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Appraisal of species composition and ecology of Drosophilids in the Lower Swat Valley, Pakistan

Hamid Ur Rahman[1], Gulalai Hadi[2]

[1]Department of Zoology, Hazara University Dhodial, Mansehra, Khyber Pakhtunkhwa, Pakistan
[2]Centre for Animal Sciences and Fisheries, University of Swat, Swat, Khyber Pakhtunkhwa, Pakistan

Abstract

The drosophilid fauna of Pakistan remains largely understudied with numerous cities in the country yet to be thoroughly surveyed. This study aimed to investigate the drosophilid fauna of tehsil Babuzai in the Lower Swat Valley, Pakistan. The aerial net was used to collect flies from ten distinct locations within the tehsil. The specimens underwent dry preservation and subsequently analyzed using a digital microscope. Five distinct species were identified, all classified into two distinct genera within the family Drosophilidae. The taxa mentioned include *Drosophila melanogaster*, *Drosophila immigrans*, *Drosophila willistoni*, *Drosophila hydei*, and *Zaprionus indianus*. The present study reports that *D. melanogaster* exhibits a wider distribution than that of *Z. indianus*. The subsequent species in the distribution sequence was *D. willistoni*, whereas *D. hydei* and *D. immigrans* exhibited equivalent distribution patterns. The daily study of the flies' habitat ecology revealed that their population peaked during low temperatures and high humidity. Dipteran insects were gathered from diverse fruit sources, and it was noted that they exhibited no preference for specific hosts. *Drosophila melanogaster* and *D. immigrans* were widely distributed and documented in all surveyed areas of Pakistan. The three remaining species showed a non-cosmopolitan distribution, and their inclusion in the present study indicates that the Swat Valley possesses favorable climatic conditions for their proliferation.

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Introduction

The Drosophilidae family is one of the most diverse and widely distributed dipteran families, comprising over 4,300 species. It includes 75 genera and two subfamilies: Steganinae and Drosophilinae (Brake and Bächli, 2013; Dias et al., 2020). Although they are sometimes referred to as 'fruit flies', they are in fact not true fruit flies; they belong to another Dipteran family, Tephritidae, and breed in fruits that are still on trees (Green, 2002; Mazzon et al., 2022). Drosophilids, on the other hand, do not typically attack fresh fruits; they wait until the fruits have started to rot (Brake and Bächli, 2013; Dias et al., 2020). Drosophilids are known as vinegar flies or pomace flies because of their strong attraction to fermented products (Green, 2002; Tolwinski, 2024).

Drosophilids were not initially noticed as pests, but during the past few decades, some of their

***CONTACT** Hamid Ur Rahman, hamidhu@hu.edu.pk; Department of Zoology, Hazara University Dhodial, Mansehra, Khyber Pakhtunkhwa, Pakistan.

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species were observed in damaging unwounded ripen fruits, that resulted in economic loss (White andElson-Harris, 1992; Dias et al., 2020). Thus, it caught the attention of researchers towards agroeconomic projects. For the development of pest-management strategies, it is necessary to study the entomological and ecological aspects of the drosophilids (Brake and Bächli, 2013; Garcia and Oliveira, 2024).

Zaprionus indianus is a serious pest of figs in Brazil, where it caused 40-50% production loss of fig fruits (Stein and Novo, 2003; EFSA Panel on Plant Health, 2022). *Drosophila suzukii* is an invasive pest, that attacks on fruits with soft and thin skin such as raspberries, cherries, etc. (Rota-Stabelli et al., 2013; Asplen et al., 2015; Wang et al., 2022). The female *D. suzukii* oviposits in healthy ripen fruits (unlike other drosophilids that attack on overripen fermented fruits) and cause physical damage to the host (Hauser, 2011; Walsh et al., 2011; Wang et al., 2022).

The drosophilid fauna of Pakistan is poorly known as very few reports can be deciphered from the literature. For example, a survey was conducted in 15 cities of Khyber Pakhtunkhwa (KP), that yielded 11 species of *Drosophila* (Shahjehan et al., 2004). During a survey conducted in Islamabad, 10 *Drosophila* species were identified (Amin Ud Din et al., 2005). From Rabwah, 7 species of *Drosophila* had been reported (Tahir, 2013). For studying the diversity of *Drosophila* in Lahore, a survey was conducted, during which 10 species were reported (Tahir, 2013). Based on the information presented in all these reports, the objective of the present study was to investigate the diversity, distribution, and ecology of drosophilids in tehsil Babuzai (Lower Swat Valley) of district Swat, Pakistan, because this habitat differs from the other habitats in terms of environmental factors including temperature, sunlight intensity, average rainfall, humidity, etc.

Materials and Methods

Study Area

The present study was conducted in 10 locations of Tehsil Babuzai (Figure 1) *viz.,* Odigram (34° 46′ 6.37″ N, 72° 17′ 47.8″ E), Qamber (34° 45′ 55.75″ N, 72° 19′ 40.10″ E), Mingora (34° 46′ 32.23″ N, 72° 21′ 37.224″ E), Watky (34° 46′ 56.7″ N, 72° 21′ 46.134″ E), Ingaro Dherai (34° 47' 2.27'' N, 72° 20' 25.2168'' E), Naway Kaly (34° 47' 21.3036'' N, 72° 20' 52.5372'' E), Saidu Sharif (34° 44' 52.21'' N, 72° 21' 19.9656'' E), Sharifabad (34° 46' 8.328'' N, 72° 22' 9.725'' E), Kokarai (34° 44' 20.91'' N, 72° 25' 30.7'' E), and Jambil (34° 43' 23.19'' N, 72° 26' 42.2'' E). Specimens were collected between early May and mid-October. The collection was primarily conducted during the morning hours (prior to 11:00 AM) and late afternoon hours (after 4:00 PM) due to unsuccessful collection attempts during afternoon.

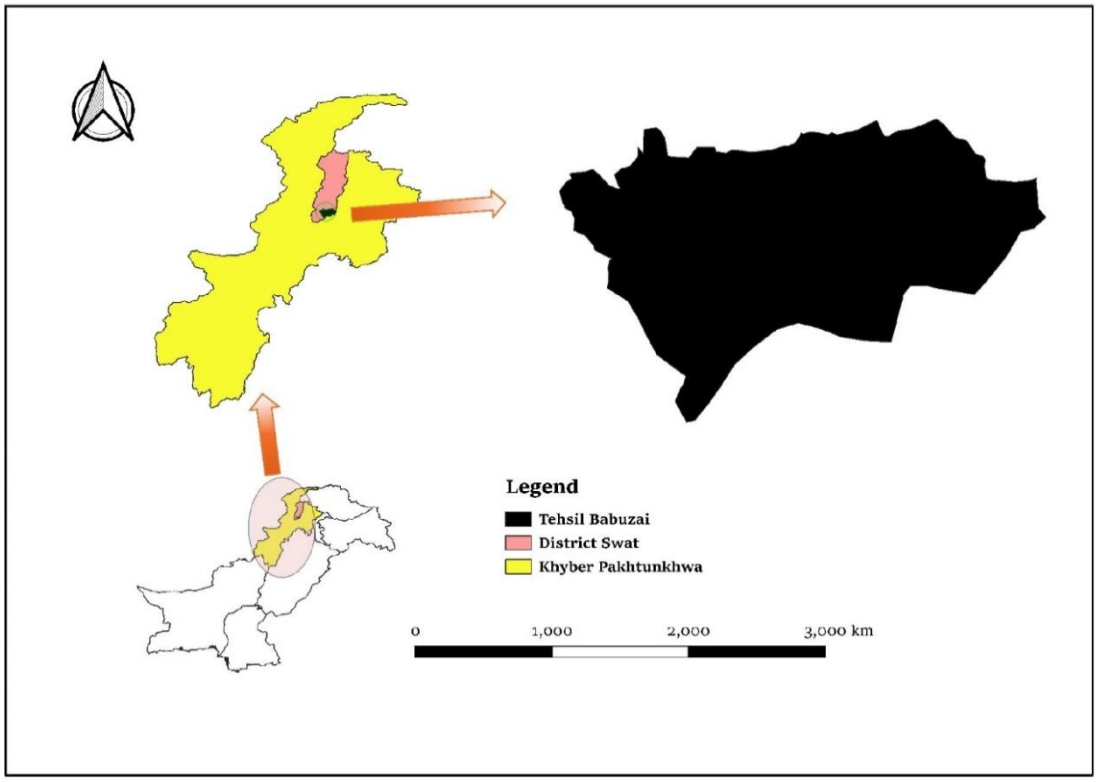

Figure 1. Map of tehsil Babuzai, district Swat

Collection and preservation of the specimens

Using an aerial net, two hundred and seventy-eight (278) specimens (Figure 2) of pieces and peelings of various fruits such as mango, melon, guava, peach, pear, apple, banana, jujube, grapes, pomegranate, persimmon, and papaya, were collected and stored in shaded areas following the instructions reported elsewhere (Whitman et al., 2019; Brown, 2021). In addition, some of the specimens were obtained from dustbins. The collected specimens were euthanized in an ethyl acetate killing jar, and subsequently relaxed using Barber's relaxing fluid to ensure proper positioning for preservation (Brown, 2021). The preservation was carried out by placing the specimens on the tip of a triangular-shaped piece of paper and affixing them using transparent nail polish (Gibb, 2014; Brown, 2021).

Figure 2. Aerial net used for collecting drosophilids

Identification of the specimens

The collected specimens were examined under a camera-equipped stereo microscope (Olympus, SZX7) and identified using authentic identification keys provided by Markow and O'Grady (2005) and Miller et al. (2017). Identification was based on morphological features, including the head, thorax, wings, abdominal tergites, and, in rare cases, legs and setae.

Statistical Analysis

The percent composition and distribution of species were calculated using Microsoft Excel 2016.

Results

A total of 278 specimens of drosophilid species were collected from various locations within tehsil Babuzai, and from them 5 species were identified, while one species remained unidentified. The collected species belonged to the subfamily Drosophilinae and two genera of the family Drosophilidae. One species *Zaprionus indianus* belonged to the genus *Zaprionus.* In contrast, four belonged to the genus *Drosophila* such as *D. melanogaster*, *D. immigrans*, *D. hydei*, and *D. willistoni*. *Zaprionus indianus* belonged to subgenus *Zaprionus*. They were categorized within the armatus species group and vittiger species subgroup.

Of the four species of the genus *Drosophila*, two belonged to the subgenus *Drosophila* such as *D. hydei* and *D. immigrans*, while the remaining two (*D. melanogaster* and *D. willistoni*) belonged to subgenus *Sophophor*a. *Drosophila hydei* was classified within the repleta species group and hydei species subgroup, whereas *D. immigrans* was categorized within the immigrans species subgroup. *Drosophila melanogaster* was placed in the *melanogaster* species group, and *melanogaster* species subgroup. Finally, *D. willistoni* was identified as a species in the willistoni species subgroup within the willistoni species group.

Description of *Drosophila melanogaster* Meigen, 1830

Female was larger than male with a body length of 2.5 mm. In the female, abdominal tergites had complete bands, possessing diffused spot in the center. The tip of the abdomen was pointed. Male was smaller than the female with a body length of 2 mm. In the male, abdominal tergites had bands that were dark and narrow at the anterior end, while the bands at the posterior end were completely darkened. Abdominal tip was rounded. Forelegs of the male carried sex combs that were present on the first tarsal segment (Figure 3).

Description of *Drosophila immigrans* Sturtevant, 1921

Drosophila immigrans had a body length 3.5 mm. Abdominal tergites have bands that were medially interrupted. The bands were triangular and do not reach the lateral surfaces. Apical tergites were almost completely dark. Cross veins and apices of wing veins were infuscated (Figure 4).

Description of *Drosophila willistoni* Sturtevant, 1916

The body length was 2.5 mm. The bands on abdominal tergites were not too darkened (Figure 4).

Description of *Drosophila hydei* Sturtevant, 1921

Drosophila hydei had a length of 3.5 mm. The base color of the scutum was pale with irregular patterns of dark spots. Abdominal tergites have characteristic dark bands that were medially interrupted. The bands on either side were narrow medially and broad at the two ends. Bands on the lateral surface lacked pale areas. The subcostal break of wing was pale (Figure 5).

Description of *Zaprionus indianus* Gupta, 1970

The body length was 3 mm. Orbital plates and mesonotum were with two white longitudinal stripes that were bordered by black lines. Fore-femur had a row of 4-6 spine-like setae. At the base of each seta, there was a short tubercle (Figure 6).

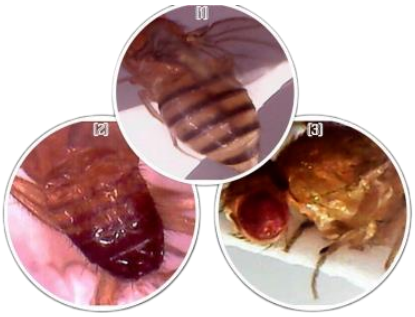

Figure 3. *Drosophila melanogaster* Meigen, 1830; (1) A pointed tip of female abdomen; (2) rounded tip of male abdomen, (3) sex combs on male forelegs

Figures 4. *Drosophila immigrans* Sturtevant, 1921, (1) infuscated cross veins and apices of wing veins, (2) dark apical tergites

Figure 4. *Drosophila willistoni* Sturtevant, 1916. Narrow bands on abdominal tergites

Figure 5. *Drosophila hydei* Sturtevant, 1921. Characteristic abdominal tergites. Pale subcostal break

Figure 6. *Zaprionus indianus* Gupta, 1970. Orbital plates and mesonotum with two white longitudinal stripes, bordered by black lines

Distribution of the explored species

Drosophila melanogaster was the most dominant species in its distribution, followed by *Z. indianus.* Next in distribution was *D. willistoni,* while *D. hydei* and *D. immigrans* were equally distributed (Figure 6).

Ecology of the Drosophilids

The specimens were collected from different hosts, and it was observed that the species did not prefer a specific host. Each species was collected from different hosts. The flies were maximum during day time when temperature was low, and humidity was high (i.e. during morning and evening), while they were fewer in the afternoon when temperature was high, and humidity low (Figure 8).

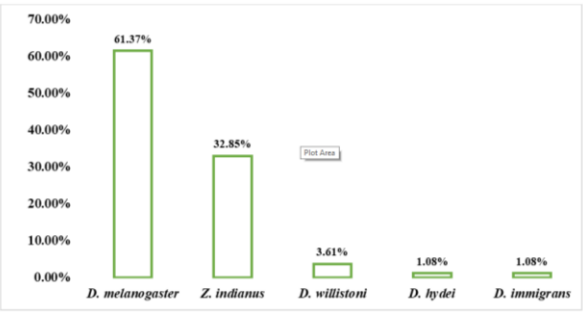

Figure 7. Percent distribution of the explored species

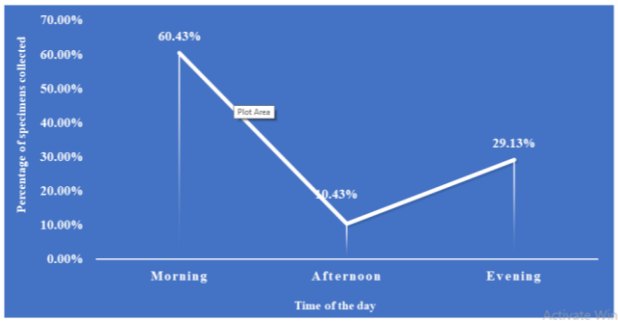

Figure 8. Percentage of the specimens collected during different times of the day

Discussion

The present study was conducted in tehsil Babuzai of the lower Swat valley. It was an attempt to explore the diversity of drosophilid species in the selected area. This study yielded 5 species of the family Drosophilidae *viz., D. melanogaster, Z. indianus, D. willistoni, D. hydei* and *D. immigrans.* Most of these species, i.e., 4 species belonged to the genus *Drosophila.* According to Amin Ud Din et al. (2005) and Tahir (2013), who concluded during their studies that the flora and climatic conditions of Pakistan are suitable for the propagation and diversification of *Drosophila* species.

It was noticed that the flies were not attracted immediately towards the fruits, rather they were attracted after some time had passed and the fruits had started rotting. This behavior has been indubitably explained elsewhere (Kandpal, 2015; Markow and O'Grady, 2005), that drosophilids are attracted towards products fermented by yeast.

It was observed that the number of flies were maximum from evening (i.e. after 4:00 PM) to next morning (i.e. before 11:00 AM). In the afternoon, there were very low number of flies noticed near the fruits. This observation evidences the findings of Zahoor et al. (2017), who observed on monthly basis that the months with high relative humidity and low temperature caused an increase in the population number of *D. melanogaster* and *vice versa*. Likewise, relative humidity is high, and temperature is low in the morning and evening, hence, the number of flies was maximum at those times. As the temperature raises and relative humidity decreases in the afternoon, so very low number of flies were collected at that time.

The specimens were collected from different hosts, and it was observed that the species did not prefer specific hosts. Each species was collected from different hosts. Perhaps some of the drosophilids that attack the fruits on trees possess host specificity. For example, *D. suzukii* attacks on fruits with soft skin like cherries, raspberries, etc. and is considered as a pest as described by Dreves and Langellotto-Rhodaback (2011) and Kandpal (2015); whereas *Z. indianus* is a pest of fig fruits as reported by Kandpal (2015), Stein and Novo (2003) and Svedese et al. (2012).

Conclusion

A total of 278 drosophilid specimens were collected in a short period of six months that yielded 5 species. Two of them were cosmopolitan, while some of the remaining were unevenly distributed in other parts of Pakistan. Yielding 5 species in such a short period within a small sample size concluded that the climate of Swat is fit for the propagation of species of family Drosophilidae, as Swat features a humid subtropical climate.

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Conceptualized and designed the study: HR. Conducted research and wrote up the first draft of the manuscript: GH. Reviewed and edited the manuscript: HR, GH.

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Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All authors contributed in designing and writing the entire article. All contributors have critically read this manuscript and agreed to publish in IJAaEB.

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It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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