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DEVELOPMENT OF LIQUID LARVAL DIET WITH MODIFIED REARING SYSTEM FOR *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE) FOR THE APPLICATION OF STERILE INSECT TECHNIQUE

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ABSTRACT

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. The quality parameters of flies reared on liquid larval diets i.e., pupal weight, pupal density, larval duration, percentage adult emergence, percentage fliers, percentage egg hatch, male-female ratios were comparatively better on modified liquid diet where baking yeast, soy bran and soy protein was used at 2:1:1 ratio. Low cost disposable plastic boxes and sponge cloths used as rearing tray and supporting substrate for larvae which reduce the rearing space and replace the need for the traditional bulking agents (wheat bran/mill feed) for mass production of *B. dorsalis* under laboratory condition. Benefits derived from liquid diet and its rearing system is discussed in relation to use of Sterile Insect Technique programmes of *B. dorsalis*.

Keywords: liquid larval diet, *B. dorsalis*, rearing system, sterile insect technique.

INTRODUCTION

The oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) is a serious pest of commercial fruits world wide especially, in South-east Asia and Pacific Rim. The fly species is reported to attack over 173 different varieties of fruit and vegetables and ranked high quarantine target lists. The control measures adopted for the fly species mainly on contact poisons or baits (Bhutani, 1975, Gupta and Verma, 1979, Lee, 1988, Perdomo *et al*., 1976). Baits and sprays of conventional insecticides have toxic effects on non-target beneficial fauna including parasitoids of *Bactrocera* spp. Sterile Insect Techniques (SIT) have been currently considered as an alternative and environmentally benign approach for suppressing or eradicating insect pests and is widely used in integrated programs against tephritid fruit flies. SIT involves the suppression of insect population through the release of sterile insects rendered infertile by gamma radiation. Cost-benefit analysis have shown that SIT is economically feasible or even superior to conventional methods when applied over a longer period of time. Successful application of this technology will greatly enhance the production of fruit fly free commodities and increase the opportunities of international trade of agricultural produce.

However, the implementation of area-wide integrated pest management using SIT is largely depends on mass rearing of the fly species using artificial larval diets. Proper larval diet is important not only to rear good quality larvae, but also to have vigorous adults. It is important to know the exact larval nutritional demands to be able to produce such a diet. Life parameters of flies, diet consistency, distribution of nutrients in a diet, microclimatic conditions during larvae aggregation, availability of diet ingredients and amount of spent diet are key requirements for a suitable laboratory diet.

Development of larval diet for mass rearing of Tephritid fruit flies has been considered as one of the prominent areas of research for several decades. In 1949-1950, Finney pioneered development of practical rearing methods for oriental fruit fly, *Bactrocera dorsalis* (Hendel) and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Carrot larval diet was the basis of other artificial diets. The wheat-bran diet used in USDA-ARS Pacific Basin Agricultural Research Center in Honolulu has performed well more than 30 years, and, except for minor changes, (primarily in bulking agents), is used worldwide for mass rearing of Tephritid fruit flies (Tanaka *et al*., 1969). This diet contain bran or wheat products as bulking agent which adjust the free water content, and provide specific nutrient such as sterols known to promote larval growth (Ashraf *et al*., 1978, Vargas *et al*., 1994). However, wheat based bulking agents such as wheat bran also has some problems associated with microbial and pesticidal contamination (Hooper, 1987). Variability in production levels and qualities is often attributed to the unsuitability of local wheat bran. In some fruit fly mass rearing program, wheat based products have been replaced by wheat mill feed, wheat shorts, grain corncob, cane and beet bagasse (Vargas *et al*., 1983) and tissue paper (Kakinihana and Yamagishi, 1991). Fay and Wornoayporn (2002) suggested replacement of plant-derived bulking agents from larval diet with an inert reusable substrate as distinct advantage for mass production system of *C. capitata.*

Inert bulking agents such as paper and cotton wool have been used with fluid diets for *C. capitata* and *Ragoletis cerasi* L. in small-scale rearing situations

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(Monro 1968, Katsoyannos *et al*., 1977, Zumreoglu *et al*., 1979). For the olive fly, *Bactrocera oleae* (Gmelin) greater number of pupae per gram diet was achieved than with a solid diet when deep-pilled cotton toweling was applied to liquid diet (Mittler and Titsipis, 1973). However, Chang *et al.* (2004) develop a novel liquid diet and its rearing system for melon fly, *B. cucurbitae* which help to alleviate many of the problems associated with wheat based bulking agent such as spent diet management (disposal and tray cleaning), storage, space, high cost, labor, and sanitation.

The delivery matrix, sponge cloth used as larval rearing system by Chang *et al*. (2004) were highly water absorbent, easy to clean, and reusable. The sponge cloths are produced using recycled raw materials (natural cellulose and cotton fibers) and can be disposed of without any problem. Fay and Wornayporn (2002) also suggested that with some adjustment to the dietary nutrients, particularly such as diced High Density (HD) sponge can satisfactorily used for wheat bran in the larval diets for med fly. The deiced HD sponge used by the authors was washed and reused on more than five occasions without deterioration and found to be entirely suitable for mechanical separation of post feeding larvae as undertaken at the rearing facility at Metapa, Mexico (Schwarz *et al*., 1985).

In the liquid diet of Chang *et al*. (2004) 34.74% brewer's yeast was used instead of 3.55% torula yeast and 31.19% mill feed which gave lower pupal production of melon fly, *B. cucurbitae*. Later pupal production was increased up to 60% by three fold increase of brewer's yeast in diet and suggested 14.20% as the best dose of brewer's yeast in the diet. For factory scale rearing of *B. dorsalis* Chang *et al*. (2006) recommended the use of 15.06% brewer's yeast as ingredients of liquid diet for larval rearing of *B. dorsalis*. Several authors (Spishakoof and Davila, 1968, Chan *et al*., 1990) reported torula yeast as good source of protein for larval development of tephritid flies.

Chang (2004) reported that the larvae of *C. capitata* died when fed with diets free of 10 exogenous essential amino acids (arginine, isoleucine, leucine, lysine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine) or containing nine exogenous amino acids with removal of any one of the ten essential amino acids. Effects of various concentrations of wheat germ oil at different concentrations and their possible mode of action were also evaluated by Chang *et al*. (2007) and suggested that addition of wheat germ oil to fruit fly rearing diet is a novel way to improve fruit fly quality, especially in egg hatch, fliers, egg production, and pupal recovery.

The pupal weight is considered as a key factor impacting most variables of quality parameters of Queensland fruit fly, *Bactercera tryoni* (Froggatt) (Dominiak *et al*., 2010). The quality parameters usually decline with the increase of pupal yield. Adult emergence was significantly positively related to the flight ability of flies. The maintenance of high emergence levels would ensure adequate flight ability. Higher pupal weight was also noted as an important quality parameter contributing to competitiveness and flight propensity (Sharp *et al.* 1983, Churchill-Stanland *et al*., 1986).

In the present experiment we are trying to develop different formulations of liquid larval diets and to identify easily available protein sources as alternative of costly brewer's yeast used in Chang *et al*. (2004) diet for mass rearing of the oriental fruit fly, *B. dorsalis*. The modified liquid larval diet and its rearing system may help to replace the use of natural hosts or bran based diets for mass production of *B. dorsalis* to use in SIT.

MATERIALS AND METHODS

Adult flies

Adult *B. dorsalis* culture originated in 1995 from infested mango collected from Rajshahi district, North-western part of Bangladesh. Rearing of *B. dorsalis* was maintained in the laboratory of Insect Biotechnology, Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment (AERE), Savar, Dhaka for more than100 generations on diet containing wheat bran/mill feed as bulking agent. About 5,000 adult flies were maintained in steel framed cages (76.2 x 66 x 76.2 cm) covered with wired net. The flies were supplied with protein based diets both in the liquid and dry form *viz*., (i) baking yeast: sugar: water at 1:3:4 ratio, and (ii) casei: yeast extract: sugar at 1:1:2 ratio. Water was supplied in a conical flask socked with cotton ball. The temperature and the relative humidity of the rearing room maintained at 27 ± 1^0C and 75±5%, by using air conditioner (Model No. Movincool Classic Plus 26, USA).

Eggs

B. dorsalis eggs were collected from the stock culture using yellow colored egging devices perforated with 0.5 mm holes. A piece of sponge was socked with banana paste was placed inside the egging device and covered with lid. After 6 hours (9 am to 3 pm) of egg collection the egging device was immerse into water ball and eggs were collected for experiment.

Diet preparation and delivery system

The liquid diet developed for larval rearing of *B. dorsalis* was modified from that of Chang *et al*., (2004). The modified diet was composed of baking yeast, soy bran dust, soy protein, sugar, antimicrobial agent (sodium benzoate), citric acid and water at different ratios. The different types of modified diets differed mainly on the ratio and ingredients of protein sources, *viz*., diet no. i. Baking yeast (Fermipan red, Langa Fermentation Company Ltd., Vietnam), and soy bran dust (3:1), ii. Only soy bran dust, and, iii. Baking yeast: soy protein (Nature's Bounty INC., USA): soy bran dust (2:1:1) (Table-1). Initial pH value for these diets was 3.5.

The liquid diet preparation and its delivery system were also followed by Chang *et al*. (2004). A set of experiments were carried out with liquid larval starter kit

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supplied by USDA, ARS, Honolulu Tropical Pests Research Unit, Honolulu, Hawaii. We considered these as control diet for *B. dorsalis* rearing. Rests of the experiments were carried out using our modified formulations of diets and rearing materials.

The diet mixture is formulated by weighing all the diets ingredients into 250 ml polyethylene container with lid and either shaking in vigorously by hand and mixing homogeneously or using a magnetic stirrer and mixing plate for 5 min or until the diet ingredients were fully dissolved and homogeneous. In the liquid diet of Chang *et al*. (2004) sponge cloth (31.5 by 25.7 cm, Kalle USA Inc., Flemington, NJ) was the primary support matrix for feeding larvae. The sponge material was 1.5 cm away from each of the fore sides of larval tray. The sponge cloth and polyethylene screen (Home Depte, Aiera, HI) was leveled on the floor of the larval tray (18 by 12 by 3.5 cm^3, Premium Incorporated, Honolulu, HI). In the present experiment we also follow the similar procedure for rearing system using similar sized disposable box, plastic net and locally available sponge instead of plastic container, polyethylene screen, and sponge cloth, respectively used by Chang *et al*. (2004).

About 0.5 ml eggs were seeded on 150 ml each type of modified and control diet. Eggs were seeded using a 1 ml transfer pipette- onto a strip of wet sponge cloth (2 by 7 cm) placed the center top surface of the sponge cloth of the larval tray containing freshly made liquid diet. After hatching from eggs younger larvae were usually found on the top surface of the sponge. Whereas older (late second and third instars) were partially submerged under the sponge cloth, screen, or between sponge cloth and screen until ready to pupariate. After 5 d of seeding eggs on the rearing trays were transferred to plastic racks containing saw dust as a pupation substrate.

Evaluation of biological parameters

The quality of flies reared on three modified liquid as well as control larval diets was evaluated by assessing: number of pupae produced/pupal yield, larval duration (d), pupal weight (mg), adult emergence (%), adult fliers (%), sex ratio (male: female), and egg hatch (%).

Egg hatch

To estimate average number of egg hatch, four sets of 100 eggs each were spread on to a strip of wet blue coloured sponge cloth (1 x 3.5 cm, Kalle USA Inc., Flemington, NJ) and incubated into Petri dishes (55 mm)covered with lids containing each type of larval diet. The numbers of eggs that did not hatch from each 100 eggs after 3 days were counted and recorded. To calculate mean percentage of egg hatch, the number of eggs that did not hatch was subtracted from 100 and then multiplied by 100. The experiment was repeated for five times.

Larval duration

Larval duration (days) was determined by recording and collecting larvae first observed exiting from the larval diet up to three days of pupal collection, and estimated the mean larval period.

Pupal weight and total pupal yield

The number of pupae produced/pupal yield, and pupal weight was determined using the method of Chang *et al.* (2007). For each larval diet pupae were collected for at least three consecutive days after larvae popped out from the diet into saw dust. Total mass (mg) of all puparia were collected and measured using electrical microbalance. Four sets of 100puparia develop from each larval diet were weighed to obtain a mean weight (mg) per 100 puparia.

Total number of pupae was estimated by dividing total pupal weight by the mean weight from the four sets of 100 puparia and then multiplying by 100.

Adult emergence and flight ability

Adult emergence and flight ability tests were performed according to the methods of Collins *et al.* (2008). Four sets of 100 pupae were set up to assess percentage adult emergence, and percentage fliers. Two days before estimated adult emergence, four sets of 100 pupae reared from each larval diet were placed in separate 55-mm plastic Petri dish lids. The dishes of pupae were then centered on 90 mm Petri dishes lined with black paper. A 100 mm tall black plexiglass tube (94 mm inner diameter, 3 mm thickness) with a fine coat of unscented talcum powder on the interior (to prevent flies walking out) was placed over the 90 mm Petri dish lid. Each tube with pupae was placed in a 30 x 30 x 30 cm mesh cage. The cages were set up on shelves near to the light of the rearing room. To minimize fly-back, flies that escaped from the tube were removed daily. When all emergence had ceased (5 days after the first flies emerged), the remaining contents of the tubes were counted. The data were collected as five categories: i. Not emerged, ii. Part emerged (portion of adult body stuck in puparium), iii. Deformed (fly fully emerged but with damaged or deformed wings), iv. Non-fliers (fly looked normal, but could not fly out of the tube), and v. Fliers (fly looked normal and had flown out of the tube).

Calculation of percentage adult emergence, and fliers was done according to the calculation of Collins *et al.* (2008). The percentage of adult emergence ((N pupae - (N not emerged + N part emerged)/N pupae) x 100), percentage fliers ((N pupae - (N not emerged + N part emerge + N deformed + N non-fliers)/N pupae)) x 100). The calculations used are directly comparable to the corresponding methods of FAO/IAEA/USDA (2003).

Differences in the diet batch per treatment data were determined by Analysis of Variance (ANOVA). Percentage data were transformed into arcsine, Duncan Multiple Range Test (DMRT) was done by statistical software.

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RESULTS AND DISCUSSIONS

Larval diet ingredients and their relative amount for *B. dorsalis* used in the present experiment was initially based on the liquid diet recipe of Chang *et al.* (2004), i.e., control diet by excluding brewer's yeast (Table-1). The present experiment aims to identify easily available and low-cost protein sources as ingredients of larval diet for the mass production of *B. dorsalis.*

Table-1. Ingredients for liquid diet of Chang *et al*. (2004) and modified diets i, ii, and iii for larval rearing of *B. dorsalis.*

Diet Ingredients	Control diet (Chang *et al*., 2004)		Modified liquid diet					
			Diet i BY: Soy bran		Diet ii Soy bran		Diet iii BY: Soybran: Soy protein	
	%	gm	%	gm	%	gm	%	gm
Sodium benzoate	0.09	0.11	1.80	0.22	1.80	0.22	1.80	0.22
Nipagen	0.09	0.11	-	-	-	-	-	-
Sugar	5.96	7.35	5.96	7.35	5.96	7.35	5.96	7.35
Brewer's yeast	11.51	14.20	-	-	-	-	-	-
Baking yeast	-	-	11.51	11.51	-	-	11.51	7.07
Soy bran dust	-	-		3.56	11.51	14.20	-	3.56
Soy protein	-	-	-	-	-		-	3.56
Water	81.08	100.00	81.08	100.00	81.08	100.00	81.08	100.00
Citric acid	1.26	1.56	1.26	1.56	1.26	1.56	1.26	1.56

BY = Baking Yeast

In the present experiment, 11.51% protein was used in all modified liquid diets. The components of baking yeast used in the present study were natural yeast (*Saccharomyces cerevisiae*), rehydrating agent, and ascobic acid. On the other hand soy protein (200g) contains sodium (125mg), potassium (15mg), protein (12.5g), calcium (3%), phosphorus (10%). Nature's Bounty soy protein isolate powder provides soy isoflavone metabolites, genistein, daidzein and glycitein, plus phophorus, calcium and iron. Isolate powder has a high protein containing a balanced supply of nine essential and nine non-essential amino acids. Whereas used whole sale soy bran may content oil and proteins.

Although same percentage of soy bran dust was used in the modified liquid diet no. ii. The obtained quality parameters of reared *B. dorsalis* are poor. This may be due to the amount of vitamins and amino acid present in baking yeast and soy protein used in the modified diet no. i and ii, respectively. Soy protein contain essential and non-essential amino acids, but comparatively expensive than baking yeast and soy bran. Additional oils or additives were not used in the present study.

The quality parameters i.e., total number of pupae produced, pupal density (w/v), larval duration (d), pupal weight (mg), percentage adult emergence, percentage fliers, male: female ratio and percentage egg hatch of *B. dorsalis* reared on Chang *et al*. (2004) (control liquid diet) and modified diet nos. i, ii, and iii respectively shown in Table-2.

Larvae reared on control liquid diet and modified diet no. iii developed at approximately the same rate. The mean duration of the larval period in the control diets were significantly shorter (less than two days) than that recorded for our modified liquid diet no. i and ii, respectively. In the present experiment *B. dorsalis* larvae were ready to pupariate at seventh day on both the control and modified diet no. iii. Pupation occurred on eighth day. However, comparatively longer larval duration was observed on modified liquid diet no. i and ii which ranged from eight to 10 days.

Mean pupal production was almost the same among control diet and modified diet no. i and diet no. iii. Only soy bran dust based liquid diet (diet no. ii) produce statistically low numbers of pupae compare to other modified liquid diets no. i and iii. Again mean pupal weight from the present modified liquid diet no. i and iii were lower than that of control diet. Pupal density was calculated using the total pupal weight (gm) divided by the total pupal volume (milliliters). There are significant differences among the pupal density recorded from control liquid diet and our modified diet no. i, ii, and iii, indicated that pupal quality may vary among diets.

Mean percentage emergence of adult flies produced from control liquid diet ranked highest and differ significantly from those reared on liquid diet no. ii. The male and female ratios were 51: 49, 50.5: 49.5, 49: 51 and 50.5: 49.5 for control diet, diet nos. i, ii, and iii, respectively. Mean flight ability among flies reared on liquid larval diet does not differ significantly except for

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those reared on liquid diet no. ii. Although there were no apparent effects on adult fly emergence or flight ability, further research would be required to improve the quality of mass reared fly over several generations to make a sound judgment. However, on the basis of quality parameters modified diet no. iii seems to be suitable for mass production of *B. dorsalis.*

In the present experiment we used locally purchased sponge and reused for more than six times. Present experiments do not require caution to ensure whether the liquid larval diet over flow the sponge cloths, causing larval drowning as soy bran dust, and baking yeast provide some shelter for newly emerge larvae on the sponge cloths. However, the storage space would be reduced as similar size rearing trays/boxes were used as Chang *et al.* (2004).

Table-2. Quality parameters of *B. dorsalis* reared on liquid diet of Chang *et al*. (2004) and modified liquid diets i, ii, and iii., respectively.

Parameters	**Control diet** (Chang *et al*., 2004)	**Modified Liquid diets**		
		Diet i BY:Soy bran	**Diet ii Soy bran**	**Diet iii BY:Soybran: Soy protein**
No. Pupae produced	3350 ± 250a	2950 ± 175a	2100 ± 150b	3000 ± 200a
Pupal density (w/v)	0.59 ± 0.004a	0.46 ± 0.007b	0.45 ± 0.0041c	0.47 ± 0.002b
Larval duration (d)	7 ± 0.149a	9 ± 0.182bc	8.5 ± 0.129b	7.5 ± 0.105a
Pupal weight (mg)	12.5 ± 0.223a	9.5 ± 0.149d	8.5 ± 0.166c	11.5 ± 0.210b
Emergence (%)	98 ± 0.333a	95 ± 0.258b	80 ± 0.577c	96 ± 0.421b
Flier (%)	79 ± 0.421a	74 ± 0.614b	72 ± 0.577bc	76 ± 0.918b
Sex ratio (male: female)	51:49 a	50.5:49.5a	49:51a	50.5:49.5a
Egg hatch (%)	88.5 ± 1.21a	87.0 ± 0.699a	83 ± 1.819b	84 ±1.282ab

Means among rows followed by different letters differ significantly (P<0.005) by DMRT; BY = Baking Yeast.

CONCLUSIONS

In conclusion, liquid diet no.iii where baking yeast, soy bran and soy protein (2:1:1) was used as sources of protein seems to be highly promising for mass rearing of *B. dorsalis* under laboratory condition. The quality parameters of adult flies reared on the liquid diet *viz*, the hatchability, pupal yield, pupal weight, adult emergence, percent fliers and male-female ratios were determined and the flies were found to be competitive. The modified liquid diet help us to replace the bran based semi-liquid larval diet. The advantage of this liquid diet is to obviate the need to use a starter diet as described in Fay and Wornoayporn (2002) and a bulking agent. It also promotes a savings in labor and storage space. The liquid based diet with recyclable substrate system is cost effective and also maintains the quality of mass produced tephritid fruit flies. The modified liquid diet and its rearing system are suitable for further nutritional and genetic research. Moreover, with the basic knowledge and technology learned from this liquid diet may be applied to rear other insects such as parasitoid of fruit flies as reported by Chang *et al*. (2004). More detail studies will be required to examine the soundness of the rearing system and improvement of the nutritional ingredients of larval diet.

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