

Potential of artificial larval diets for mass rearing of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae)

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ABSTRACT: The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) is a major polyphagous pest of various agricultural and horticultural crops worldwide, especially in tropical areas. The larval stage of this pest is most dangerous to fruits and vegetable crops. To identify the preferable artificial diet, the larvae of *B. dorsalis* were reared on four different artificial diets under standard laboratory conditions at temperature $28\pm 2^{\circ}\text{C}$, relative humidity $60\pm 10\%$ and 14:10 hours (L:D) photoperiod. The biological parameters and progeny performance were tested and the results showed that liquid diets support biological parameters and progeny performance more as compared to solid diets. The average adult emergence on liquid and solid diets was $78.3\pm 1.59\%$ and $67.9\pm 3.26\%$, respectively. The pre-oviposition period was recorded as 7.7 ± 0.33 days and 11.3 ± 0.88 days for a liquid and solid diet. The fecundity and female sex ratio were also recorded to be significantly higher on liquid diets than solid diets. The average total life cycle (egg-adult) was 17.7 ± 0.88 days on liquid diets while 22.3 ± 0.33 days on solid diets. The results showed that liquid diets were more suitable for fruit flies' larval growth. The inclusion of liquid larval diets in the mass rearing system can be a viable replacement for using natural hosts in mass rearing protocols.

Keywords: Larval diets, *Bactrocera dorsalis*, mass rearing

INTRODUCTION

The family Tephritidae (Diptera) is one of the largest evolutionary within the true flies, are among the most economically vital pest species attacking a vast range of fruits and fleshy vegetables throughout tropical and sub-tropical areas. These species are such devastating crop pests that major control and eradication programs have been developed in various parts of the world (Roger, 2015).

However, the fruit flies tephritid include approximately 100 species which are agricultural insect pests of global importance (White, 1992) and fruit fly management incurs huge costs to agriculture production, including hundreds of millions of dollars spent annually on their control and eradication (Clarke et al., 2005).

The *Bactrocera dorsalis* was first recorded from Taiwan in 1912, and then from Tahiti Island in July 1996. It is well-known in Asia and now in the pacific region. *B. dorsalis* occurs in a broad swath from Pakistan and India east to

southern China, Taiwan, and Southeast Asia, including Malaysia and the great island groups of Sumatra, Java, Borneo and the Philippines. It has been introduced and established in some Pacific islands, such as the Hawaiian Islands since about 1945, and Guam since 1947. It invaded east Africa in about 2003 and spread very rapidly throughout large parts of the continent. *Bactrocera dorsalis* is a frequent invader (Gary, 2017). *B. dorsalis* is polyphagous in nature; it has about 250 host plants. They mostly attack ripened as well as unripened fruits. At the same time, it also infests vegetables and many other fruits of tropical and subtropical regions (Hui and Lui, 2005).

The *Bactrocera dorsalis* population peak appeared in July and August and maximum decline was observed in October depending on the host fruit maturity, temperature and rainfall. Availability of host fruits was another essential factor affecting population fluctuation (Mahmood and Mishkatullah, 2007). *Bactrocera dorsalis* species are one of the greatest barriers to fresh produce exports worldwide. In the Asia-Pacific region they lose 40% to 100% are experienced everywhere from villages to large area farming systems (Chang, 2009; Yan Shi et al., 2017; Dong Wei et al., 2017; Clarke et al., 2005). In Asia the favorable hosts of *Bactrocera dorsalis* are mango, guava, peach as well as other fruits (Gafoor et al., 2010).

Mass production of flies depends on the use of artificial diets (Rivera, 2012). Among many factors to consider when developing the mass rearing strategies, artificial diets may be considered as one of the most important components and, together with labor, they constitute the main costs. Therefore, in mass rearing, the search for cost reduction of diet is constant, always aiming a balance between the costs and insect quality. The use of local ingredients may guarantee the supply and, generally, reduce the production costs (Parker, 2005).

Artificial diets are foods synthesized from one or more ingredients that may be completely defined chemically. Artificial diets must fulfill sensory requirements and be nutritious for animals within a framework of economic feasibility (Cohen, 2004). In reality, the production of artificial diets is one of the most substantial direct input costs in many areas related to animal breeding (Jefferies, 2000; Chaudhury, 2007; Woyengo, 2014).

Several diets have been used in the large-scale commercial production of fruit flies worldwide (ALUJA et al., 2001). A good nutrition in the larval phase is necessary for a proper development, affecting survival and adult size (Cesoni-Pereira and Zucoloto, 2009).

A liquid larval diet and its rearing system have been developed for mass rearing of *Bactrocera dorsalis* (Hendel). Baking yeast, soy bran, soy proteins were used at different combinations for the formulation of liquid diet. Sugar, anti-microbial agent (sodium benzoate) and citric acid were also included in the diet (Mahfuza et al., 2011). Advantages of a fruit fly (Diptera: Tephritidae) liquid diet (without mill feed as a biological bulking agent) over the conventional mill feed diet used in Hawaii (Tanaka, 1969) include simplified spent diet management and reduced labor and space costs (Chang et al., 2004). The first fruit fly liquid diet without a biological bulking agent was successfully developed for small scale melon fly, *Bactrocera cucurbitae* Coquillett, larval rearing (Schroeder et al., 1971; Chang et al., 2004).

The objectives of this study were identified easily available protein sources and diet for mass rearing of the oriental fruit fly, *B. dorsalis*. The artificial larval diet and its rearing system may help to replace the use of natural hosts.

MATERIALS AND METHODS

Colony Origin and Maintenance

The *Bactrocera dorsalis* were collected from guava orchard at Sharaqpur and Haripur, and established colony since from 2014. Maintained the culture under control condition 25 ± 2 °C, 65 – 75 % RH, and 14:10 (L:D) photoperiod in Biological Control Laboratory at CABI Rawalpindi, Pakistan (Farooq et al., 2019).

In this experiment, *B. dorsalis* cultures were maintained separately in rearing cages (60×60×48 cm) with a cloth sleeve opening at front and capacity of 500 pairs per cage.

Diet Preparation ingredients

The experiment was based on liquid and solid diets formulation. We tested liquid diet ingredients such as sugars, Sodium benzoate, Yeast, Water, Vitamin B Complex and Citric acid in (Chang et al., 2004). In solid diet used Carrot powder, Sodium benzoate, Yeast, Water, Nipagen and Hydrochloric acid (HCL) 4. Table 1.

Table 1: Ingredients of diets used for larval feeding and off spring performance

T1 (Liquid Diet)		T2 (Solid Diet)	
Ingredient	Quantity	Ingredient	Quantity
Sugar (g)	48.72	Carrot powder (g)	75
Sodium benzoate (g)	0.8	Sodium benzoate (g)	1.25
Yeast (g)	81.6	Yeast (g)	35
Water (ml)	400	Water (ml)	385
Vitamin B Complex (ml)	1	Nipagen (g)	1
Citric acid (g)	9.24	Hydrochloric acid (HCL) (ml)	4

Experimental Procedure

In liquid diet experiments using 500 ml plastic containers. The liquid diet were prepared as described by (Chang et al., 2004). Used block sponge sheet and plastic boxes, 150 ml of diet was added to each box. The sponge were dipped in diet. A plastic cup with punctures on the wall, were used as an artificial oviposition device. The cup is placed upside down on a piece of sponge soaked in a mixture of guava juice and water to stimulate flies to lay eggs in the punctures. Circular black sheets were put above the sponge for collecting deposited eggs. The eggs were sprayed gently with distilled water to spread them evenly across the sponge surface. The sponge was then placed on top of the diet. The plastic boxes were covered with plastic lids to raise the temperature. The collected eggs were seeded on the liquid and solid artificial diets on sponge inside plastic container and on direct on solid diet. Containers lids were ventilated with holes during larval duration period. Before larval maturation, larval trays were put in ventilated boxes provided with wet sand on the bottom. After one week the mature larvae begin to

leave the diet and jump to the sand for pupation. The Pupae were sieved from the sand and held in the adult rearing cages until emergence.

Recording Biological Parameters

The various biological parameters studied in this experiment included the percent hatching, larval stage, percent pupation, pupal weight, pupal duration, adult emergence, egg to adult period, pre-oviposition period, fecundity and female ratio was measured in liquid diet and solid diet.

Statistical Analyses

Descriptive statistics as mean values \pm standard error (SE) was calculated. The differences among the diets quality control parameters were determined by analysis of variance (ANOVA). LSD test at $P = 0.05$ level of significance was used to determine separation and significance of means (SAS, 1999).

RESULTS AND DISCUSSION

In the current study, the biological parameters such as egg duration, percent hatching, larval period, percent pupation, pupal weight, pupal duration, adult emergence, life span (egg-adult), pre-oviposition period, fecundity and female ratio of oriental fruit fly, *B. dorsalis* were recorded by using different artificial diets under controlled conditions.

a. Egg duration, percent hatching and larval stage

The results regarding egg duration showed that incubation period significantly varied with diets. It was observed that T1 reduce the egg duration (4.33 ± 0.33 days) as compared to T2 (5.67 ± 0.33 days) ($F_{1,5} = 8.00$, $P = 0.04$). The percent hatching of *B. dorsalis* on different diets (solid and liquid) were showed significant variations. The liquid diet (T1) was showed maximum percentage of egg hatching ($75.22 \pm 3.39\%$) while solid diet (T2) ($53.44 \pm 5.83\%$) ($F_{1,5} = 10.4$, $P = 0.03$). The larval growth and development were highly affected with diets. In addition, data regarding larval development showed that solid diet (T2) delayed the larval developmental stage (8.67 ± 0.33) (days) as compared to liquid diet (T1) (7.33 ± 0.33) (days) ($F_{1,5} = 8.00$, $P = 0.04$) (Table 2).

b. Percent pupation, pupal weight and duration

The maximum percent pupation ($86.14 \pm 1.35\%$) of *B. dorsalis* was observed on liquid diet (T1) while solid diet T2 showed ($71.42 \pm 3.65\%$) ($F_{1,5} = 14.3$, $P = 0.02$). The pupal weight was significantly higher in the liquid diet T1 (11.77 ± 0.18) (days) as compared to solid diet T2 resulted in reduced pupal weight (9.53 ± 0.34) (days) ($F_{1,5} = 34.3$, $P = 0.004$). The pupal duration showed significant variations as shorter pupal developmental time (4.33 ± 0.33) (days) was observed for liquid diet T1 as compared with T2 (6.67 ± 0.33) (days) ($F_{1,5} = 24.5$, $P = 0.01$) (Table 2).

c. Adult emergence and life span

Among tested diets, liquid diet (T1) was showed significantly higher adult emergence percentage ($78.29 \pm 1.59\%$) than solid diet (T2) ($67.86 \pm 3.26\%$) ($F_{1,5} = 8.25$, $P = 0.04$). The total developmental time from egg to adult was 17.67 ± 0.88 days on liquid diet while 22.33 ± 0.33 days on solid diet ($F_{1,5} = 24.5$, $P = 0.01$). The solid diet was not suitable for pest growth as compared to liquid diet (Table 2).

d. Pre-oviposition period and fecundity

The minimum pre-oviposition period on T1 and T2 were recorded (7.67±0.33 days) and (11.33±0.88 days) ($F_{1,5}=15.1$, $P=0.02$), respectively. Moreover, maximum fecundity (216.00±18.36) was observed in T1 in comparison to T2 (109.67±10.68) ($F_{1,5}=25.1$, $P=0.01$) (Table 2).

e. Female ratio

It was observed that T1 showed higher female ratio (55.67±0.88) in comparison to T2 (45.00±1.53) ($F_{1,5}=36.6$, $P=0.003$) (Table 2).

Table 2: Effect of different diets on biological parameters and off spring performance of *Bactrocera dorsalis*

Biological Parameters	Liquid Diet (T1)	Solid Diet (T2)	P value (F, HSD)
Egg duration (d)	4.33±0.33b	5.67±0.33a	0.04(8.00.1.31)
Larval stage (d)	7.33±0.33b	8.67±0.33b	0.03(8.00.1.31)
Percent pupation (%)	86.14±1.35a	71.42±3.65b	0.01(14.3.10.81)
Pupal weight (mg)	11.77±01.8a	9.53±0.34b	0.004(3.42.1.06)
Pupal duration (d)	4.33±0.33b	6.67±0.33b	0.008(24.5.1.31)
Adult emergence (%)	78.29±1.59a	67.86±3.26b	0.04(8.25.10.1)
Egg to adult period (d)	17.67±0.88b	22.33±0.33b	0.008(24.5.2.62)
Pre-oviposition period (d)	7.67±0.33b	11.33±0.88a	0.02(15.1.2.62)
Fecundity (no.)	216.00±18.36a	109.67±10.68b	0.08(25.1.59.03)
Female ratio (%)	55.67±0.88a	45.00±1.53b	0.004(36.6.4.9)

The current study was conducted to check the most preferable artificial diet for rearing of oriental fruit fly, *B. dorsalis* under artificial conditions or controlled. The liquid diet consists of 48.72g sugar, 0.8g sodium benzoate, 81.6g yeast, 400ml water, 1ml vitamin B complex and 9.24g citrus acid while solid diet contains 75g carrot powder, 1.25g sodium benzoate, 35g yeast, 385ml water, 1g nipagin and 4ml HCL as shown (Table 1). The potential of the artificial diet was recorded or analyzed based on their effectiveness on life cycle of tested insect such as *B. dorsalis*. The biological parameters which recorded during the study were highly affected by the artificial diets tested in the study period. The productivity of *B. dorsalis* is highly associated with various biotic and abiotic factors. The growth and developmental stages such as egg, larva, pupa and adults of insects especially *B. dorsalis* are highly linked with food components. The similar findings had been reported by (Chaudhury and Skoda, 2007). The incubation period can vary with composition of diets. In the current study liquid diet was recorded most preferable diet for larval development as compared to solid diet. Our current study results are similar to previous studies performed by various researchers in the globe. The results showed significantly higher percent hatchability of the eggs in T1 as compared to T2. The maximum and minimum development of larvae to pupae formation was recorded in treatments where the larvae fed on T1 and T2 diets, respectively. The study concluded that solid diet (T2) is not suitable for development of fruit fly larvae. (Paskova, 2006) had investigated the similar findings. He has

reported that larval weight and growth found high at liquid diet as compared to larvae reared on solid diet. The similar findings had been recorded by various early researchers. They had recorded the larval performance on different artificial diets (Chang et al., 2006; Vera et al., 2014; Ekesi et al., 2014).

The pre-oviposition period, fecundity and female ratio was recorded high on liquid diet as compared to solid diet. The fertility of insect pests can increase with preferable host. The mortality rate of pest can decrease with the availability of suitable diet or food. Many scientists had reported the similar results. Our findings are also in line with other studies such as (Chang et al., 2009). It had been reported that high protein is not suitable for larval growth and development. The larvae can't survive at high protein even die.

The larval growth and development are highly affected with nutritional composition like cellulose, agar and protein. The diet containing low protein percentage is most preferable and suitable for pest growth. The maximum adult emergence was recorded in T1 than T2. results were in accordance with those of (Chang et al., 2006; Fay, 2009). It is shown that liquid diet (T1) has potential to develop larval growth and development. Larval diet ingredients and their relative amount for *B. dorsalis* used in the present experiment was based on the liquid diet recipe of (Chang et al., 2004) and solid diet based on (Allwood, 1996).

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