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Research Article

Dietary Preferences and Their Impact on the Life Cycles of *Bactrocera Zonata* and *Bactrocera Dorsalis*

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Abstract: This study aimed to contribute to the biological control of two economically significant fruit flies species, *Bactrocera zonata* and *Bactrocera dorsalis*. The research examined the effects of different host fruit species and artificial larval diets on their development.. Experiments were conducted under controlled laboratory conditions (28 ± 2 °C, 60-65% relative humidity). Specified five fruit varities as host including apple (*Malus sylvestris*), guava (*Psidium guajava*), mango (*Mangifera indica*), persimmon (*Diospyros kaki*), and pomegranate (*Punica granatum*). Key biological parameters taken into consideration were egg production, larval and pupal development time, survival rate, adult emergence percentages, and sex ratios. Comparisons were made between liquid and solid artificial diets under different host conditions. Results revealed that *P. guajava* was the most favorable host, yielding optimal egg production (297.3 ± 9.3), larval numbers (261 ± 3.2), pupal counts (237.6 ± 27.1), and adult emergence rates (86.9 ± 3.9%). Similarly, *P. granatum* exhibited the longest egg hatching duration (2.3 ± 0.3 days) and larval development time (7.6 ± 0.3 days). While *M. sylvestris* and *D. kaki* showed maximum pupal duration (4.3 ± 0.3 days). Solid artificial diets produced higher egg numbers (408 ± 108.3) and extended developmental periods compared to liquid diets, though liquid diets achieved superior adult emergence rates (80 ± 2.8%). It is concluded that *P. guajava* serves optimally for *Bactr ocera* mass rearing. Moreover, solid diets enhance reproduction while liquid diets improve adult emergence. This can help and inform existing and future biological control programs.

Keywords: Biological Control, Fruit Flies, Mass Rearing, Artificial Diet, Cucurbitaceae, Fruit Preference, Larval Diet.

1. INTRODUCTION

B. zonata is a polyphagous tephritid with its native habitat in tropical Asia before spreading to southern and Southeast Asia, the Middle East, and northern Africa and infesting more than 50 species of wild and cultivated fruits [1, 2]. Similarly, the oriental fruit fly *B. dorsalis* is characterized by a large host range, which covers a wide range of fruits and vegetables, which allows it to spread among territories worldwide and representing a long-lasting challenge to production systems [3, 4]. This broad host specification not only contributes to their pest status but also causes difficulties in their control due to the diversity of nutritional needs required during life cycles [5, 6]. These two species share a specific preference for fleshy fruits like mangoes, guava, citrus, peach, apricot, and figs since these give the best substrates on oviposition and larval development in temperate, subtropical, and tropical zones [7].

The flexibility of adaptation in different agro-climatic conditions and the high reproduction rate make it harder to monitor and control, as the larvae can hide in the fruit pulp impossible to detect at an early stage and treated with chemicals [8]. As a result, their hidden larval growth in the tissues

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of fruits makes them the priority of combined surveillance and management strategies over areas to contain their growth in the tropical and subtropical fruit systems [9]. These qualities explain why area-specific monitoring guidelines are urgently required, because the recent invasion of the *B. dorsalis* on Reunion Island has exacerbated mango infestations alongside *B. zonata* [10].

B. zonata and *B. dorsalis* cause significant economic damages to commercial crops in different countries due to direct decreases in yield and difficult to import fruits due to quarantine against infected hosts [11, 12]. The pests are especially costly since they destroy more than 40 species of fruit crops and require expensive phytosanitary measures, and in the case of fruit flies, crops losses are estimated at about 353.2 million US dollars [13, 14]. The economic losses caused by these infestations are 144.6 million dollars per capita and have caused the host production, which is popular in the southern areas, to be abandoned; this is because of the heavy losses and high management costs [2, 15]. Fruit flies are also difficult to manage because of their behavioral flexibility at different development stages, and possess resistance against several chemical pesticides, thus there is a need of alternative control solutions [16, 17]. As a result, integrated pest management approaches, including the use of fruit bags, protein baits with ammonium-based synergist, and installing traps are promoted to reduce these economic costs and minimize environmental risks of conventional eradication measures [18, 19].

Polyphagous fruit flies such as *B. zonata* and *B. dorsalis* possess distinct ecological characteristics of contrasting developmental times and survival rates of all life cycles, with *B. zonata* completing its cycle comparatively faster than *B. dorsalis* which can confer competitive advantages during comparable hosts [12]. Host fruit preferences also moderate these competitive dynamics with *B. dorsalis* showing different fitness behaviors in guava, papaya, and banana which affect the potential to increase a population in different agroecosystems [20]. This dietary plasticity highlights the underlying evolutionary importance of phenotypic plasticity in such species, in which alterations in gene expression in connection to metabolic and stress-related pathways allow adaptation to changing nutritional qualities of host fruits [21]. Such plasticity is not only beneficial to survive throughout the changes in the availability of resources but also leads to evolutionary trade-offs in the aspects of life-history due to the modulated stress resistance and reproductive plasticity to dietary limitations [21]. As a result, the specified trade-offs are reflected in opposite larval survival and developmental intervals of the species under standardized conditions, which determines their invasion success and interspecific relationships within fruit orchards [12, 21]. The interspecific interactions are also favored by the high variation in the developmental timing of the host fruits, such as the reduction of the third stage of the instar stage and the pupal period of *B. dorsalis* on guava compared with mango and apple; which influence the relative fitness and has the ability to nurture in shared niches [12, 15].

Although there are fundamental gaps in comprehending the research on the pupal growth during different soil regimes, demonstrating how environmental factors affect *B. dorsalis* assessment and stage length, and how all three factors interact with dietary host preferences, and subsequent life cycle fitness are yet to be examined [22]. Additionally, the molecular processes underlying diet-based changes in gene expression and phenotypic plasticity, and their occurrence in related insects, are not yet understood in either *B. zonata* or *B. dorsalis*, which restricts understanding of adaptive responses to nutritional gradients [21]. Moreover, genetic and biotechnological management research is obstructed by the inability to rear both species in mass culture due to their difficulties in culturing at different developmental stages and the absence of standardized artificial diets with diverse nutritional profiles [23].

Artificial diets allow biological parameters in *B. zonata*, which supports parasitoid production to a higher level of pest management [24]. The presence of polypaganthic, high-fecundity, and multivoltine life cycles makes it extremely difficult to mass rear these fruit flies using standard artificial diets, and alternative diets are required to facilitate adaptation in the laboratory and enable advanced pest control programs involving these fruits flies [23]. New artificial diets demonstrated a higher percentage of pupation, adult expression, and ability to fly in mass-reared *B. dorsalis* including liquid diets with mixture of substrates and demonstrated to overcome the limitations of traditional bran-based media [3, 25]. Such recipes also enhance high flight capacity and longer adult life in *B. dorsalis*, which is well above the international standards of sterile insect releases [3, 26]. These advancements have been extended to *B. zonata*, in which artificial feeding approaches open continuous laboratory culture to overcome seasonal limitations on host availability to research and application of sterile insect techniques [27]. As a result, the development of semi-solid artificial diets with yeast, sucrose, and preservatives has been very effective in mass rearing *B. dorsalis* under controlled laboratory environments [3]. This effectiveness focuses on increasing the use of artificial rearing of *B. doralis* aiding biological control and sterile insect approaches by aiding quality-in-large-scale production proven through a test of larval food substrates in the laboratory [28]. The present study aims to evaluate the ovipositional preference and larval success of *B. zonata* and *B. dorsalis* under choice and no-choice conditions. This information will help to assess the host vulnerability and optimize mass rearing protocols.

2. MATERIALS AND METHODS

2.1. Sample and Sampling

Adult flies of *B. zonata* and *B. dorsalis* were collected from established cultures (originally from a field at Gilgit (35°55′11.36” N, 74°22′47.44” E). Pakistan. Flies were kept in rearing cages (60 × 60 × 48 cm) with cloth sleeves opening at the front for handling. Adults were treated using standard laboratory conditions [29]. Adults were given water-based diet and water on dripping cotton *ad libitum*. For egg laying, adults were offered fresh fruit, and infected fruit were later detached and put into a plastic container with sand at the bottom for pupation. Pupae were separated from sand by using a mesh screen and kept in a jar (15 × 6 × 6 cm) until adults emerged. Temperature was maintained at 25 °C - 30 °C for 24 hours under light conditions.

2.2. Host Fruits Used

Apple (*Malus sylvestris*), guava (*Psidium guajava*), mango (*Mangifera indica*), persimmon (*Diospyros kaki*), and pomegranate (*Punica granatum*) were available at market during studied period at Gilgit. A no-choice test was used to evaluate host exposure, preference, and progeny of *B. zonata* and *B. dorsalis* separately. Adult *B. zonata* and *B. dorsalis* (5-7 days old) were used separately for additional experiments (male to female ratio 1:1). The experiments were conducted at a research laboratory setup [29]. Each host treatment had three replications. Randomly Fifty pairs of *B. zonata* and *B. dorsalis* adults were kept separately in plastic containers (15 × 6 × 6 cm) and provided 300 g of each host fruit for 48 hours. The exposed host was later exchanged with fresh fruits and infected hosts were checked for oviposition punctures per host for each species. Oviposited fruits were put into plastic boxes with sand substratum. Pupae were sieved, and the number was recorded. The pupae were put into plastic jars to observe adult appearance and the male-to-female ratio.

2.3. Artificial Larval Diet

Liquid artificial larval diet was prepared by mixing 100 mL of distilled water with sugar, sodium benzoate, vitamin B complex, citric acid and streptomycin to make a homogenous solution (Table 1). A beaker containing the mixture of ingredients was put into a freezer. The prepared liquid artificial diet was poured into a container. An egg-laying device instead of a fruit was used. Ten eggs were put into the container with the artificial diet, and the lid was closed to make it airtight. Treatment was replicated three times. The lid of the container was perforated at two days interval. After the third larval instar, the container was opened and pupae were collected [30].

Ingredients of solid artificial larval diet for *B. zonata* and *B. dorsalis* are listed in Table 2. The constituents of group ‘A’ were mixed carefully in a mixer with 550 ml of deionized water. Agar-agar

Table 1. Composition of liquid artificial larval diet.

Ingredient	Quantity
Distilled water	400 ml
Sugar	+48.7 g
Sodium benzoate	0.8 g
Vitamin B complex	+1 ml
Citric acid	+9.2 g
Streptomycin	+1 ml
Baker’s yeast	5 g

Table 2. Composition of solid artificial larval diet.

Group	Ingredient	Quantity (g)
Fraction A (main ingredients)	Wheat germ	26.0
	Kidney bean *Phaseolus vulgaris* flour	51.3
	Chickpea *Cicer arietinum* var. Kabuli flour	56.0
Fraction B	Dried yeast powder	31.6
	Casein	15.2
	L-Ascorbic acid	3.2
	Cholesterol	0.5
	Multivitamin multi-mineral capsule	2 capsules
	Vitamin E capsule USP	1 capsule
	Castor oil USP	1 ml
	ABDEC drops	2 ml
Fraction C	Methyl-p-hydroxybenzoate	1.8
	Sorbic acid	1.3
	Streptomycin sulfate	0.25
	Formaldehyde solution	2 ml
Fraction D	Agar-agar	16.4
Distilled water		820 ml

(Group 'D') weighed dissolved in 270 ml of warm water, heated in a glass beaker, cooled and mixed for about one minute with the other constituents at fast speed in mixer. The components of groups 'B' and 'C' were mixed well. The solution was transferred to disinfected Petri dishes (15-cm diameter). The diet was cooled until it solidified in a refrigerator. The diet was removed from the refrigerator and kept at room temperature for 2-3 hours before use. The solid diet was divided and transferred to plastic rearing containers for development of larvae [31]. Mean percentage of adult fly emergence was calculated using a formula given by Gupta *et al.* [29]:

$$\text{Adult emergence (\%)} = \frac{\text{Number of adults emerged}}{\text{Total number of pupae}} \times 100$$

Sex ratio (female) was calculated using a formula specified by Farooq and Freed [32]:

$$\text{Sex ratio (female)} = \frac{\text{Number of females emerged}}{\text{Total (male + female)}} \times 100$$

Data was subjected to Statistic 8.1 [33]. Mean data on parameters (number of eggs, number of larvae, larval development time, number of pupae, percentage of adults emerged, and sex ratio.

3. RESULTS

Host preferences by life stages of *B. zonata* and *B. dorsalis* are shown in Figures 1 and 2, respectively. Eggs laid by *B. zonata* (297.3 ± 16.1) and *B. dorsalis* (253.6 ± 44.6) were maximum on *P. guajava.* Days for larval development of *B. zonata* and *B. dorsalis* were maximum on *P. granatum* (11.6 ± 0.5 and 12.3 ± 1.1 respectively and fewest on *P. guajava* (7.6 ± 0.5 and 7.3 ± 0.5). The maximum number of larvae produced on *P. guajava.*by *B. zonata* (261 ± 5.5) and by *B. dorsalis* showed (244.3 ± 41.4) were similar compared to other hosts.

Most pupae were produced by *B. zonata* (233 ± 27.6) and *B. dorsalis (*237.6 ± 46.9) on *P. granatum*. Pupal duration was minimum on *P. guajava,* but equally maximum (4.3 ± 0.5) on *P. granatum, M. sylvestris,* and *D. kaki*. *B. dorsalis* had maximum pupal duration (4 ± 1) on *P. granatum* but equally minimum on *P. guajava* and *D. kaki* compared to other hosts. Percentages of adults' emergence of *B. zonata and B. dorsalis* were similar and greatest (82 ± 7.8 and 86.6 ± 6.8, respectively) on *P. guajava.*

P. granatum resulted in the greatest female-to-male ratio **(**female = 1.2 ± 0.17, male = 0.84 ± 0.13)

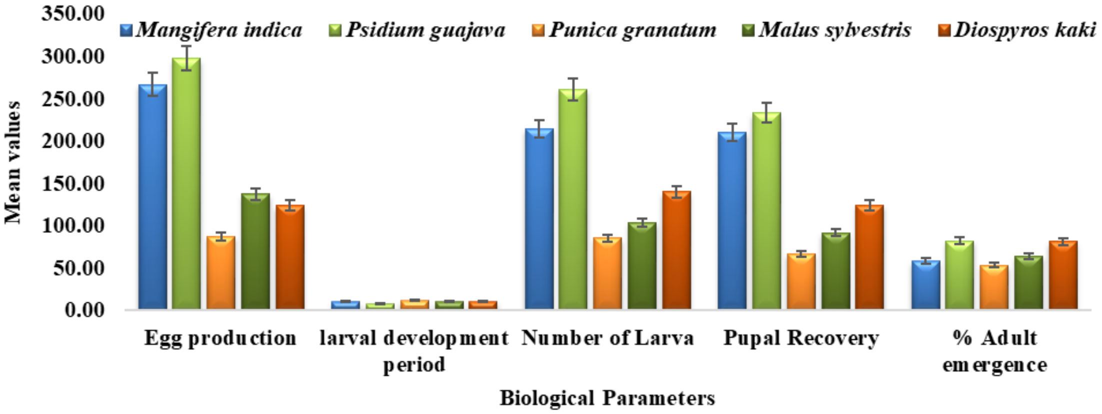

Fig. 1. Biological parameters of *B. zonata* fruit flies on different host fruits.

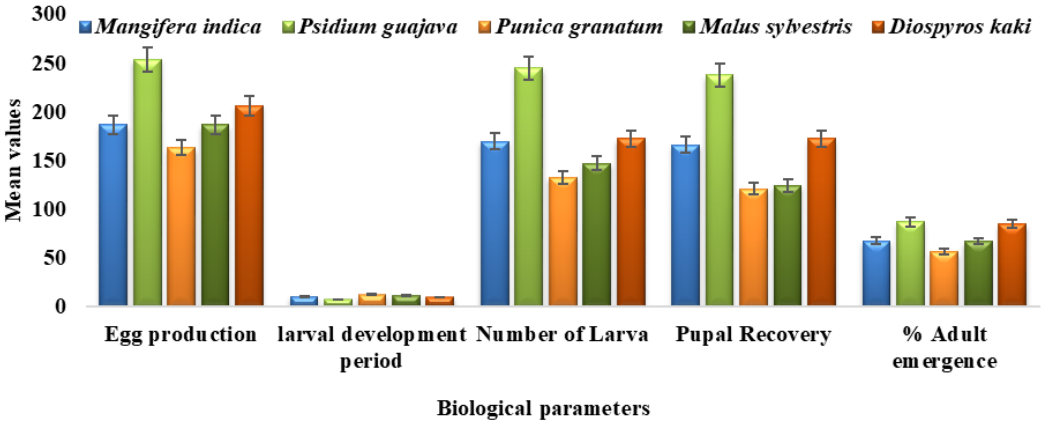

Fig. 2. Biological parameters of *B. dorsalis* fruit flies on different host fruits.

of *B. zonata*, followed by *M. sylvestris* (female = 1.17 ± 0.02, male = 0.86 ± 0.14)). *D. kaki* resulted in the greatest ratio of males to females (male = 1.19 ± 0.23, female = 0.85 ± 0.15), followed by *P. guajava* (male = 1.16 ± 0.03, female = 0.85 ± 0.02).

The greatest female-to-male ratio for *B. dorsalis* was on, *M. indica* (female = 1.61 ± 0.09, male = 0.62 ± 0.06), followed by *P. guajava* (female = 1.18 ± 0.05, male = 0.84 ± 0.04), as shown in Table 3. *P. granatum* resulted in the greatest male-to-male ratio for *B. dorsalis* (male = 1.09 ± 0.39, female = 0.99 ± 0.33), followed by *M. sylvestris* (male = 1.03 ± 1.17, female = 0.98 ± 0.18).

B. zonata produced most eggs (218.3 ± 15.8), as did, *B. dorsalis* (408 ± 108.3) on solid rather than liquid artificial larval diet (Figures 3 and 4). Development duration of *B. zonata* and *B. dorsalis* larvae were similar (20.6 ± 0.57) on solid compared with liquid artificial larval diet. The number of larvae of *B. zonata* and *B. dorsalis* were 182 ± 2 and 258.6 ± 50.1, respectively, on solid compared with liquid artificial larval diet, see Figures 3 and 4.

More pupae of *B. zonata* (142.33 ± 22.5) and *B. dorsalis* (187.6 ± 41.6) developed on solid compared to liquid artificial larval diet (Figures 3 and 4). Larval developmental period was longest for *B. zonata* (9.3 ± 0.57) and *B. dorsalis* (9.67 ± 0.57) on solid artificial larval diet. Percentage of adults emerged *B. zonata* (80 ± 2.8) and *B. dorsalis* (66.6 ± 3.3) was greater on liquid artificial larval diet.

Liquid artificial larval diet resulted in more female than male *B. zonata* adults (female = 1.2 ± 0.02, male = 0.8 ± 7.8), compared with solid artificial larval diet (female = 1.08 ± 0.05, male = 0.9 ± 0.04). For *B. dorsalis*, solid artificial larval diet resulted in a greater female-to-male ratio (female = 1.07 ± 0.14, male = 0.97 ± 1.14) than did liquid artificial larval diet (female = 1.02 ± 0.14, male = 1.0 ± 0.13); these results are presented in Table 4.

Table 3. Ratios of male and female of fruit flies on studied fruits.

Host treatment	*B. zonata*		*B. dorsalis*	
	Male ratio	Female ratio	Male ratio	Female ratio
Mango, *Mangifera indica*	1.08 ± 0.03	0.92 ± 0.04	0.62 ± 0.06	1.61 ± 0.09
Guava, *Psidium guajava*	1.16 ± 0.03	0.85 ± 0.02	0.84 ± 0.04	1.18 ± 0.05
Pomegranate, *Punica granatum*	0.84 ± 0.13	1.2 ± 0.17	1.09 ± 0.39	0.99 ± 0.33
Apple, *Malus sylvestris*	0.86 ± 0.14	1.17 ± 0.02	1.03 ± 1.17	0.98 ± 0.18
Persimmon, *Diospyros kaki*	1.19 ± 0.23	0.85 ± 0.15	0.94 ± 0.04	1.06 ± 0.05

Table 4. Male and female ratio of fruit flies on artificial larval diet.

Treatment given	*B. zonata*		*B. dorsalis*	
	Male ratio	Female ratio	Male ratio	Female ratio
Liquid artificial larval diet	0.8 ± 7.8	1.2 ± 0.02	1.0 ± 0.13	1.02 ± 0.14
Solid artificial larval diet	0.9 ± 0.04	1.08 ± 0.05	0.97 ± 1.14	1.07 ± 0.14

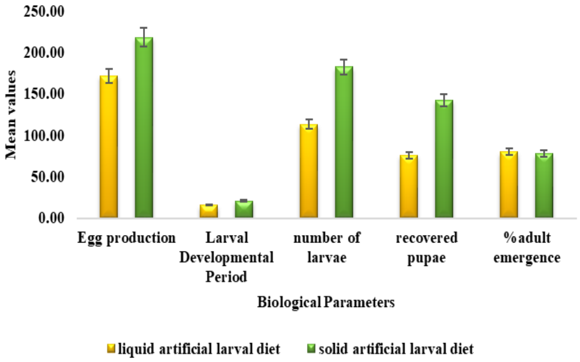

Fig. 3. Biological parameters of *B. zonata* fruit flies on liquid and solid artificial larval diet.

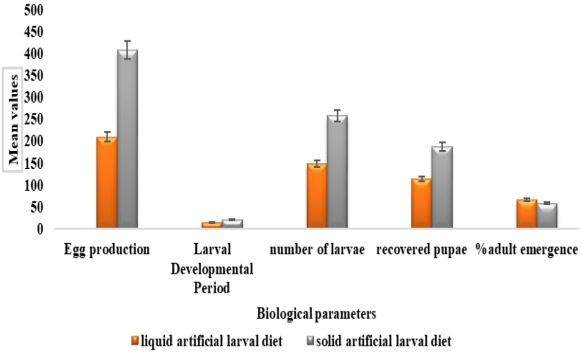

Fig. 4. Biological parameters of *B. dorsalis* fruit flies on liquid and solid artificial larval diets.

4. DISCUSSION

Host preferences of *B. zonata* and *B. dorsalis* fruit flies for five types of fruit apple, guava, mango, persimmon and pomegranate were tested at controlled laboratory conditions. The study showed significant differences among development of immature stages until adults emerged on different host fruits.

Aroma receptors are important for fruit flies from distant areas to find food sources Reisenman and Scott [34]. In present study most pupae were recovered on *P. guajava* and fewest on apple; Rauf *et al.* [31], Kalia and Yadav [35] also found most eggs produced on *P. guajava*. In contrast to our study, *M. indica* was most favorable for egg production in studied by Sarwar *et al.* [36]; but these researchers did not include *P. guajava* in their studies, perhaps because *M. indica* was most preferred by fruit flies. In a current study overall development was maximum on *P. guajava* and minimum on *D. kaki*. In similar study Kalia and Yadav [35] found larval development was longest on *P. guajava* followed by *M. indica*. Most pupae were developed from *P. guajava* and least from *P. granatum.* In contrast to our investigation *M. indica* was most favored, while *M. sylvestris* was least favored for pupae [35, 36].

The impact of guava fruit on longevity, growth and development of fruit fly was established during a similar study as the skin of guava fruit being very thin and soft played vital role in palatability and consumption by fruit flies [15]. A similar pattern was observed in present study. In contrast to our study, *M. indica* was preferred, followed by *M. sylvestris* for percentage emerging reported by Sarwar *et al.* [36]. Kalia and Yadav [35] found most adult emerging on *P. guajava* and lowest by *M. indica*.

During present study two artificial diets (solid and liquid) were tested at laboratory conditions to evaluate biological parameters of *B. zonata* and *B. dorsalis.* Solid artificial larval diet resulted in a greater number of eggs (218.3 ± 15.8) than did liquid diet (171.6 ± 15.8). According to Sookar *et al.* [38] significant eggs were produced on liquid artificial larval diet. Current Study showed solid artificial larval diet resulted in a greater number of larvae (182.3 ± 15.9) than did liquid diet (113 ± 7.5). According to Sookar *et al.* [37], Momen *et al.* [38], and Shinwari *et al.* [30] 95%, 90.98%, and 43.67% of larvae, respectively, developed on artificial larval diets.

Present study showed duration of larval development was longer on solid artificial larval diet (20.6 ± 0.3) than liquid diet (15.6 ± 0.3). According to Abro *et al.* [39], larval duration was 4.95 ± 0.35 days on diet. The present study investigates solid artificial larval diet that resulted in a greater number of pupae (142.3 ± 12.9) compared to liquid larval diet (76 ± 14). According to Gupta *et al.* [29] 89.2% of pupae developed on solid artificial larval diet. According to Sookar *et al.* [37] and Shinwari *et al.* [30] 95%, and 48.07% of pupae developed from artificial liquid larval diet.

In a present research Solid diet resulted in maximum pupal duration (9.3 ± 0.3) compared with liquid larval diet (5.3 ± 0.3). According to Gupta *et al.* [29] pupal duration was 9.8 days on solid larval diet, respectively. According to Abro *et al.* [39], pupal duration was 7.25 ± 0.25 days on larval diet.

Present study, liquid artificial larval diet resulted in greater percentage of adults emerging (80 ± 2.8) than on solid diet (77.6 ± 5.3). According to Gupta *et al.* [29] 97% of adults emerged on solid larval diet, while Shinwari *et al.* [30] and Sookar *et al.* [37] found more than 80% of adults emerged on liquid larval diet. The present study found a greater female-to-male ratio on liquid than solid diet. Rauf *et al.* [31] found a 1:2.3 male-to-female ratio on solid larval diet. Shinwari *et al.* [30] found 43.5:56.5 male-to-female ratio on a liquid larval diet.

The selection of host fruits by *Bactrocera* species is a key factor that affects their reproductive fitness and demographics because nutritional quality differences have a direct effect on oviposition selection and development rates of different geographical strains. *B. dorsalis* frequently prefers oviposition on *P. guajava* as this fruit has a soft and thin skin than other fruits, which is easily penetrated by females [15]. In addition to physical features, volatile organic compounds released by ripe *P. guajava* are effective chemical attractants which act as indication of high nutritional value to gravid females hence it correlates with much greater visits and oviposition punctures as compared to other hosts such as papaya and banana [20].

Methodologies used to evaluate these host preferences include standardization of the fruit condition of the host by selecting disease-free specimens freshly washed, pesticides free and rearing pests under controlled laboratory conditions of 25 ± 1 °C and 65 ± 5% relative humidity [40-41]. Moreover, experimental design needs to use fruits must be same stage of maturity for the consistent volatile profiles and the texture of the fruits because differences in maturity levels can drastically change oviposition behavior and the survival rates of the larvae [42].

These dynamics also become complicated by variations in the geographic strain sensitivity as different populations showing variation in developmental plasticity in response to the same fruit sources or rearing strategies [43, 15]. For instance, comparative study found that developmental stages of larval instar and pupal duration are shorter when larvae fed on *P. guajava* compared to mango or apple, again reflects that the nutritional content of *P. guajava* more effective to supporting the development of larvae and the successful eclosion period [15]. The exceptional nutritional composition of *P. guajava* consisting of unique pH and water content provides an ideal condition to larval growth as compared to other mango varieties such as Kent and apple mango [15, 42].

5. CONCLUSIONS

This investigation revealed that the host selection and larval diet composition influence significantly on the performance of *B. zonata* and *B. dorsalis*. Research showed that *P. guajava* guava was most preferred as a host by *B. zonata* and *B. dorsalis* fruit flies. Guava fruit was preferred for mass rearing fruit flies in a laboratory as well as rearing for biological control agents as larval and pupal parasitoid of fruit flies. Solid diet was preferred over liquid artificial diet for larval and pupal development of fruit flies during winter due to unavailability of liquid diet for mass rearing in laboratory-controlled conditions. Mass rearing fruit flies under laboratories are useful for developing larva and pupae and their parasitoids on large scale. The larva and pupal parasitoids are released to control fruit flies in fields and farms. Comparative analysis of artificial diets revealed that solid diets enhanced egg production, larval survival, and pupal development. Whereas liquid diets promoted higher adult emergence and more balanced sex ratios. Findings suggest that diet selection can be used deliberately to improve parasitoid production and achieving efficient adult emergence for colony maintenance. In a nutshell, the study provides practical insights for improving mass rearing protocols of *B. zonata* and *B. dorsalis.* These are critical for integrated pest management and biological control programs. The results contribute valuable baseline data for optimizing cost-effective rearing systems and enhancing the effectiveness of area-wide fruit fly management strategies.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest.

8. ETHICAL STATEMENT

Ethical approval was obtained from the Bio-Ethics committee of Karakoram International University (Approval No. KIU/2023/021).

9. FUNDING

No funding was received to conduct this research.

10. AUTHORSHIP CONTRIBUTION

Chandni Kiran and Maisoor Ahmed Nafees conceived and designed the experiments. Chandni Kiran, Saif Ud Din, and Nasreen performed the experiments. Tika Khan and Akbar Khan analyzed the data, while Saif Ud Din and Akbar Khan wrote the paper.

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