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Evaluation of artificial larval diets for rearing of fruit fly *Bactrocera zonata* (Diptera: Tephritidae) under laboratory condition

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Abstract
Impact of five different larval diets on various growth parameters of fruit fly Bactrocera zonata was studied in the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. Each diet was composed of basic ingredients (sugar, sodium benzoate, wheat bran, nipagen, HCL) and variable components i.e. (i) yeast extract, (ii) Protein Hydrolysate +Baker's yeast, (iii) Torula yeast, (iv) Protein Hydrolysate, (v) Baker's yeast. Among these Baker's yeast enhanced the efficacy of protein hydrolysate and resulted in highest egg hatchability (58%), development of larvae to pupal stage (50.53%) and fecundity (211.9) of female B. zonata. However, this treatment did not show significant statistical difference with others regarding adult emergence (92.35%) from pupae except sole protein hydrolysate treatment. The impact of these diets on sex ratio was not significant. The other treatments/larval diets gave variable results regarding the effect on various parameters of B. zonata.

Keywords: Artificial larval diets, Rearing, *Bactrocera zonat*a

1. Introduction
The fruit flies of the family Tephritidae are amongst the world's serious pests of fruit with over 4,000 species distributed throughout the world [7]. Feeding by fruit fly larvae (maggots) damages the fruit internally, causing it to ripen prematurely and rot. Up to 100% of fruits may be damaged by fruit fly when left uncontrolled [7]. As such, their infestation not only reduces the fruit yield but also affects the quality and commercial value of the crop.
In Pakistan, the important fruit flies of fruits and vegetables are peach fruit fly, *Bactrocera zonata* (Saunders); Oriental fruit fly, *Bactrocera dorsalis* Hendle; Melon fruit fly, *Bactrocera cucurbitae* Coq; Ethiopian melon flies, *Bactrocera ciliatus* Loew; and Ber fruit fly, *Carpomyia vesuviana* [9]. These flies cause losses to fruits and vegetables up to an estimated cost of $200 million, annually [8]. Guava productions by small farmers suffer 25 to 70% losses owing to the susceptible varieties and expensive management measures [8].
The occurrence of fruit flies mutually affects yield and quality of fruits. The larvae or maggots channel into the fruits for feeding on the pulp and make them unhealthy for human use. In certain fruits, rotting starts at the penetrated points. The fruit flies cause various losses according to the host plant species. In the scientific literature the most serious pest species reported is the oriental fruit fly (*B. dorsalis*), which cause 5-100 percent loss to various fruits [8]. As many as 22 eggs per day are laid by *B. zonata* and 300 to 800 eggs during her lifetime. The female fruit flies insert eggs into the skin of ripening fruits. Eggs hatch in 1.5 to 3 days during warm weather. The hatching period of eggs is significantly increased by lower temperatures [16]. Larvae upon hatching start eating, and make channel at first, but might remain close together in feeding until nearly full grown. Larva completes its development in 6-15 days depending on the temperature. Larvae depart the fruit and preferably pupate in the soil. Pupal stage lasts from 6-19 days depending on temperature. During warm weather adults emerge in largest numbers early in the morning and during cool weather emerge more infrequently [16]. Most of the adults emerge between 8 to 10 a.m. Attainment of sexual maturity depends upon eating by adults of food having important nutrients during post emergence period. Adults may feed on various foods with glandular secretion of plants, nectar, plant sap exudates from trunk/stem leaf, fruit injuries and honeydew secretes of insects, or by food sources. Adult flies are more active at sunrise and their activity declines gradually towards midday with rise in temperature and fall in relative humidity [1].

The preservation of biodiversity, undesirable chemical residues and environmental pollution enforce a need of new strategies and technologies for combating fruit flies. Among biological control methods, the sterile insect technique (SIT) is more target-specific and safe pest control method [5]. Through SIT a large population of sterile males are reared in a ''factory'' by exposing them to low doses of radiation. The infested area is then treated with these sterile males, whereby they perform mating with wild females which outnumber a large population of fertile wild males and thus quickly dies out the wild fly population [4].

Mass production of fruit fly at low cost for male sterility technique is essential. Proteins are the essential nutrients for cellular growth of life on Earth and thus at the same time is major components of fruit fly eggs. Female fruit fly needs a protein source to mature their oocytes to the vitellogenic stage and mate. Total egg laying capacity of a female is mainly determined by the amount of protein intake [2]. In SIT programs, the main nutritional/protein component of diet used for mass-rearing of adults and larvae of fruit flies are yeast products. [3, 11, 14].

Most commonly used yeasts in mass rearing fruit flies are Brewer's yeast and torula yeast (*Candida utilis*). The imported Brewer's yeast makes the mass rearing of fruit fly expensive due its high cost. Natural baker's yeast (*Saccharomyces cerevisiae*) and yeast extract can be obtained conveniently. Natural yeast and yeast extract contain all the vital microbial growth factors for fruit fly such as vitamins, high level of amino nitrogen, peptides, and minerals. These are economical and good alternatives of Brewer's yeast [15]. The yeasts have direct impact on the ultimate fecundity and fertility of the adult fruit flies. These important food ingredients increase the cost of production of artificial larval diet of fruit flies depending on its availability and formulations. The present study was therefore, planned to workout effective, locally available and inexpensive formulations of yeast for the application as the artificial diets.

Materials and Methods

Two experiments were conducted during summer 2009 at the Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar to evaluate economical artificial larval diets for the rearing of *B. zonata* under laboratory conditions.

The various parameters studied in this study included the percent eggs hatchability, larval development into pupae, emergence rate of adults from pupae, sex ratio and fecundity of the female. Percent hatchability was measured in liquid medium/liquid diets. The other parameters were studied in second experiment wherein solid diets/ treatments were applied.

Eggs Culture and Quantification in ml

Eggs were collected from fruit flies rearing cages through an egg collection device at laboratory of NIFA, Peshawar. The fruit flies eggs were quantified by using standard 1ml =2500 eggs. For eggs collection, plastic bottle (collection device/dummy fruits) having 1 mm holes throughout and smeared inside with the diluted juice of guava was kept in the rearing cages of fruit flies for eggs laying. After ensuring the presence of eggs (1-2 days), the collection device was rinsed with the small amount of tap water and the diluted solution thus collected at the bottom of the bottle including eggs was poured into 500 ml beaker. This water containing eggs was left for 20 to 30 minutes in beaker to allow the viable eggs to settle down. The unviable eggs that float on the surface of water were discarded. The settled eggs at the bottom of the beakers were spread on graded Petri dish and counted under a stereo microscope for the known volume, quantification and experimentation. The rearing room conditions were 23 ^{0}C ± 2 ^{0}C temperature and 60-70% humidity with a photoperiod of 12:12 (L: D).

Liquid diets experiment

In this experiment the effect of various liquid diets/treatments on the percent hatchability of eggs were studied. The proportions of various ingredients used in preparation of these diets are shown in Table 1.

Each diet was made by filling 100 ml of distilled water in a beaker. Then in each beaker known quantity of other ingredients (Table 1) were added and stirred thoroughly to prepare a homogenous solutions. Afterwards 100 eggs in each experimental unit (beaker) were added. Each treatment was replicated thrice. The beakers were then placed in incubator at 23±2 ^{o}C, 60-70% R.H and 12:12 (D: L) for 3-5 days. The solution from the beaker was poured into Petri dishes and examined under stereo microscope for percent eggs hatched.

Table 1: Compositions of various liquid diets applied to measure the percent hatchability of the eggs of *B. zonata.*

Treatments	Basic ingredients	Variable ingredients
Diet 1	100 ml water + 18.8 g sugar + 0.07 g sodium benzoate + 0.15 g Nipagen + 0.25 ml HCl	5 g Yeast extract
Diet 2	---------- do ----------	5 g Protein hydrolysate + baker's yeast
Diet 3	---------- do ----------	5 g Torula yeast
Diet 4	---------- do ----------	5 g Protein hydrolysate
Diet 5	---------- do ----------	5 g Baker's yeast

Solid diets experiment

In this experiment the effect of various solid diets/treatments on the development parameters of fruit fly *B. zonata* from egg hatching to egg laying were studied. The proportions of various ingredients used in preparation of these diets are shown in Table 2.

Diet preparation

Measured quantity of distilled water was taken in a plastic tub and sugar was added. After the dilution of sugar, other solid ingredients like yeast (variable ingredient), sodium benzoate, and nipagen along with HCl were added. At the end, wheat shorts/bran was added and mixed thoroughly for making a homogenous diet.

Percent pupae formation

Known number of eggs (500/per experimental unit) was put in a tray; containing larval diet. These trays were then shifted to a wooden box (91 x 6l x 91 cm3) containing saw dust as a medium for pupation and for subsequent collection of the pupae. The pupae thus formed in each treatment were sieved using mesh-10 and counted. Percent pupa formation was then calculated.

Table 2: Compositions of various solid diets applied to measure the percent hatchability of the eggs of *B. zonata*.

Treatments	Basic ingredients	Variable ingredients
Diet 1	1 lit. water + 188 g sugar + 0.7 g sodium benzoate + 1.5 g Nipagen + 2.5 g HCl + 388 g wheat shorts	50 g Yeast extract
Diet 2	---------- do ----------	50 g Protein hydrolysate + baker's yeast
Diet 3	---------- do ----------	50 g Torula yeast
Diet 4	---------- do ----------	50 g Protein hydrolysate
Diet 5	---------- do ----------	50 g Baker's yeast

Percent adult emergence

The collected pupae were shifted to adult rearing cage (1.21 x 0.45 x 1.52 cubic meter) containing casein + protein hydrolysate + sugar in the Petri dishes as adult diets for fruit flies. Clean cotton soaked in distilled water was placed in a small jar with distilled water for continuous supply of water and for the maintenance of moisture content inside the rearing cage. The number of adults emerged were calculated by visually observation. The percent adult's emergence and sex ratio of adults from the pupae was determined on daily basis till all of adults emerged.

Fecundity of female

Known number of males (50) and females (50) was put in a rearing cage. The fecundity/number of eggs laid by 50 females was determined through method explained earlier under heading "Eggs culture and quantification in ml".

Data analysis

All the experiments were conducted in Randomized Complete Randomized Design (CRD). There were five treatments and each treatment was replicated three times. The data were analyzed using one way ANOVA test. The means were separated using LSD test at 5% level of significance ($P<0.05$).

Results and Discussion

The effects of five different artificial larval diets on the hatchability of eggs of *B. zonata* are presented in Table 3. Significantly high egg hatchability was shown by eggs put in diet-2 (58.00%) followed by diet-3 (50.67%). Diet-4 and diet-1 showed low egg hatchability 34.67% and 38.00%, respectively with no significant difference. Diet-5 showed moderately significant egg hatchability (43.67%).

Table 3: Effect of larval diets on the eggs hatchability of *B. zonata*.

S. No.	Treatments	Mean percent eggs hatchability
1	Diet-1	38.00c
2	Diet-2	58.00a
3	Diet-3	50.67ab
4	Diet-4	34.67c
5	Diet-5	43.67bc

Mean followed by different letters in columns are significantly different from each other at 5% level of probability.

The effects of five different artificial larval diets on the percent pupae formation of *B. zonata* are presented in Table 4. Significantly high pupae formation was recorded in treatment where larvae were fed with diet-2 followed by diet-5 with no significant difference from diet-2 and diet-3. Diet-4 resulted in significantly low pupae formation followed diet-1.

Table 4: Effect of various diets on the percent pupae formation.

S. No.	Treatments	Mean percent pupae recovered
1	Diet-1	42.67c
2	Diet-2	50.53a
3	Diet-3	45.40bc
4	Diet-4	36.73d
5	Diet-5	48.07ab

Mean followed by different letters in columns are significantly different from each other at 5% level of probability.

The effects of five different artificial larval diets on the percent adult emergence from pupae of *B. zonata* are presented in Table 5. Significantly high adult emergence was revealed on diet-1, diet-2, diet-3 and diet-5. However, significantly low adult emergence was recorded in treatment where larvae were fed with sole protein hydrolysate. All the other treatments were statistically equal in adult emergence from pupae; however, highest emergence was recorded in protein hydrolysate + baker's yeast treatment followed by baker's yeast treatment.

Table 5: Effect of different artificial larval diets on the percent adult emergence from pupae of *B. zonata.*

S. No.	Treatments	Mean adult emergence	Mean percent adult emergence
1	Diet-1	197.0a	92.35
2	Diet-2	219.7a	86.94
3	Diet-3	201.0a	88.55
4	Diet-4	161.0b	87.34
5	Diet-5	215.0a	89.44

Mean followed by different letters in columns are significantly different from each other at 5% level of probability.

The effects of five different artificial larval diets on the percent sex ratio of *B. zonata* are presented in Table 6. Significantly high number of females with low number of males was produced by female *B. zonata* when fed with diet-4 treatment followed by diet-2 & diet-5 treatments. The lowest number of females with highest number of males was recorded in Torula yeast treatment.

Table 6: Effect of different artificial larval diet on the sex ratio of *B. zonata.*

S. No.	Treatment	Mean percent sex ratio	
		Male %	Female %
1	Diet-1	45.02a	54.98ab
2	Diet-2	43.32a	56.68b
3	Diet-3	47.55a	52.45ab
4	Diet-4	42.55b	57.45a
5	Diet-5	43.50a	56.50b

Mean followed by different letters in columns are significantly different from each other at 5% level of probability.

The effects of five different artificial larval diets on the mean fecundity of female *B. zonata* are presented in Table 7. Significantly high mean fecundity of female was recorded in treatment where larvae were fed with protein hydrolysate +baker's yeast (diet-2) followed by diet-1 and diet-3 with significant difference from diet-2, diet-4 & diet-5. Significantly low mean fecundity of female was recorded on Baker's yeast and protein hydrolysate (diet-5 & diet-4, respectively).

Table 7: Effect of different larval diets on the fecundity of female *B. zonata*.

S. No.	Treatments	Mean fecundity
1	Diet-1	206.1ab
2	Diet-2	211.9a
3	Diet-3	202.0ab
4	Diet-4	195.4b
5	Diet-5	193.2b

Mean followed by different letters in columns are significantly different from each other at 5% level of probability.

The data (Table 8) showed that Baker's yeast is more economical and easily available than the other protein sources. It is also significant for all biological parameters i.e hatchability, pupal recovery, adult emergence, sex ratio and for fecundity. It is therefore, recommended that Baker's yeast may be as a substitute for other expensive artificial larval diets.

Table 8: Estimated cost of protein sources used in artificial diets.

Treatments	Types of Protein sources	Market Price / 500gm (PKR)	Cost of 50gm (PKR)
D-1	Yeast Extract	7800	780
D-2	Protein hydrolysate + Baker'sYeast	1250 + 40 = 1290	129
D-3	Torula Yeast	11500	1150
D-4	Protein hydrolysate	2500	250
D-5	Baker's Yeast	80	8

Male sterility technique is one of the important management techniques used in IPM of insects. For male sterility technique, mass production of males at low cost in laboratories is the key component of SIT. Larval diets are mostly imported and these in result increases the cost of production of males or rearing of insects in laboratories. The research study in hand was an attempt to find larval diets that are locally available at comparatively low costs.

The efficacy of the larval diets was assessed on the basis of their impact on various developmental periods of *B. zonata* in its life cycle. The results revealed that percent hatchability of the eggs of *B. zonata* in protein hydrolysate was enhanced by the addition of baker's yeast. Baker's yeast though was lower than Torula yeast in eggs hatchability yet it was significantly higher than sole protein hydrolysate diet. The maximum development of larvae to pupae formation was recorded in treatments where the larvae were fed with protein hydrolysate + baker's yeast and sole baker's yeast diets. It showed that protein hydrolysate efficacy was enhanced by baker's yeast, as sole protein hydrolysate did not perform well. Similar results were achieved by Paskova (2006) [10] while reporting that larvae reared on new agar-based diet reached better results and higher pupal rearing than larvae reared on bran diet. Chang *et al.* (2006) [4] used different artificial liquid larval diet composed of brewer's yeast, sugar, antifungal agents (sodium benzoate and nipagen), citric acid, and distilled water. Larval rearing of fruit fly diet resulted in 20% less pupal production and 10% lighter pupal weight than from the control diet. Pupal recovery increased with yeast concentrations up to 14.2%.

The significantly lowest adult emergence was recorded in treatment where larvae were fed with sole protein hydrolysate. All the other treatments were statistically equal in adult emergence from pupae; however, maximum adult emergence was recorded in protein hydrolysate + baker's yeast treatment followed by baker's yeast treatment. These results showed that protein hydrolysate efficacy was enhanced by baker's yeast, as sole protein hydrolysate did not perform well. These results were in accordance with those of Chang *et al.* (2006) [4] and Fay (2009) [6] who observed no significant difference in the percent emergence of *Ceratitis capitata* on Torula yeast-based starter diets and bran reference diet.

The sex ratio due to all the five artificial larval diets was not significantly different, which proved that artificial diets have no effect on the male and female sex ratio. These results were in conformity with those of Qureshi *et al*. (2004) [13] who found adult emergence ranged from 89-99% having sex ratio of 1:1.

The highest mean fecundity of female was recorded in treatment where larvae were fed with protein hydrolysate +baker's yeast and yeast extract diets, both of which did not differ significantly. Torula yeast diet followed; however, it was statistically equal to yeast extract and sole protein hydrolysate diets. The lowest mean fecundity of female was recorded on Baker's yeast and protein hydrolysate diets. These findings are in line with those of Poramarcom and Mitchell (1983) [12] who observed no statistical difference observed in fecundity and fertility of flies reared in all media, wheat germ flakes and torula yeast. They found the media containing banana as the most suitable one for rearing fruit fly larvae; and all the three media containing rice bran as unsuitable for rearing this insect.

Conclusion

Protein hydrolysate + Baker's yeast yielded the highest number of eggs, hatchability, percent pupae formation, percent adult emergence, percent female production and fecundity. Baker's yeast was statistically equal to Protein hydrolysate + Baker's yeast diet in mean percent pupae formation, percent adult emergence and percent female production. While for mean percent hatchability and mean fecundity it was statistically equal to all other treatments. Cost on Baker's yeast diet is quite lower then that all other diets. On the bases of the observed results, it is recommended that Baker's yeast is the most economical diet for the mass rearing of *B. zonata* in the laboratory and could be the substitute for other expensive diets.

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