OPEN ACCESS Journal of Innovative Biology and Environmental Sciences

Research Article

Rearing Techniques of Peach Fruit Fly, *Bactrocera zonata* (Diptera: Tephritidae) using Natural and Artificial Diets

Inam Ullah*[1], Fawad Khan[2], Fazal Said[1], Khan Bacha[1], Sara Naeem[1], Meer Arif Ullah Khan[1], Kamran Khan[1], Muhammad Waqas[1]

[1]Department of Entomology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, Pakistan; [2]Medical Entomologist, Health Department KPK, Pakistan

Abstract | Fruit flies are responsible for significant agricultural losses, reducing crop values and creating challenges for exporters. To address these issues, establishing a robust laboratory-rearing system is crucial for conducting effective research and development activities, both in controlled environments and field settings. A key aspect of laboratory rearing is developing an economical and suitable artificial diet that supports the sustainability and vitality of fruit fly colonies. The two primary nutritional components essential for fruit fly development are proteins and carbohydrates. Proteins, in particular, play a vital role as they contain essential amino acids responsible for various metabolic functions. The availability of an appropriate protein source in the diet significantly influences the performance, survival, and longevity of fruit flies. Current study was designed to rear fruit flies on their natural hosts and evaluate the most suitable artificial diet comprising different protein sources: torula yeast, instant yeast, and hydrolyzed soybean, to determine their impact on life history traits of *Bactrocera zonata*. Results indicated that torula yeast is the most effective protein source for the development of *B. zonata*. The agar-based diet with instant yeast also showed promising results, though not as effective as torula yeast. The diet containing hydrolyzed soybean, however, was found to be the least effective compared to the other two diets.

Received | April 25, 2024; **Accepted** | August 10, 2024; **Published** | September, 2024
***Correspondence** | Inam Ullah, Department of Entomology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, Pakistan
Email: inamullahpk15@gmail.com
Citation | Ullah, I., Khan, F., Said, F., Bacha K., Naeem, S., Khan, M.A.U., Khan, K. and Waqas, M. 2024. Rearing Techniques of Peach Fruit Fly, *Bactrocera zonata* (Diptera: Tephritidae) using Natural and Artificial Diets. *Journal of Innovative Biology and Environmental Sciences*, 4(2): 11-17
Keywords | Fruit flies, *Bactrocera zonata*, Laboratory rearing, Artificial diet, Protein sources, Torula yeast

Introduction

Fruit flies (Diptera: Tephritidae) are considered a major agricultural pest worldwide, causing severe yield losses, reducing crop quality, and disrupting the

export of horticultural produce (McPheron and Steck, 1996; Clarke *et al*., 2005; Vargas *et al*., 2015) They pose a significant threat to a wide range of horticultural crops (Sarwar, 2015). Due to their destructive nature, fruit flies are classified as key quarantine pests and are often transported across borders through the international trade of fruits and vegetables (Permalloo, 1998; McQuate, 2015). The damage inflicted by these pests leads to a considerable reduction in farm output, resulting in limited export potential (Khan *et al*., 2020).

Among the fruit flies, the genus *Bactrocera* is comprised of around 651 documented species, making it one of the most economically significant fruit fly genera. Approximately 50 species within this genus are considered highly destructive, acting as major polyphagous pests of various horticultural and vegetable crops (Ahmad *et al*., 2005; Vargas *et al*., 2015; Saeed *et al*., 2022). In Pakistan, species such as *Bactrocera zonata*, *B. dorsalis*, and *B. cucurbitae* are responsible for significant losses in a broad range of fruits and vegetables (Ali *et al*., 1999; Ahmad *et al*., 2019; Sarwar *et al*, 2023).

The peach fruit fly, *Bactrocera zonata*, is widely distributed in tropical, subtropical, and temperate regions across the world. It infests a variety of host plants and is regarded as a key pest of fruits including apricot, mango, pear, plum, peach, banana, and apple. Depending on crop species, environmental conditions, and severity of infestation, losses can range from 30% to 100% (Dhillon *et al*., 2005). The abundance of *B. zonata* tends to increase when the temperature falls below 32°C and the relative humidity ranges around 60±10% (Bara, 2013). In holometabolous insects, variation in diet quality during developmental stages significantly influences key life-history traits (Ben-Yosef *et al*., 2023).

Two essential dietary components responsible for fruit fly growth are carbohydrates and proteins. Proteins, in particular, are crucial for metabolic processes, as they contain essential amino acids required for various physiological functions. It has been demonstrated that protein-rich diets enhance the performance, longevity, and overall fitness of insects (Cangussu and Zucoloto, 1997). In oviparous insects, proteins are directly linked to egg production (Braga and Zucoloto, 1981). In *Ceratitis capitata* (Mediterranean fruit fly), an increase in brewer's yeast concentration in the diet significantly enhanced egg production and longevity (Cangussu and Zucoloto, 1997). Similarly, the dietary protein content has a marked impact on the larval development of fruit flies (Lemos *et al*., 1992). Shelly and Kennelly (2002) reported that the addition of protein hydrolysate to the diet improved male mating success in fruit flies. Furthermore, imbalances in dietary amino acids can lead to adverse effects on growth and overall health (Dadd, 1985; Nash and Chapman, 2014).

To optimize fruit fly rearing for the production of parasitoids and facilitate effective pest management strategies, it is critical to develop suitable artificial diets that promote high pupal production. Present study focused on evaluating the effects of various protein- based diets on the mass rearing of *Bactrocera zonata*, by identifying the most effective dietary formulations.

2. Materials and Methods

The present studies were conducted on the fruit fly (*Bactrocera zonata*) at the laboratory of the Plant Protection division, Nuclear Institute for Meals and Agriculture (NIFA), Peshawar. *Bactrocera zonata* was maintained on natural hosts under managed laboratory conditions (27±1ºC, 60±5% relative humidity).

2.1. Handling of larvae and pupae

The host fruits included guava, banana, mango and peach. Fruit flies were kept in pre-maintained fly cages for *B. zonata* infestation and oviposition by gravid females. After forty-eight hours, the infested fruits were accumulated and shifted to the pupal chamber for larval development and subsequent pupal formation. After completing three instars inside the host fruits the third instar larvae emerged out of the end result to the pupation substrate (noticed dust or sand) for pupal development. The newly formed pupae were collected after noticed dust turned into sieved. The pupae had been saved in 10cm diameter petri dishes (50 pupae/petri dish) covered with filter paper and held in the grownup rearing cages till emergence of adults.

2.2. Rearing of adult flies

The newly emerged flies were maintained inside the rearing cages (35×30×35cm). Each rearing cage had wire mesh on 3 sides and a round sleeve door within the center of the front to facilitate the collection of adult flies and feeding. The male and female flies were fed seasoned-tein hydrolysate and sugar in petri dishes as a meal supplement. Water was provided in a 250ml glass jar and a thumb-sized water-soaked cotton swab was laid in immersed in water while the remaining half of stayed above the rim of the glass jar to preserve the water.

The artificial diet (provided in Table 1) was evaluated against infested guava and peach fruits. Healthy clean specimens of male and female *B. zonata* adults were collected from the pupae colony maintained inside the laboratory. The adult flies used for egg laying inside the laboratory-tailored cages measuring (45×30×35cm) with nylon mesh covers. The flies were reared on a weight-reduction plan containing sugar, and yeast placed in Petri dishes as meal complements. Water was changed frequently and cotton wicks were replaced inside a conical flask to prevent microbial infection, especially mildew. Flies were allowed to lay eggs inside plastic jars installed inside the adult fruit fly cages, having holes and internally smeared with banana juice. The eggs were gathered from the jar in a beaker full of water 24 hours after the flies had been allowed to lay eggs. The eggs separated from the holes and settled within the bottom while the sterile eggs floated on the surface. The eggs were then cautiously collected, counted and used for seeding on an artificial weight-reduction plan.

Table 1: Artificial diets used for *Bactrocera zonata*

Diets	Ingredients	Amount (g/ml)
Diet 1: Yeast Torula	Wheat bran	400g or as per need
	Sugar	40g
	Torula yeast	80-90g
	HCl	5ml
	Sodium benzoate	1.5g
	Nipagen	1g
	Water	250ml
Diet 2: Instant Yeast	Sugar	60g
	Yeast instant	60g
	Wheat flour	60g
	Water (distilled)	800ml
	Methylparahydroxybenzoate (Nipagen)	8ml
	Sodium benzoate	1g
	HCl	3ml
Diet 3: Soybean	Sugar	50g
	Soybean (crushed)	70g
	Wheat flour	60g
	Water (distilled)	800ml or as needed
	Methylparahydroxybenzoate (Nipagen)	7ml
	Sodium benzoate	1.5g
	HCl	3ml

Figure 1: Insect rearing lab at NIFA Peshawar

3. Results

The effects of numerous non-natural larval diets on the *B. zonata* pupae formation are shown in Figure 3. The results revealed that a higher percentage of pupae emerged as adults in the larvae fed with diet 1, followed by the larvae fed with diet 2, whereas larvae fed with diet 3 recorded considerably low pupae formation and recovery rates.

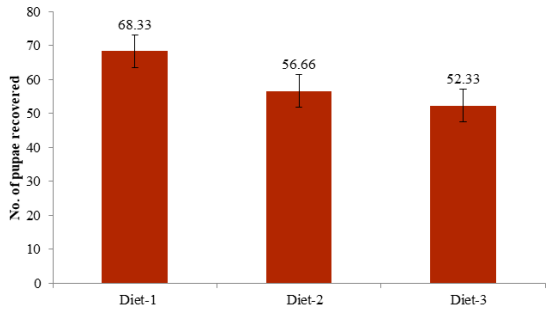

Figure 3: Effects of non-natural larval diets on the pupae formation and recovery of *B. zonata*

The impact of three synthetic larval diets on the percentage of adult fly emergence from pupae of *B. zonata* is offered in Figure 4. The results showed that there was a better adult emergence percentage in diet 1 larvae i.e., food regimen having Torula yeast as a protein source, followed by the diet 2 food regimen having instant yeast, whereas the lowest percentage was recorded in larvae had been fed diet 3 food regimen having soybean hydrolysate.

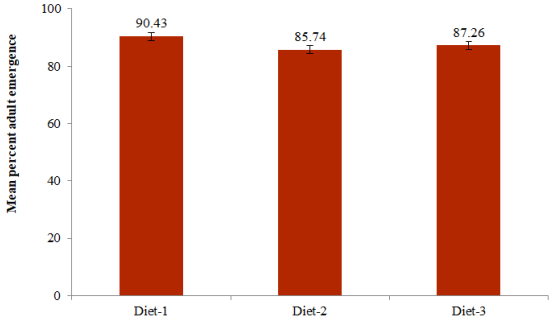

Figure 4: Effects of non-natural larval diets on the percentage of adult emergence from pupae of *B. zonata*

The impact of three distinct synthetic non-natural larval diets on the percentage ratio of *B. zonata* intercourse is supplied in Figure 5. It was observed that the intercourse ratio of *B. zonata* exhibited variations among male and female flies. The intercourse percentage ratio of female flies was slightly higher than that of males in all diet regimens.

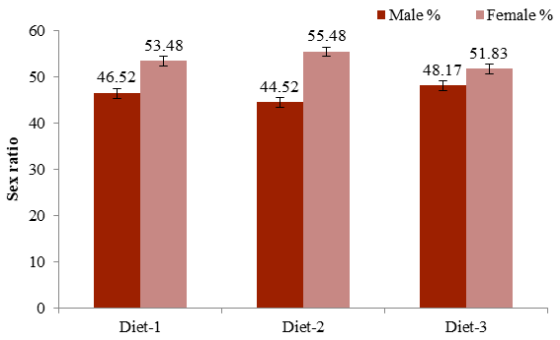

Figure 5: Effects of non-natural larval diets on the percentage of intercourse ratio of *B. zonata*.

The impact of three distinct synthetic non-natural larval diets on the durability and adult longevity of *B. zonata* is presented in Figure 6. Amongst all of the three diet treatments, diet 1 produced promising outcomes by showing better durability of *B. zonata* male and female flies as compared to the rest of the diets.

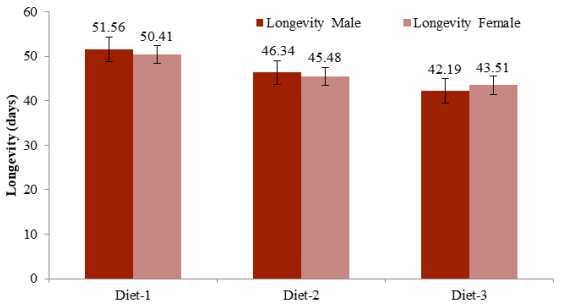

Figure 6: Effects of non-natural larval diets on the percentage of durability and adult longevity of *B. zonata*

4. Discussion

Present study revealed that troula yeast is the perfect best protein source for the

laboratory rearing of *B. zonata* larvae. The agar-based food plan in which yeast was used as a protein source also produced promising outcomes. These results are consistent with Paskova (2007), who reported larvae reared on agar-based total food regimen with superior outcomes and higher pupal rearing. Chang and Vargas (2014) used numerous artificial liquid larval diets consisting of products of brewer's yeast, sugar, antifungal entrepreneurs (sodium benzoate and nipagen), citric acid, and distilled water. Fruit fly diet plan larval rearing resulted in a 10% decrease in pupal weight and a 20% less pupal output. Similarly, Shelly *et al*., (2005) determined that the inclusion of protein inside the oriental fruit fly diet complemented the male mating performance. Such studies might be useful in mass rearing to provide the economically efficient, extensive variety of healthy fruit flies without difficulty available all through the year. In addition, these findings of food components can be greatly beneficial in the low-cost mass rearing of fruit flies.

5. Conclusion

It can be concluded from the prevailing research findings that diet 1 containing torula yeast as a predominant ingredient is the best diet plan for laboratory scale rearing of peach fruit fly *B. zonata* as compared to the other two diets evaluated in current work. Subsequently, based on present results, it is advocated that implementation of diet regimen 1 i.e., primarily torula yeast based synthetic diet plan can be used for laboratory rearing and renovation of peach fruit fly *B. zonata* under controlled laboratory conditions.

6. Author's Contribution

Inam Ullah was responsible for the conceptualization, methodology, data curation and original draft preparation. Fawad Khan supervised the project, secured funding, managed project administration and contributed to the writing through review and editing. Fazal Said and Meer Arif Ullah Khan conducted formal analysis, investigation and visualization. Khan Bacha provided resources, validated findings and contributed to data analysis. Sara Naeem managed data collection, conducted the literature review and prepared references. Kamran Khan handled software development, data management and interpretation. Muhammad Waqas performed statistical analysis, interpreted results and proofread the manuscript.

7. Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

8. References

Ahmad, B., Anjum, R., Ahmad, A., Yousaf, M.M., Hussain, M. and Muhammad, W., 2005. Comparison of different methods to control fruit fly (*Carpomyia vesuviana*) on ber (*Zizyphus mauritiana*). *Pakistan Entomologist*, *27*(2), pp.1-2.

Ahmad, J.N., Sharif, T., Ahmad, S.J., Maqsood, S. and Zafar, F., 2019. Molecular identification and characterization of fruit flies of genus *Bactrocera* (Diptera: Tephritidae) in Pakistan. *Pakistan Journal of Zoology*, *51*(6), p.2275.

Bara, G.T., 2013. *Preliminary studies on the winter season abundance and fitness of tephritid fruit flies in the Harare area, Zimbabwe* (Doctoral dissertation, University of Zimbabwe).

Ben-Yosef, M., Altman, Y., Nemni-Lavi, E., Papadopoulos, N.T. and Nestel, D., 2023. Larval nutritional-stress and tolerance to extreme temperatures in the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae). *Fly*, *17*(1), p.2157161.

Braga, M.A.S. and Zucoloto, F.S., 1981. Estudos sobre a melhor concentração de aminoácidos para moscas adultas de Anastrepha obliqua (Díptera, Tephritidae). *Revista Brasileira de Biologia*, *41*, pp.75-79.

Cangussu, J.A. and Zucoloto, F.S., 1997. Effect of protein sources on fecundity, food acceptance, and sexual choice by *Ceratitis capitata* (Diptera, Tephritidae), *Revista Brasileira de Biologia*, *57*, pp. 611-618.

Chang, C.L. and Vargas, R.I., 2014. Wheat germ oil and its effects on a liquid larval rearing diet for oriental fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology*, *100*(2), pp.322-326.

Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K. and Yeates, D.K., 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology.*, *50*(1), pp.293-319.

Dadd, R.H., 1985. Nutrition: organisms. *Regulation: digestion, nutrition, excretion*, pp.313-390.

Dhillon, M.K., Singh, R., Naresh, J.S. and Sharma, H.C., 2005. The. melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *Journal of Insect Science*, *5*(1), p.40.

Ishtiaq Ali, I.A., Farman Ullah, F.U. and Shah Alam Khan, S.A.K., 1999. Efficacy of various insecticides and trap heights in methyl eugenol baited traps against fruitflies (*Bactrocera* spp.). *Sarhad Journal of Agriculture. 15*(6), pp. 589-594.

Khan, M.H., Khuhro, N.H., Awais, M., Memon, R.M. and Asif, M.U., 2020. Functional response of the pupal parasitoid, *Dirhinus giffardii* towards two fruit fly species, *Bactrocera zonata* and *B. cucurbitae Entomologia Generalis*, *40*(1).

Lemos, F.J., Zucoloto, F.S. and Terra, W.R., 1992. Enzymological and excretory adaptations of Ceratitis capitata (Diptera: Tephritidae) larvae to high protein and high salt diets. *Comparative Biochemistry and Physiology Part A: Physiology*, *102*(4), pp.775-779.

McPheron, B.A. and Steck, G.J. eds., 1996. *Fruit fly pests: a world assessment of their biology and management*. CRC Press.

Mcquate, G.T., Follett, P.A., Liquido, N.J. and Sylva, C.D., 2015. Assessment of navel oranges, clementine tangerines, and rutaceous fruits as hosts of *Bactrocera cucurbitae* and *Bactrocera latifron*s (Diptera: Tephritidae). *International Journal of Insect Science*, *7*, pp.IJIS-S20069.

Nash, W.J. and Chapman, T., 2014. Effect of dietary components on larval life history characteristics in the Medfly (*Ceratitis capitata*:

Diptera, Tephritidae). *PLoS One*, *9*(1), p.e86029.

Pašková, M., 2007. New larval agar-based diet for laboratory rearing of Mediterranean fruit fly *Ceratitis capitata* (Diptera, Tephritidae). *Biologia*, *62*(4), pp.477-481.

Permalloo, S., Seewooruthun, S.I., Joomaye, A., Soonnoo, A.R., Gungah, B., Unmole, L. and Boodram, R., 1998, March. An area wide control of fruit flies in Mauritius. In *Proceedings of the 2 nd Annual Meeting of Agricultural Scientists, Food and Agricultural Research Council*, pp. 203-210.

Saeed, M., Ahmad, T., Alam, M., Al-Shuraym, L.A., Ahmed, N., Alshehri, M.A., Ullah, H. and Sayed, S.M., 2022. Preference and performance of peach fruit fly (*Bactrocera Zonata*) and Melon fruit fly (*Bactrocera Cucurbitae*) under laboratory conditions. *Saudi Journal of Biological Sciences*, *29*(4), pp.2402-2408.

Sarwar, M., 2015. Quarantine treatments for mortality of eggs and larvae of fruit flies (Diptera: Tephritidae) invading fresh horticulture perishable produces. *International Journal of Zoology and Animal Biology*, *1*(5), pp.196-201.

Sarwar, M., Rasool, B., Shah, M.M. and Ahmad, N., 2023. Effects of environmental variables and role of food attractants for management of *Bactrocera zonata* (Saunders, 1842) and *Bactrocera dorsalis*, (Hendel, 1912) (Diptera: Tephritidae). *Journal of the Entomological Research Society*, *25*(3), pp.563-578.

Shelly, T.E. and Kennelly, S., 2002. Influence of male diet on male mating success and longevity and female remating in the Mediterranean fruit fly (Diptera: Tephritidae) under laboratory conditions. *Florida Entomologist*, *85*(4), pp.572-579.

Shelly, T.E., Edu, J. and Pahio, E., 2005. Influence of diet and methyl eugenol on the mating success of males of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *Florida Entomologist*, *88*(3), pp.307-313.

Vargas, G., Gómez, L.A. and Michaud, J.P., 2015. Sugarcane stem borers of the Colombian Cauca River Valley: current pest status, biology, and control. *Florida Entomologist*, *98*(2), pp.728-735.

Vargas, R.I., Piñero, J.C. and Leblanc, L., 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific region. *Insects*, *6*(2), pp.297-318.