing of melon fly larvae on natural fruit. Larval rearing was carried out at laboratory conditions of 26°C, 65% RH and a photoperiod of 12:12 (L:D) h.

Liquid diet rearing system was made up of stainless steel tray ($25 \times 20 \times 2.5 \text{ cm}^3$), screen ($23 \text{ cm} \times 18 \text{ cm}$;

[illegible][illegible]

0.1 cm mesh) layered on the floor of larval rearing tray and sponge cloth (24 cm × 19 cm) as primary support matrix for feeding larvae. Sponge material was placed on top of the screen by leaving 0.5 cm space away from four sides of larval rearing tray (Fig. 1). Thoroughly blended liquid diet was poured over the sponge cloth. 500 eggs were inoculated on a small piece of sponge cloth in the rearing trays containing large sponge cloth saturated with 250 mL of liquid medium. The hatched larvae were allowed to feed *ad libitum* (Fig. 2A).

In fruit based rearing system 1000 eggs were directly seeded on 500gm fruit based diet placed in stainless steel trays. This rearing system was devoid of the net screen and sponge cloth (Fig. 2B). Upon egg hatching, larvae fed on the respective diet by making galleries. Matured larvae started popping out of the different diet trays for pupation in the sand provided at the bottom of the larval rearing container.

In natural fruit rearing system, small pieces (250 g) of fully ripened pumpkin (*Cucurbita moschata*) were inoculated with 500 eggs. Inoculated pumpkin fruit pieces were placed in a container with a perforated lid. The sterilized sand at its bottom was given as pupation medium (Fig. 2C).

Recording of biological parameters

Total pupal yield was worked out by dividing the total number of pupae produced by initial numbers of eggs seeded on the diet. Pupae recovered from different diets were weighed 2 days after collection and expressed as a pupal mass in gram/100 pupae. Three lots of 100 pupae were randomly selected from the second day pupal collection and each lot was placed in a petri dish



Fig. 1 — Rearing of melon fly larvae on a liquid diet. (A) Liquid larval diet rearing system, (B) Sponge material saturated with a liquid diet, and (C) Eggs inoculated on small sponge strip

cover. A tube of black plastic polyvinyl chloride pipe (20 cm in length and 8.5 cm in diameter) coated with talcum powder was placed over the dish in an adult rearing cage without food or water. Observations were recorded daily for all of the flies emerged and flies which could not cross the tube. To minimise fly back, flies that escaped from the tube were removed daily. When emergence was ceased, the remaining flies inside the tubes were counted. Adult emergence was calculated by dividing the number of flies emerged from an initial number of pupae. Adult flying ability was calculated by the number of flies that flew through a pipe out of the total number of emerged flies. Sex ratio was expressed as a number of females to males.

For recording the fecundity, twenty pairs of freshly emerged male and female adult flies of uniform age were confined to ovipositional cages, provided with a mixture of sugar and yeast hydrolysate (3:1) and water soaked cotton swabs. After the pre-oviposition period of 14 days, Petri dishes containing semi-solid fruit substrate covered with paraffin membrane were provided as eggging devices for collection of eggs. Egg production was recorded for each cage for seven consecutive days. Fecundity was expressed as a number of eggs/female/day. For observing the fertility, the eggs collected on the first day were used. Three sets of 100 eggs from each individual cage were sampled. The numbers of unhatched eggs were recorded 4 days later.

Statistical analysis

Data in Table 2 were presented as a mean ± standard error (SE). Values were obtained from an average of three replications for each treatment. Differences among the diets were determined by analysis of variance (ANOVA), and means were

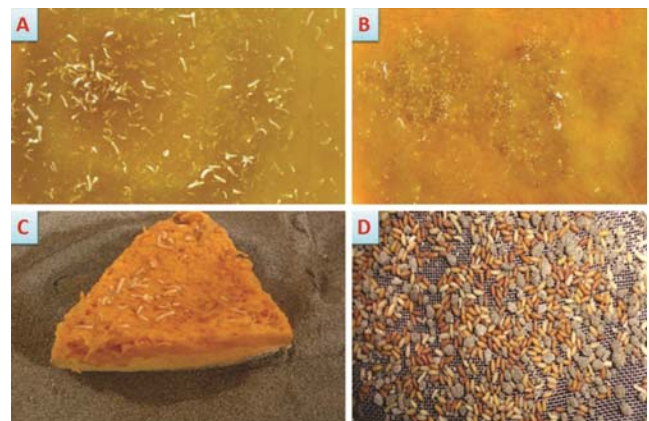


Fig. 2 — Melon fly larvae reared on (A) Liquid diet, (B) Fruit-based diet, (C) Natural fruit, and (D) Pupae collected from diets

Table 2 — Comparison of quality parameters of melon fly, *Bactrocera cucurbitae* reared on liquid diets, fruit-based diet, and natural fruit

Parameter	LD-C (Control)	LD-I	LD-II	LD-III	LD-IV	LD-V	LD-VI	LD-VII	LD-VIII	Fruit-based diet	Natural fruit	ANOVA
P R (%)	66.82 ^{bcd} (42.02±3.55)	69.50 ^{bc} (44.02±1.39)	68.85 ^{bcd} (43.49±0.68)	58.22 ^f (35.59±1.10)	71.03 ^b (45.25±0.93)	58.96 ^{ef} (36.13±1.96)	61.00 ^{def} (37.58±1.33)	62.57 ^{cdef} (38.72±0.50)	63.00 ^{bcd} (39.03±0.51)	83.20 ^a (56.34±1.51)	68.00 ^{bcd} (42.85±1.73)	F=21.48;df=10,22; P< 0.0001
L D (days)	8.33 ^{ab} (8.33±0.33)	8.00 ^b (8.00±0.00)	8.33 ^{ab} (8.33±0.33)	8.50 ^{ab} (8.50±0.29)	8.00 ^b (8.00±0.00)	8.00 ^b (8.00±0.00)	8.67 ^{ab} (8.67±0.33)	8.50 ^{ab} (8.50±0.29)	8.50 ^{ab} (8.50±0.29)	9.33 ^{ab} (9.33±0.33)	9.67 ^a (9.67±0.33)	F=3.93;df=10,22; P= 0.0036
P W (g)	1.32 ^d (1.32±0.02)	1.50 ^a (1.50±0.01)	1.41 ^b (1.41±0.01)	1.22 ^c (1.22±0.02)	1.51 ^a (1.51±0.00)	1.54 ^a (1.54±0.01)	1.30 ^{de} (1.30±0.02)	1.32 ^d (1.32±0.02)	1.34 ^{bcd} (1.34±0.02)	1.34 ^{bcd} (1.34±0.02)	0.91 ^f (0.91±0.03)	F=106.82;df=10,22; P< 0.0001
A E (%)	77.00 ^{ab} (50.51±3.21)	81.00 ^a (54.23±2.52)	78.33 ^{ab} (51.56±0.88)	70.67 ^{bcd} (44.97±1.45)	78.00 ^{ab} (51.25±1.00)	76.67 ^{ab} (50.07±1.45)	71.67 ^{bcd} (45.77±1.20)	74.00 ^{abc} (47.72±1.00)	73.00 ^{abc} (46.87±0.58)	62.67 ^d (38.79±0.88)	66.00 ^{cd} (41.31±1.73)	F=9.95;df=10,22; P< 0.0001
S R (f/m)	1.16 ^a (1.16±0.05)	1.09 ^a (1.09±0.04)	1.13 ^a (1.13±0.02)	1.14 ^a (1.13±0.01)	1.06 ^a (1.06±0.02)	1.04 ^a (1.04±0.04)	1.02 ^a (1.02±0.08)	1.10 ^a (1.10±0.01)	1.11 ^a (1.11±0.02)	1.01 ^a (1.00±0.04)	1.08 ^a (1.08±0.03)	F=1.82;df=10,22; P= 0.1162
A F (%)	80.03 ^{bc} (53.17±1.27)	85.58 ^a (58.86±1.07)	81.68 ^{ab} (54.78±1.23)	73.05 ^d (46.93±1.38)	77.33 ^{bcd} (50.65±1.20)	76.53 ^{bcd} (49.91±0.32)	76.33 ^{bcd} (49.77±1.45)	76.77 ^{bcd} (50.14±0.91)	75.99 ^{cd} (49.44±0.84)	54.33 ^c (32.90±1.20)	53.67 ^c (32.45±1.45)	F=65.76;df=10,22; P< 0.0001
Fecundity (e/f/d)	22.92 ^{bc} (1.36±0.11)	32.01 ^a (1.50±0.04)	26.39 ^b (1.42±0.32)	20.24 ^{cd} (1.30±0.29)	33.26 ^a (1.52±0.43)	35.15 ^a (1.54±0.04)	21.53 ^c (1.33±0.34)	21.89 ^c (1.34±0.56)	21.79 ^c (1.33±0.88)	17.33 ^{de} (1.23±1.45)	16.67 ^c (1.21±1.20)	F=47.68;df=10,22; P< 0.0001
Egg hatch (%)	69.00 ^{cde} (43.63±1.53)	78.67 ^a (51.88±1.20)	74.33 ^{abc} (48.02±1.33)	73.00 ^{bcd} (46.86±0.00)	78.00 ^{ab} (51.24±0.58)	76.67 ^{ab} (50.04±0.88)	67.33 ^c (42.32±1.45)	67.00 ^c (42.06±1.15)	68.00 ^{de} (42.83±0.58)	68.67 ^{bc} (43.35±0.88)	55.67 ^f (33.82±1.20)	F=35.80;df=10,22; P< 0.0001

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values.

PR= pupal recovery; LD= larval duration; PW= pupal weight; AE= adult emergence; SR= sex ratio; AF= adult fliers; e/f/d = eggs/female/day

separated using Tukey's test at $\alpha = 0.05$ (SAS, version. 9.4, available at ICAR-IASRI, New Delhi, India).

Results

Quality parameters such as pupal recovery, larval duration, pupal weight, adult emergence, adult flying ability, fecundity and egg hatchability on a standard liquid diet (control), 8 modified liquid diets, fruit-based diet, and natural fruit shown in Table 2.

Pupal recovery

The substantial difference among the diets in pupal recovery was recorded, being highest in the fruit-based diet (83.2%), followed by LD-IV (71.03%) and LD-I (69.50%). Larvae reared on LD-II, natural fruit and LD-C resulted in a pupal yield of 68.85, 68.00 and 66.82%, respectively. Pupal recovery was in the range of 61.00-63.00% in LD-VI, LD-VII, and LD-VIII.

Larval duration

The duration from egg seeding until pupation was considered as a larval developmental period. It was significantly longer on natural fruit (9.67 days) and fruit-based diet (9.33 days) compared to liquid diets. No significant difference in the larval developmental period was observed in liquid diets as it ranged from 8.00 to 8.67 days.

Pupal weight

Mean weight of 100 pupae was significantly higher and at par in LD-V (1.54 g), LD-IV (1.51 g) and LD-I (1.50 g) followed by LD-II (1.41 g). In remaining

diets, mean pupal weight was not significantly different and ranged between 1.30-1.34 g except LD-III and natural fruit, where pupae were lighter in weight (1.22 and 0.91 g).

Adult emergence with active fliers

Liquid diet with WGO (LD-I) performed best among all the diets tested, as 81.0 percent adults emerged, with the highest percentage of fliers (85.58%). The adult emergence in LD-II, LD-IV, LD-C, and LD-V were on par as the emergence recorded was 78.33, 78.00, 77.00 and 76.67 percent with flight ability of 81.68, 77.33, 80.03 and 76.53%, respectively. The diets, LD-III, LD-VI, LD-VII and LD-VIII recorded adult emergence and adult fliers in the range of 70.67-74.00 and 73.05-76.77 percent, respectively. Adult emergence was low in natural fruit (66.00%) and fruit-based diet (62.67%) with less number of adult fliers (53.67 and 54.33%).

Sex ratio

There was no significant difference in mean sex ratio (females to males) for all the diets tested. However, females were in greater proportion (1.16) in control diet (LD-C) in comparison to the remaining diets. In LD-VI and fruit-based diet, the sex ratio of nearly 1:1 was observed.

Fecundity and Fertility

Egg production was significantly higher in LD-V (35.15), LD-IV (33.26) and LD-I (32.01) compare to control diet (22.92). The next best diet was LD-II that

recorded egg production of 26.39 eggs/female/day. Lowest fecundity was observed in fruit-based diet and natural fruit with 17.33 and 16.67 eggs/female/day, respectively. In the diets LD-III, LD-VI, LD-VII, and LD-VIII, the egg production was in the range of 20.24-21.89 eggs/female/day. Percentage egg hatching of F1 generation was highest in LD-I (78.67%) followed by LD-IV (78.00%), LD-V (76.67%) and LD-II (74.33%) and was not significantly different. Lowest egg hatching was recorded in natural fruit (55.67%). In the remaining diets, the percentage of egg hatching ranged from 67-68.67%.

Discussion

Impact on developmental period, growth, and survival of the larvae affecting the quality of flies produced in different diets was observed. The most noticeable parameters were the pupal recovery and weight. Pupal recovery increased with the increase of brewer's yeast in LD-IV but its further increase in LD-V did not increase the pupal recovery. Although a slight increase in pupal weight was recorded in LD-V as compared to LD-IV, adult emergence and adult fliers reduced, may be expressing the fact that excessive amount of protein is detrimental to the insect. The diets LD-IV and LD-V were not as suitable for the melon fly as LD-I, in particular owing to the low adult emergence and poor flight performance. Chang *et al.*¹⁶, have also shown that larvae of a med fly, *Ceratitis capitata* (Wiedemann) failed to develop in diets containing a high percentage of protein. Pupae formed from larvae reared on a diet with WGO (LD-I) was heavier compared to control. Pupal weight is considered a key quality parameter as it is a valuable indicator of the overall viability of pupae¹⁷ and associated with the adult emergence and flight propensity in the mass rearing of tephritid fruit flies for sterile insect technique^{18,19}. In liquid diets, larval duration was reduced by nearly two days compared to natural fruit and fruit-based diet. In fruit-based diet, mean pupal weight was also below the standard acceptable weight which should range between 1.4-1.5g/100 pupae (FAO/IAEA/USDA 2014). Similar results were found by previous workers that diets produced more pupae resulted in pupae with lower weight²⁰. Dominiak *et al.*²¹, reported a positive significant relationship between pupal weight and adult emergence. In their further study, they found that fly emergence was not affected up to 15 mg/pupae²² and beyond which there is no further increase in quality of insect. Consistent with this

interpretation, Meza *et al.*²³, reported that once larvae of *Anastrepha ludens* (Loew) reached a critical weight, adult performance depends more on genetic quality than on size. Collins *et al.*²⁴, reported that heavier pupae may not emerge successfully if they are long and thin. Larva of *Bactrocera invadens* reared on a liquid diet with WGO performed well than those reared on the carrot-based solid diet²⁵. Shinwari *et al.*²⁶, found that artificial diet with protein hydrolysate and brewers' yeast resulted in a highest pupal recovery in *Bactrocera zonata* (Saunders). Vera *et al.*²⁷, evaluated various supports, sugar contents and protein proportions in the larval diet for the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) and found high pupal recovery in sponge cloth based liquid diet.

The addition of WGO to liquid diet increased the percentage of adult emergence as well as adult fliers. The influence of WGO in insect nutrition was first reported by Fraenkel & Blewett²⁸. They explained that saponifiable fraction (linoleic acid) of wheat germ oil is necessary for emergence, growth effect, and good wing scales, and the unsaponifiable fraction (vitamin E) is necessary for proper growth. Kahlon²⁹ studied the nutritional composition of wheat and oat kernel oil; fatty acid composition of WGO include 42-59% linolenic acid, 12-28% oleic acid, 11-19% palmitic acid, 2-11% α -linolenic acid, and stearic acid 1%, and 0.14% of vitamin E. In the present study also diet with 14.2 g of brewer's yeast and 0.2 mL of WGO (LD-I) gave high adult emergence and adult fliers. The improving effects of WGO on insect performance derive from its high content of nutritionally essential and non-essential fatty acids, and from physiologically active tocopherols. It is also reported that polyunsaturated fatty acids, including oleic acid, linoleic acid, alpha-linolenic acid and gamma-linolenic acid are essential nutrients for many insect species¹². The larvae of *Ephestia kuehniella*, *E. elutella* and *E. cautella* grow well on artificial diets that contain wheat germ oil. In the absence of wheat germ oil growth is slow, mortality is high, and moths fail to emerge from the pupae²⁸. Incorporation of WGO into larval diets of the house cricket, *Acheta domesticus* (L.), has improved survival and growth³⁰. Diet containing high fat showed a significant rise in serum and hepatic total cholesterol and triglyceride³¹. The recommended specifications (FAO/IAEA/USDA)³² of adult emergence and adult flight ability for *Bactrocera cucurbitae* produced for SIT programmes is 80-90% and 75-80%, respectively.

Sex ratio was skewed in favour of females in all liquid diets except LD-VI in which the sugar was reduced. It was also possible that some larval diets provide better nutrients for metamorphosis of one sex compared to the other. Hence females are more in ratio and larger than males³³. Egg production by females from WGO-fortified liquid diet was more than those from WGO-free control diet. Percentage of egg hatch also increased with the addition of WGO to the diet. Chang & Vargas¹¹ have also noted that as brewer's yeast contains a small proportion of fatty acids and the addition of wheat germ oil (WGO) to the standard liquid diet formulation increased fly fertility in oriental fruit fly, *B. dorsalis*. Chang *et al.*³⁴, generated the hypothesis that mode of WGO action in insect development is through its influence on gene expression and they reported that the presence of WGO in larval diet substantially alters expression of genes encoding a range of proteins in the corresponding adults. WGO led to increased expression of genes encoding cuticular and musculature proteins in males and females in *B. dorsalis*. These include chitin-binding proteins and the muscle contraction protein tropomyosin. In males, WGO supplementation led to increased expression of genes for cell protection proteins, specifically glutathione reductase and thioredoxin reductase. Naghii *et al.*³⁵, found that consumption of diet consisting of fatty acids, Vit-D, calcium, and boron appeared to have beneficial effect on physical activity and corresponding metabolic hormones. Addition of omega-3 PUFA (α -linolenic acid) rich components in the diet may counter the adverse effect of oxidative stress in biological systems³⁶.

In the present study, the quality parameters of melon fly performed with varied quantities of brewer's yeast as a protein source indicates that, diets with 11.51% of brewer's yeast and 0.15% of WGO (LD-I) and 14.38% of brewer's yeast (LD-IV) were at par except for the production of active adult fliers. For the diets with brewer's yeast concentration higher than 14.38% (i.e. LD-V) and lower than 11.51% (i.e. LD-III) had a negative impact, as the quality parameters of melon fly such as pupal recovery, adult emergence, adult fliers, and fertility were decreased. It indicates that 11.51 and 14.38% of brewer's yeast in the liquid larval medium was sufficient and optimal for larvae to develop. Brewer's yeast quantities between 14.2 g and 17.77 g in 100 mL of liquid larval diet can be considered good for the rearing of melon

fly larvae in the laboratory. The addition of 0.15% of WGO to the liquid diet significantly improved pupal recovery, pupal weight, the percentage of adult emergence, adults capable of flying, egg production and percentage of egg hatch when compare to the liquid diet without wheat germ oil (control). The results recorded with LD-I in agreement with the findings of Chang & Vargas¹¹, Chang *et al.*³⁴, and Chang *et al.*¹⁰, as they have reported that WGO in larval-rearing media increased pupal recovery, percentage fliers, egg production and egg hatch in *B. dorsalis* and *C. capitata*. Hence, this study demonstrated the potential for the rearing of melon fly using liquid diet supplemented with wheat germ oil similar to those that have been adopted in small and large-scale mass rearing of other tephritids. We also conclude that when compared with recommended standards of FAO/IAEA/USDA³².

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