



STUDIES ON LIFE HISTORY PARAMETERS OF MELON FRUIT FLY, *Zeugodacus cucurbitae* (Coquillett) REARED ON FRUIT-BASED DIETS

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ABSTRACT

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Maggots of melon fruit fly, *Zeugodacus cucurbitae* were mass reared on seven semi-synthetic fruit based diets. Biological parameters of melon fruit fly were recorded and compared with control diet to develop an effective semi-synthetic fruit based diet for mass production of pupae in support of sterile insect technique. Seven semi-synthetic diets with different quantities of protein powder (7.5, 10.0, 15.0 and 20.0 g) and sucrose (7.0 and 10.0 g) were formulated. Among the seven diets evaluated, semi-synthetic fruit based diet-IV (SSFD-IV) composed of pumpkin fruit (1000 g), yeast extract (7.5 g), brewer's yeast (7.5 g), Sucrose (7.0 g) and 2.0 ml of wheat germ oil was found as the most appropriate diet with significantly higher pupal recovery (77.8%), pupal weight (1.61 g), adult emergence (80.2%), active fliers (75.87%), sex ratio (1.23) fecundity (28.0 eggs/female) and fertility (71.81%). The diets, SSFD-III and SSFD-VII are the next best diets for mass rearing of melon fly.

KEYWORDS: Biological parameters, Melon fruit fly, Pumpkin fruit, Semi-synthetic diet, Yeast extract

INTRODUCTION

Melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) is considered as the foremost important fruit fly that attacks 61 plant species belonging to 19 different families, of which twenty-eight of them are cucurbits and remaining are non-cucurbit hosts (De Meyer *et al.*, 2015). The pest damages the crop by means of ovipositional injury, larval feeding on ovaries, fruit pulp, and rotting of fly-damaged fruits (Viraktamath *et al.*, 2003). Both major and minor cucurbits are being cultivated in India and sharing about 7.0 per cent of the total vegetable production (Anonymous, 2020). Melon fruit fly damages over 60 per cent of cucurbit crops in India (Kapoor, 2005) and the extent of losses vary from 30 to 100 per cent (Shooker *et al.*, 2007).

Mass production of insects on improved diets provide large supply of insects for various studies in methods of pest management such as release of parasitoids, predators and sexually sterile insects *etc.*, (Cohen, 2015). Hence, majority of pest management strategies largely rely on establishment of effective mass rearing methods. The Laboratory rearing of insects on solid based diets may results in undesirable changes in insect biology, behavior and physiology which could undermine the overall viability of adult insects. Hence, there is a need to develop suitable diet for mass rearing of insect without compromising with quality of insects.

In the present study, pumpkin fruit was used as natural fruit substrate, to which different sources of protein, fatty acid, and carbohydrate were fortified at different concentrations to develop a suitable semi-synthetic fruit based diet for mass rearing of melon fly. The performance of these diets were assessed by recording biological parameters of melon fly such as larval duration, pupal recovery, pupal weight, adult emergence, adult flying ability, sex ratio, fecundity egg hatch and were compared with diet-I (Control).

MATERIAL AND METHODS

The studies on dietary effect of semi-synthetic fruit-based diets on biology of melon fruit fly, *Zeugodacus cucurbitae* was conducted during 2020-21 at Insectary, Department of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh. Diets were evaluated based on pupal recovery, larval duration, pupal weight, adult emergence, active fliers, fecundity and egg hatch.

Raising and maintenance of melon fruit fly culture

Melon fly culture was raised by collecting infested fruits from farmers' field and horticulture garden of S.V. Agricultural College, Tirupati. Infested bitter melon fruits were brought to the laboratory and were kept at controlled conditions (25-27°C, 65-75% RH) in 8"×6" glass jars provided with 5 cm thick sterilized sand for pupation. The fully-grown larvae pop out from the fruit for pupation

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into soil. Male and female pupae were collected and transferred to adult rearing cages (30×30×30cm) provided with a mixture of sugar and yeast hydrolysate (3:1) as adult food and water-soaked cotton swabs in 100 ml conical flask as source of water. Male and female pupae were differentiated by observing the colour, size, and shape of pupa. Male pupae were dark brown, round, spherical with blunt tip at posterior region. Whereas, female pupae were light brown in colour, elongated with tapering or pointed at posterior end and larger in size than male pupa. After pre-oviposition period of 12 days, equal number of males and females of same age were confined to ovipositional cages. Petri dishes containing semi solid pumpkin fruit substrate covered by a thin parafilm were provided as ovipositional substrate to the females to collect the eggs required for the experiment. Cages were cleaned and replaced with adult food and water as and when necessary.

Diet formulation and rearing system

The diets were formulated using pumpkin fruit (*Cucurbita moschata*), protein source (yeast extract and brewer's yeast), fatty acid (wheat germ oil), sucrose and antimicrobial agents (sodium benzoate and methyl paraben) as basic ingredients obtained from HiMedia Laboratories. Seven SSFDs were formed with different quantities of protein (7.5, 10.0, 15.0 & 20.0 g), sucrose (7.0 & 10.0 g) and wheat germ oil (2.0 ml) as given in Table 1. The effectiveness of all the diets were assessed by recording the life history parameters of melon fly reared as larvae and compared with artificial liquid diet (Panduranga *et al.*, 2018). Sliced pieces of well refined pumpkin fruit (*Cucurbita moschata*) were grinded in an electric mixture. Remaining ingredients were added into semi-solid pumpkin fruit substrate and the entire mixture was again blended till it forms homogeneous mixture. Ingredients of all the seven semi-synthetic fruit based diets are given in the table 1. Collected eggs were inoculated at the rate of 500 eggs per 250 g of SSFD in a stainless-steel tray (25×20×2.5 cm). Diet trays inoculated with eggs were kept in plastic container (60×40×7.5 cm) provided with 5-6 cm thickness of sterilized sand. These containers were covered with black coloured muslin cloth secured by rubber band. Each diet was replicated thrice.

Recording of biological parameters

Larval duration: Larval duration (days) was arrived based on the period between egg hatch and initiation of pop out of maggots from each larval diet.

Pupal recovery: Pupal recovery is the number of pupae produced out of total number of eggs inoculated.

Pupal recovery (%) =

$$\frac{\text{Number of pupae harvested}}{\text{Initial number of eggs inoculated}} \times 100$$

Pupal weight: For each larval diet, pupal weight was measured by taking the weight of 100 pupae (2-days old) from daily collection and expressed as gram/100 pupae.

Adult emergence: 100 pupae from each diet were taken in glass Petri dish and were kept in adult rearing cage provided with adult food and water. After complete ceasing of adult emergence, number of adults emerged out of 100 pupae were worked out.

Adult emergence (%) =

$$\frac{\text{Number of adult emerged}}{\text{Initial number of pupae kept for test}} \times 100$$

Adult flying ability: Sample of 100 pupae from each diet were randomly selected and placed in a glass Petri dish. A tube of black PVC pipe (20 cm in length and 8.5 cm in diameter) coated with talcum powder was placed over the Petri dish. An entire set up was kept in adult rearing cages for fly emergence. The flies emerged and flew out of PVC pipe were recorded daily. When emergence was ceased, the remaining flies inside the tubes were counted and adult flying ability was calculated by the number of flies that flew through pipe out of the total number of pupae kept for adult emergence.

Adult flying ability (%) =

$$\frac{\text{No. of adult flew out of 20 cm height pipe}}{\text{Total No. of pupa kept for emergence}} \times 100$$

Sex ratio: It is the ratio of number of females to number of males emerged out of pupae kept for emergence test.

Fecundity: Twenty pairs of freshly emerged male and female flies of same age were confined to ovipositional cages provided with adult diet (3:1 ratio of sugar and yeast hydrolysate) and water. After the pre-oviposition period of 12 days, Petri dishes containing semi-solid pumpkin 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 per female.

Egg Hatch: Three sets of 100 eggs were collected from each diet and inoculated on to the moistened black coloured cotton muslin cloth held in Petri dishes. Number of hatched and unhatched eggs was worked out after 4-days of inoculation.

$$\text{Egg hatch (\%)} = \frac{\text{No. of egg hatched}}{\text{Total no. of eggs inoculated}} \times 100$$

Statistical analysis

Data collected on biological parameters of melon fly reared as larvae on semi-synthetic fruit-based diets were subjected to ANOVA using and mean of three replications for each parameter were separated by using DMRT analysis ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Biological parameters viz., larval duration (days), pupal recovery (%), pupal weight (g/100pupae), adult emergence (%), adult flying ability (%), sex ratio(females/males), fecundity (no. of eggs/female) and F1 egg hatch (%) of melon fly reared as larvae on artificial liquid diet and semi-synthetic fruit-based diets (SSFD) are presented in table 2.

Pupal recovery and pupal weight

Statically significant and higher pupal recovery (77.8%) was recorded in SSFD-IV composed of 7.5 g of yeast extract, 7.0 g of sucrose and 2.0 ml of WGO with pupal weight of 1.61 g per 100 pupae followed by SSFD -III and SSFD -VII with pupal recovery of 75.76 and 74.67 per cent and pupal weight of 1.60 and 1.58 g respectively. In comparison to diet I (control); SSFD -V, SSFD -VI and SSFD -II diets were recorded with lowest pupal recovery (73.67, 73.35 and 68.45%) with pupal weight of 1.55, 1.52 and 0.82 g, respectively which are inferior over the control diet.

Larval duration

Maggots of melon fly able to complete its larval stage in 6.90 days on SSFD -IV followed by SSFD -III and SSFD -VII with 6.50 and 7.77 days, respectively. While, relatively longer larval duration (8.32 days) was recorded in SSFD -V followed by control diet-I, SSFD -II and

SSFD -VI with 7.60, 7.56 and 7.21 days, respectively which were statistically on par.

Adult emergence and adult flying ability

Semi-synthetic fruit-based diet-IV (SSFD-IV) showed significantly higher suitability for melon fly with high percentages of adult emergence (81.8%) and adult flying ability (75.87%) compared to diet-I (control). Diets; SSFD-III and SSFD-VII are next best diets with 80.2 and 78.1 per cent of adult emergence with flying ability of 74.23 and 73.43 per cent, respectively which were statistically on par. Semi-synthetic fruit-based diet-V, V I and II were found inferior with lower adult emergence (72.3, 70.37 and 68.72%) and least adult fliers (71.87, 69.2 and 55.9%) compared to control diet I (75.23 and 72.56%, respectively).

Sex ratio

There is no significant difference with respect to sex ratio (female to male) among the fruit-based diets ranging from 1.17 to 1.23.

Fecundity and egg hatch

Significantly higher egg production (28.0 eggs/female) was recorded in SSFD-IV with 71.81 per cent of egg hatch. Egg production of females reared on SSFD-III, SSFD-VII and control diet-I are ranging from 26.82 to 23.12 eggs/female with egg hatchability of 68.45 to 64.23 per cent. Lowest fecundity was recorded in SSFD-II (17.92 eggs/female), SSFD-VI (21.42 eggs/female) and SSFD-V (22.37 eggs/female) with egg hatchability of 46.62, 56.74 and 60.22 per cent, respectively.

Pupal weight is the key quality parameter and significant indicator of overall viability of pupae (Sharp *et al.*, 1983). Pupal recovery and pupal weight were significantly increased with addition of protein, sucrose and WGO to semi-synthetic fruit-based diet. When compared to diet-I (control), SSFD-IV with equal concentration of protein (7.5 g of yeast extract and brewer's yeast), sucrose (7.0 g) and WGO (2.0 ml) was found superior with significantly higher pupal recovery and pupal weight over the remaining diets. Next best diets are SSFD-III (15.0 g yeast extract, 7.0 g sugar and 2.0 ml WGO) and SSFD-VII (10.0 g brewer's yeast, 10.0 g yeast extract, 10.0 g sugar and 2 ml (WGO).

Yeast extract and brewer's yeast are the products of *Saccharomyces cerevisiae* used as protein source in

Table 1. Composition of experimental diets for mass rearing of *Z. cucurbitae*

Diet Ingredients	Diet-I (Control)	Diet-II (SSFD-II)	Diet-III (SSFD-III)	Diet-IV (SSFD-IV)	Diet-V (SSFD-V)	Diet-VI (SSFD-VI)	Diet-VII (SSFD-VII)
Methyl paraben (g)	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Sorbic acid (g)	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Yeast extract (g)	15.0		15.0	7.5	-	20.0	10.0
Brewer's yeast (g)	-	15.0		7.5	20.0		10.0
Sugar (g)	-	7.0	7.0	7.0	10.0	10.0	10.0
Wheat germ oil (ml)	-	0.2	0.2	0.2	0.2	0.2	0.2
Pumpkin fruit (g)	1000	1000	1000	1000	1000	1000	1000

SSFD : Semi synthetic fruit based diet

Table 2. Life history parameters of melon fly, *Zeugodacus cucurbitae* reared as larvae on fruit-based diets

Parameters	Diet I (Control)	Diet-II (SSFD-II)	Diet-III (SSFD-III)	Diet-IV (SSFD-IV)	Diet-V (SSFD-V)	Diet-VI (SSFD-VI)	Diet-VII (SSFD-VII)
Pupal recovery (%)*	72.32 ^b (58.27)	68.45 ^c (55.83)	75.76 ^{ab} (60.52)	77.8 ^a (61.90)	73.67 ^b (59.14)	73.35 ^b (58.93)	74.67 ^{ab} (59.79)
Pupal weight (g)**	1.57 ^a (1.60)	0.82 ^b (1.35)	1.60 ^a (1.61)	1.61 ^a (1.61)	1.55 ^a (1.60)	1.52 ^a (1.59)	1.58 ^a (1.61)
Larval duration (days)**	7.60 ^b (2.92)	7.56 ^b (2.91)	6.50 ^a (2.72)	6.90 ^b (2.80)	8.32 ^{ab} (3.04)	7.21 ^b (2.85)	7.77 ^a (2.95)
Adult emergence (%)*	75.23 ^{cd} (60.16)	68.72 ^f (56.00)	80.2 ^{ab} (63.60)	81.8 ^a (64.77)	72.3 ^d (58.25)	70.37 ^{ef} (57.03)	78.1 ^{bc} (62.11)
Sex ratio (f/m)**	1.19 ^a (1.48)	1.17 ^a (1.47)	1.21 ^a (1.49)	1.23 ^a (1.49)	1.18 ^a (1.48)	1.18 ^a (1.48)	1.20 ^a (1.48)
Adult fliers (%)*	72.56 ^{abc} (58.42)	55.9 ^d (48.39)	74.23 ^{ab} (59.50)	75.87 ^a (60.59)	71.87 ^{bc} (57.98)	69.2 ^c (56.30)	73.43 ^{ab} (58.92)
Fecundity*** (eggs/female)	23.12 ^{bc} (1.38)	17.92 ^d (1.28)	26.82 ^{ab} (1.44)	28.00 ^a (1.46)	22.37 ^c (1.37)	21.42 ^c (1.35)	24.71 ^{abc} (1.41)
F1 Egg hatch* (%)	64.23 ^c (53.27)	46.62 ^c (43.06)	68.45 ^b (55.83)	71.81 ^a (57.94)	60.22 ^d (50.90)	56.74 ^d (48.88)	66.72 ^{bc} (54.77)

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; DMRT). Figures in the parentheses are retransformed values (*Arc sign transformation; **Square root transformation; ***Logarithmic transformation), SSFD : Semi synthetic fruit based diet.

various diets for mass culturing of organisms. They are rich source of amino acids, proteins, vitamins and growth factors which are essential components of nutritional diets. Yeast extract is composed of 50 per cent of protein (20% glutathione), 18 amino acids, 0.5 per cent of vitamin-B complexes and 3.1 per cent of minerals. Yee (2010) reported the role of yeast extract in enhancing fecundity and survivability of western cherry fruit fly. Brewer's yeast is essential dietary component for many fruit flies, composed of 24.80 g of amino acids (includes essential amino acids (20.76 g) and non-essential amino acids (4.0 g)), vitamins (501.89 mg) and minerals (24.35 mg) in 100 g of brewer's yeast. Chang *et al.* (2004) reported the significant improvement of life history parameters of melon fly reared on brewer's yeast supplemented diets. Significant positive effects of Brewer's yeast (17.77 g) was observed in larval liquid diet with respect to pupal recovery, pupal weight, adult emergence, adult flying ability, fecundity and fertility of melon fly.

Wheat germ oil (WGO) is a rich source of fatty acid responsible for enhancement of quality of flies. Nutritional composition of WGO was reported by Kahlon (1989) that WGO is composed of linolenic acid (42-59%), oleic acid (12-28%), palmitic acid (11-19%), α -linolenic acid (2-11%), stearic acid (1%) and vitamin E (0.14%). Fraenkel and Blewett (1946) reported the role of linoleic acid and vitamin E of wheat germ oil in enhancing emergence, growth and wing scales development. Coudron *et al.* (2010) reported the significant influence of WGO on expression of various genes encoding for range of proteins and lipids. Chang *et al.* (2006) reported the substantial improvement of flying ability, fecundity and fertility with unsaturated fatty acids (linolenic acid and oleic acid); pupal recovery and flying ability with saturated fatty acid (Palmitic acid) in med fly.

Non-significant differences was noticed with respect to larval duration though melon fly completes their larval stage early on semi-synthetic fruit-based diets with higher concentrations of protein, sucrose and WGO (SSFD-V, VII and II) compared to the diets with lower concentrations (SSFD-IV and III). The larvae of *C. capitata* fed with artificial host fruit containing protein and sugar was heavier with faster development (Kapsi *et al.*, 2002). Nestel and Nemny-Lavy (2007) reported the reduced larval period of *C. capitata* on the diet with increased protein concentration.

Performance of Semi-synthetic fruit-based diet on adult emergence and adult fliers was enhanced through increased addition of protein, sucrose and wheat germ oil into the diet. SSFD-IV with 7.5 g of yeast extract, 7.5 g of brewer's yeast, 7.0 g of sucrose and 2.0 ml of WGO performed superior with higher adult emergence and active fliers followed by SSFD-III and SSFD-VII over the liquid diet (Control). Semi-synthetic fruit-based diets with lower concentration of protein, sucrose and WGO (SSFD-II, V and VII) were found inferior than control diet. Fraenkel and Blewett (1946) for the first time reported the nutritional impact of WGO on adult emergence, growth and wing development. Panduranga *et al.* (2018) reported the increased performance of larval diet with higher adult emergence and adult fliers of melon fly by the addition of brewer's yeast, sucrose and WGO to diet. Satisfactory adult emergence of *C. capitata* was recorded in diets with higher brewer's yeast and sucrose. Higher adult emergence was recorded in diet with higher proportion of WGO and sucrose.

Males and females have equal chances to produce but due to their differential nutritional requirement, some diets may favour metamorphosis of one sex than others (Khan, 2013). Negative correlation between male proportion and sugar content was reported by Vera *et al.* (2014). Similarly, Panduranga *et al.* (2018) reported the higher male production from the diet with reduced sugar content. Shinwari *et al.* (2015) reported the significantly higher male from the tarula yeast-based diet.

Adult survival is mainly depending on amount of food accumulated during larval period. Larvae fed on diet with higher nutritional quality was able to accumulate more protein and fatty acid which helps to survive under food stress, which reflects the nutritional status of the diet. Melon flies reared on SSFD with higher nutritional level has the significantly higher percentages of survival. Similar findings were reported by Nestel and Nemny-Lavy (2007) that the med fly larvae reared on diet with increased brewer's yeast to sucrose ratio resulted in increased ability of larvae to accumulate the protein, lipid and produced pupae with higher pupal weight. Whereas, the diet with decreased brewer's yeast to sucrose ratio resulted in decreased ability of larvae to accumulate the protein, lipid with lower pupal weight.

For higher fecundity and egg hatchability, females and males must be of quality flies and reach their maturity in time for coupling. Maturity of flies is significantly

was significantly higher in SSFD-III followed by SSFD-II and SSFD-V. The present result was supported by Ashraf *et al.* (1978) who reported the increased rates of fecundity and fertility of oriental fruit fly on sugarcane bagasse diet with increased wheat germ. Vargas and Mitchell (1987) found that the *Dacus latifrons* reared on wheat based larval diet resulted in high fecundity and fertility. Vargas *et al.* (1994) reported the significantly higher fecundity and egg hatch by increasing wheat germ oil concentration to 3 per cent with constant yeast and sucrose. Similar findings were reported by Panduranga *et al.* (2018) that addition of wheat germ oil has increased the adult emergence, adult fliers, fecundity and egg hatchability in *B. cucurbitae*.

An effective diet can be developed for mass production of quality flies by using natural host fruits externally supplied with protein, carbohydrates and fatty acids to meet the nutritional requirement of fly. Finney (1956) a pioneer in development of fresh and fortified carrot diet supplied with brewer's yeast as source of protein for rearing of oriental fruit fly, *Dacus dorsalis* and reported 80 per cent of egg hatch and matured maggots, 95 per cent of pupal recovery and adult emergence. Similarly, Abadin *et al.* (2014) reported the reduced larval duration, higher pupal recovery, adult emergence, egg viability and egg hatchability of *B. cucurbitae* reared on natural host fruit (bottle gourd) without any supplements of protein and fatty acids.

Panduranga *et al.* (2018) reported the increased pupal recovery (83.20%) and pupal weight (1.34 g) of *B. cucurbitae* larvae reared on fruit-based diet supplied with yeast extract (15.0 g). Present findings are supported by Kirti *et al.* (2018) that melon fly reared as larvae on fruit-based diet composed of 15 g of yeast extract resulted in significantly higher pupal recovery (80.93%), pupal weight (1.41 g), adult emergence (69.00%), adult flying ability (74.36%), fecundity (15.84 eggs/female) and fertility (65.3%). Therefore, supplement of nutritional elements *viz.*, protein, sugar and fatty acid in fruit-based diet in adequate quantity is crucial to produce good quality flies.

Based on performance of melon fly reared as larvae on semi-synthetic fruit-based diets with differential concentration of protein, sucrose and WGO revealed that, the SSFD-IV with 7.5 g of yeast extract, 7.0 g of sucrose and 2.0 ml of WGO was the most suitable diet for mass rearing of melon fly followed by SSFD-III with 15.0 g of

yeast extract, 7.0 g of sucrose and 2.0 ml of WGO followed by SSFD-VII with 10.0 g of yeast extract, 10.0 g of brewer's yeast, 10.0 g of sucrose and 2.0 ml of WGO which were statistically on par with respect to quality standards recommended by FAO/IAEA/USDA for sterile insect technique.

CONCLUSION

Semi-synthetic fruit based diet-IV (SSFD-IV) composed of 7.5 g of yeast extract (protein source), 7.0 g of sucrose (carbohydrate source) and 2.0 ml of WGO (fatty acid source) is the most appropriate diet as it fulfilled the quality standards for sterile insect technique recommended by FAO/IAEA/USDA. Hence, it is concluded that SSFD-IV as suitable larval medium for mass rearing of melon fly larvae to produce enough pupae required for irradiation in support of sterile insect technique.

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