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Bionomics of *Bactrocera* fruit flies (Diptera: Tephritidae) in Khyber Pakhtunkhwa, Pakistan; exploring performance of various trap types and their characteristics

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Abstract

This study investigated spatio-temporal fluctuations and population dynamics of *Bactrocera* fruit flies (Diptera: Tephritidae) in Khyber Pakhtunkhwa, Pakistan, in relation to selected climatic variables. Additionally, infestation rate of *Bactrocera* species and trapping efficiency of different trap types and characteristics were explored. Fruit flies were collected from 14 selected localities of 9 districts in 4 agro-ecological zones of Khyber Pakhtunkhwa using pheromone traps (methyl eugenol and raspberry extract), food-baited traps, and from rearing of infested fruits. A total of 12,058 fruit flies belonging to nine species: *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera signata* (Hering), *Bactrocera cucurbitae* (Coquillett), *Bactrocera tau* (Walker), *Bactrocera zahadi* (Mahmood), *Bactrocera scutellaris* (Bezzi), and *Bactrocera nigrofemoralis* (White and Tsuruta) (all Diptera: Tephritidae) were collected. The first 3 species were categorized as dominant. Highest flies per trap per d was recorded from district Kohat (Zone-D) while the lowest was in Nowshera (Zone-C). Havelian, district Abbottabad (Zone-B), was the most diverse locality. Fruit fly population peaked in May and were at a minimum in Dec. There was a significant positive correlation between rainfall and *Bactrocera* species abundance, and a negative association between relative humidity and population abundance. Highest flies per trap per d were recorded at elevations ranging from 285 to 855 masl in semi-arid and cool zones, in sub-humid zones of Khyber Pakhtunkhwa, and in rangelands south of Khyber Pakhtunkhwa. Fruit flies showed host specificity with respect to certain plant families. Methyl eugenol-raspberry extract traps and food-baited traps displayed species-specific trapping patterns. South-facing and green or yellow colored traps were the most attractive. Numbers of *Bactrocera* were higher in methyl eugenol-raspberry extract mixture traps compared to other pheromone trap combinations. In food-baited trials, peach and guava-baited traps attracted the highest number of *Bactrocera* flies. Adding sugar and yeast increased trapping efficiency.

Key words: *Bactrocera*; spatial distribution; trapping patterns; Khyber Pakhtunkhwa

Resumen

Este estudio investigó las fluctuaciones espacio-temporales y la dinámica poblacional de las moscas de la fruta del género *Bactrocera* (Diptera: Tephritidae) en Khyber Pakhtunkhwa, Pakistán, en relación con las variables climáticas seleccionadas. Además, se exploró la tasa de infestación de especies de *Bactrocera* y la eficiencia de captura de diferentes tipos y características de trampas. Se recolectaron moscas de la fruta en 14 localidades seleccionadas de 9 distritos en 4 zonas agroecológicas de Khyber Pakhtunkhwa utilizando trampas de feromonas (metil eugenol y extracto de frambuesa), trampas con cebo alimentario y cría de frutas infestadas. Un total de 12.058 moscas de la fruta pertenecientes a nueve especies: *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera signata* (Hering), *Bactrocera cucurbitae* (Coquillett), *Bactrocera tau* (Walker), *Bactrocera zahadi* (Mahmood), *Bactrocera scutellaris* (Bezzi), y *Bactrocera nigrofemoralis* (White and Tsuruta) (todas Diptera: Tephritidae) fueron colectados. Las primeras 3 especies fueron categorizadas como dominantes. Se registró la mayor cantidad de moscas por trampa por día en el distrito de Kohat (Zona-D), mientras que la menor fue en Nowshera (Zona-C). Havelian, distrito de Abbottabad (Zona-B), fue la localidad más diversa. La población de moscas de la fruta alcanzó su punto máximo en mayo y alcanzó un mínimo en diciembre. Hubo una correlación positiva significativa entre la lluvia y la abundancia de especies de *Bactrocera*, y una asociación negativa entre la humedad relativa y la abundancia de la población. La mayor cantidad de moscas por trampa por día se registró en elevaciones que oscilan entre 285 y 855 msnm en zonas semiáridas y frías, en zonas subhúmedas de Khyber Pakhtunkhwa y en pastizales al sur de Khyber Pakhtunkhwa. Las moscas de la fruta mostraron especificidad de hospedero con respecto a ciertas familias de plantas. Las trampas con extracto de frambuesa y eugenol de metilo y las trampas cebadas con alimentos mostraron patrones de captura específicos de la especie. Las trampas orientadas al sur y de color verde o amarillo fueron las más atractivas. El número de *Bactrocera* fue mayor en las trampas de mezcla de metil eugenol y extracto de frambuesa en comparación con otras combinaciones de trampas de feromonas. En los ensayos

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con cebo alimentario, las trampas cebadas con durazno y guayaba atrajeron la mayor cantidad de moscas *Bactrocera*. La adición de azúcar y levadura aumentó la eficiencia de captura.

Palabras Claves: *Bactrocera*; distribución espacial; patrones de captura; Khyber Pakhtunkhwa

Fruit flies (Diptera: Tephritidae) are significant horticultural insect pests (White & Elson-Harris 1992; Norrbom 2010). Tephritids include more than 5,000 species worldwide, approximately 1,400 species of which develop in fleshy fruits. The most destructive of these fruit fly species belong to genera *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis*, and *Anastrepha* (White & Elson-Harris 1994). Among these, the principal pests belong to the Dacine group with *Bactrocera* being the predominant genus (Clarke et al. 2005).

Nearly 250 species of fruit flies are capable of achieving pest status by feeding on and causing severe damage to plants and crops of economic importance (Thompson 1998). Many of these fruit fly species are polyphagus, very mobile, and can effectively search for food and oviposition sites. This combination of attributes makes these insects very successful at colonizing new areas, achieving large populations relatively quickly, and causing enormous losses (Allwood 1997). Apart from direct damage to fruit crops, these insects also impact international trade because of their quarantine significance (Ekesi et al. 2009).

Tephritid population dynamics greatly vary with changing abiotic and biotic factors (Vayssières et al. 2009). For instance, the diversity and faunistic indices (i.e., frequency, dominance, and constancy) of fruit fly populations are affected by mating behavior, oviposition behavior, dispersal, nutrition, type of host or vegetation, moisture, temperature, light, and competition (Hendrichs & Prokopy 2019). Host selection behavior may be affected by size, skin, odor, color, fruit shape, and foliage characteristics of plants (Jaleel et al. 2018). Fruit fly activity also varies considerably depending on prevailing climatic conditions and diversity of hosts in a particular agro-ecosystem. Additionally, the nutritional level of a host may affect fruit fly development (Gripenberg et al. 2010).

Bactrocera poses a serious risk to the horticultural crops in Pakistan due to its wide range of hosts and its invasive nature (Clarke et al. 2005). Among these fruit flies, *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders), and *Bactrocera cucurbitae* (Coquillett) (all Diptera: Tephritidae) are polyphagous pests of international significance (Clarke et al. 2005). In Pakistan, the preferred hosts of these fruit flies are apple, peach, mango, bitter gourd, and musk melon (Khan & Musakhel 1999; Khan et al. 2005).

The success of fruit fly integrated management programs depend on elucidating ecological and biological aspects of these tephritids (Aguiar-Menezes et al. 2008). The present study is one of the first extensive surveys of fruit flies in Khyber Pakhtunkhwa that provides a comprehensive record of fruit flies and their bionomics in the province. We aimed to faunistically characterize *Bactrocera* fruit flies in different ecological zones of Khyber Pakhtunkhwa in addition to exploring their seasonal population dynamics in relation to climatic factors, host preferences, trap characteristics, and their infestation rates. There is a shortage of elaborate studies that focus on the effects of these factors on population dynamics of fruit flies in Pakistan, particularly Khyber Pakhtunkhwa. Understanding these factors for pests is essential in devising effective management strategies (Baskauf 2003).

Materials and Methods

STUDY AREA AND SURVEY DESIGN

Fruit flies were collected from 14 selected localities of 9 districts in 4 agro-ecological zones of the Khyber Pakhtunkhwa region (Fig. 1). Khy-

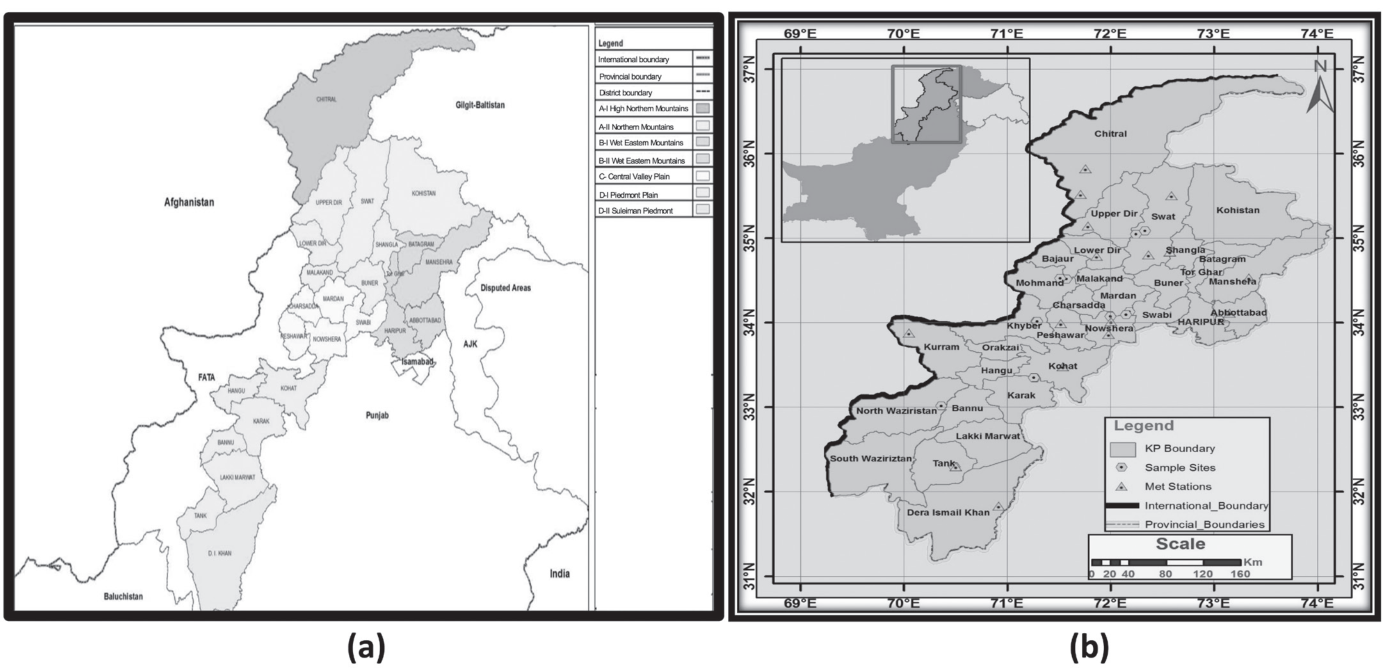

Fig. 1. Map of Khyber Pakhtunkhwa showing (a) agro-ecological zones and (b) sampling sites.

ber Pakhtunkhwa is a topographically diverse region in the northwest of Pakistan. It is divided into 4 major agro-ecological zones, named A, B, C, and D, in its Environmental Profile developed by the Environmental Protection Agency of Khyber Pakhtunkhwa (Khan et al. 2019).

Samples were collected during 2018 to 2019 in a variety of mixed and individual orchards comprising different host plant families (depending on seasonal and regional availability). Global positioning system (GPS) coordinates and weather parameters including mean monthly minimum and maximum temperature, relative humidity, rainfall, and wind speed were acquired using weather devices (Kestrel 4500NV Pocket Weather Tracker; Kestrel, Boothwyn, Pennsylvania, USA) or collected from the Meteorological Department in Peshawar, Pakistan.

COLLECTION METHODS AND TRAP CHARACTERISTICS

Adult flies were collected using pheromone traps (methyl eugenol and raspberry extract-baited traps) and food-baited traps (fruit pulp extract) installed at different heights on the selected host plants (peach, apple, plum, apricot, raspberry, citrus, guava, persimmon, gourd, mango, tomato, orange, olive, mulberry, and banana) during the study period. These traps were inspected every wk, every 2 wk, or for seasonal analysis on a monthly basis, for a period of 1 yr (in Peshawar only) (i.e., Jan–Dec 2019).

Adult flies also were reared from infested fruits collected for the purpose of studying infestation rates. Field sampling of infested fruits was conducted in selected guava orchards in agricultural farms in Peshawar based on the availability of this particular host plant during the study period. Fruit was sampled and processed as described by Ekesi et al. (2007). Ripe fruits plucked from plants and fallen on the ground were collected randomly on each sampling and tested for larval infestation. The collected infested fruits were counted, washed, and weighed to determine fruit fly infestation indices (Rwomushana et al. 2008). For this purpose, flies were reared from fruits over a pupation medium (moistened fine sand) in plastic containers with netting material fitted on top for proper ventilation. Containers were kept under lab conditions (24 °C, 28% RH, and 11:13 h [L:D] photoperiod) for 2 to 3 wk until larvae left fruits and pupated in pupation media (Ekesi et al. 2007). Pupae were placed in Petri dishes provided with moist filter paper, counted, and kept until emergence of adult flies. Emerging flies were given diet (1 part yeast and 3 parts sugar) and water (cotton wool soaked in water). Adult were kept alive for about 1 wk until maturation and development of adult features to facilitate easy taxonomic identification.

Different characteristics of pheromone-baited traps were assessed to determine their effectiveness in fly capture including trap color and direction. For this purpose, white, red, yellow, and green colored traps of both attractant types (methyl eugenol and raspberry extract-baited traps) were installed facing north, south, east, and west at a height of 2 m above ground level for a period of 4 wk. The white trap served as control whereas the other traps were covered with yellow, green, and red colored papers. In addition, the response of fruit fly species was assessed towards methyl eugenol traps, raspberry extract traps, mixture traps (methyl eugenol + raspberry extract) and methyl eugenol–raspberry extract traps placed side-by-side in citrus orchards for 15 d.

Food-baited traps were employed to explore characteristics and attractiveness of 3 different combinations of food baits. For this purpose, these combinations of food-baited traps were suspended on citrus trees in a single orchard in Peshawar at 1 to 2 m height and separated by a distance of 30 m from each other. All attractant traps were inspected each wk. Combinations used were: Treatment 1 – sugar solution, banana extract + sugar + insecticide (dipterex), peach extract + sugar + insecticide, guava extract + sugar + insecticide; Treatment 2 – yeast only, sugar only, sugar + yeast, grapes + sugar + yeast, banana + sugar + yeast; Treatment 3 – sugar only, yeast only, sugar + yeast, banana + sugar + yeast, guava + sugar + yeast.

Specimens from different collection methods were preserved and identified morphologically using dichotomous keys (Drew & Raghu 2002; David & Ramani 2011; Prabhakar et al. 2012; Choudhary et al. 2014; Leblanc et al. 2014).

DATA ANALYSIS

The number of fruit flies collected in traps were expressed in terms of standard relative fly density index (IAEA 2003) as fruit flies per trap per d, relative abundance (Rydzanicz & Lonc 2003; Sengil et al. 2011), distribution (Dzięczkowski 1972), and as diversity indices (Aguiar-Menezes et al. 2008). Faunistic analysis to characterize the populations regarding frequency, abundance, constancy, dominance, and evenness was performed in ANAFAU software (Marsaro Júnior et al. 2012) and PAST v4.03 (Paleontological Software, Oslo, Norway). Infestation indices in terms of incidence, infestation rate, and percentage emergence were calculated as described by Eskafi and Kolbe (1990), Cowley et al. (1992), and Copeland et al. (2002). Hierarchical clustering analysis for assessing host plant preferences was carried out in SPSS v12.0 (SPSS Inc., Chicago, Illinois, USA). ANOVA and Pearson's correlation was performed using STATA v13 software (Stata Statistical Software, vers. 13; Stata Corp., College Station, Texas, USA). For spatial analysis, GPS coordinates data for collection sites were arranged in MS Excel and analyzed in ArcGIS v10.2.0 (Environmental System Research Institute, Redland, California, USA). For this purpose, study area and land cover maps were obtained from the National Centre of Excellence in Geology, University of Peshawar, Pakistan, whereas a digital elevation model was extracted from an advanced space-borne thermal emission and reflection radiometer (Global Digital Elevation Model V003; ASTER, Sioux Fall, South Dakota, USA/Japan).

Results

SPECIES COMPOSITION AND FAUNISTIC INDICES OF *BACTROCERA* FRUIT FLIES IN DIFFERENT AGRO-ECOLOGICAL ZONES OF KHYBER PAKHTUNKHWA

A total of 12,058 fruit flies were collected through all collection methods in 14 localities of Khyber Pakhtunkhwa including 11,829 (98%) male and 229 (2%) female specimens. Nine tephritid species belonging to the genus *Bactrocera* were identified: *B. dorsalis*, *B. zonata* (Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera signata* (Hering), *B. cucurbitae* (Coquillett), *Bactrocera tau* (Walker), *Bactrocera zahadi* (Mahmood), *Bactrocera scutellaris* (Bezzi), and *Bactrocera nigrofemoralis* (White and Tsuruta). *Bactrocera zonata*, *B. dorsalis*, and *B. cucurbitae* were categorized as dominant and constant species. *Bactrocera signata* and *B. zahadi* were classified as satellite and infrequent species whereas *B. nigrofemoralis* was a satellite and sporadic species (Table 1). Locality-wise analysis also established *B. zonata*, *B. dorsalis*, and *B. cucurbitae* to be super-dominant, super-frequent, super-abundant, and constant species. These 3 species were dominant in all 4 zones except *B. zonata*, which was super-dominant in zone C. *Bactrocera nigrofemoralis* was a non-dominant, infrequent, and rare species collected only in Peshawar (Zone C) (Table 2).

Bactrocera zonata was sampled in all the selected districts. *Bactrocera cucurbitae* was reported from 8 districts whereas *B. dorsalis* was collected from 7 districts (Supplementary Table 1). The highest flies per trap per d was recorded in localities from Kohat (Zone D) whereas the lowest flies per

Table 1. Relative abundance and distribution status of collected fruit fly species in Khyber Pakhtunkhwa.

Genus	Sub genus	*N* (%)	Fruit fly species	Total number of specimens (%)	Relative abundance status[a]	Distribution status[b]
	Bactrocera		*Bactrocera dorsalis*	1,806 (14.9)	Dominant	Constant
		10,270 (85.17)	*Bactrocera zonata*	8,173 (67.8)	Dominant	Constant
			Bactrocera correcta	289 (2.4)	Subdominant	Infrequent
Bactrocera			*Bactrocera nigrofemoralis*	2 (0.02)	Satellite	Sporadic
	Zeugodacus		*Zeugodacus signata*	28 (0.2)	Satellite	Infrequent
		1,788 (14.82)	*Bactrocera cucurbitae*	1,283 (10.6)	Dominant	Constant
			Bactrocera tau	252 (2.1)	Subdominant	Frequent
			Bactrocera zahadi	38 (0.3)	Satellite	Infrequent
			Bactrocera scutellaris	187 (1.6)	Subdominant	Infrequent
	Total	12,058				

[a]Relative Abundance < 1% = Satellite species; 1% < Relative Abundance < 5% = Subdominant species; Relative Abundance > 5% = Dominant species (Rydzanicz & Lonc 2003; Sengil et al. 2011); bSporadic (0–20%); Infrequent (20.1–40%); Moderate (40.1–60%); Frequent (60.1–80%); Constant (80.1–100%) (Dzięczkowski 1972).

trap per d was in Rashakai of district Nowshera (Zone C). Diversity indices indicated Havelian, Abbottabad (Zone B) as the most diverse locality, while the least diverse were Sakhra and Behzadi in Zone A. Madyan (Swat) had a maximum value of Margalef species richness index whereas Pielou's Evenness value was highest for Kalu Khan (Swabi) (Table 3).

SPATIAL ANALYSIS OF *BACTEROCERA* FRUIT FLIES IN KHYBER PAKHTUNKHWA

Spatial analysis of the 3 most abundant species (*B. dorsalis*, *B. zonata*, *B. cucurbitae*) was performed on elevation, climatic, and land cover maps. High ranges of flies per trap per d for *B. dorsalis*, *B. zonata*, and *B. cucurbitae* were observed at elevations ranging from 285 to 855 masl. All 3 species existed and were abundant in a range of climatic zones including warm, semi-arid, and cool, sub-humid zones of Khyber Pakhtunkhwa. Rangelands in the south of Khyber Pakhtunkhwa showed the highest flies per trap per d for fruit flies followed by agricultural lands (Fig. 2).

HOST SPECIFICITY OF *BACTROCERA* FRUIT FLIES

Bactrocera species were collected from a variety of host plants belonging to 9 families: Rosaceae, Rutaceae, Anacardiaceae, Myrtaceae, Solanaceae, Cucurbitaceae, Oleaceae, Moraceae, and Musaceae. A dendogram constructed for plant families based on the absence or presence

Table 2. Faunistic analysis of the Bactrocera fruit flies in 4 zones of Khyber Pakhtunkhwa.

Agro-ecological zone	Index[a]	*B. dorsalis*	*B. zonata*	*B. correcta*	*B. signata*	*B. cucurbitae*	*B. tau*	*B. scutellaris*	*B. zahadi*	*B. nigrofemoralis*
A	N	563	105	0	0	169	87	187	0	0
	D	D	D	–	–	D	D	D	–	–
	A	Ma	C	–	–	C	C	C	–	–
	F	MF	F	–	–	F	F	F	–	–
	C	W	W	–	–	W	W	W	–	–
B	N	31	28	0	0	40	27	0	17	0
	D	D	D	–	–	D	D	–	D	–
	A	C	C	–	–	Ma	C	–	r	–
	F	F	F	–	–	MF	F	–	PF	–
	C	W	W	–	–	W	W	–	W	–
C	N	493	3,171	181	27	798	114	0	18	2
	D	D	SD	D	D	D	D	–	D	ND
	A	A	sa	C	C	ma	c	–	c	d
	F	MF	SF	F	F	MF	F	–	F	PF
	C	W	W	W	W	W	W	–	W	W
D	N	488	1,579	7	0	33	0	0	0	0
	D	D	D	D	–	D	–	–	–	–
	A	Ma	ma	Ma	–	ma	–	–	–	–
	F	F	MF	F	–	F	–	–	–	–
	C	W	W	W	–	W	–	–	–	–
Total	N	1,575	4,883	188	27	1,040	228	187	35	2
	D	SD	SD	D	D	SD	D	D	D	ND
	A	Sa	sa	C	d	sa	ma	c	c	R
	F	SF	SF	F	PF	SF	MF	F	F	PF
	C	W	W	W	W	W	W	W	W	W

[a]Number of flies captured; D = dominance: sd (super dominant), d (dominant), nd (non-dominant); A = abundance: sa (super abundant), ma (very abundant), c (common), d (dispersed), r (rare); F = frequency: sf (super frequent), mf (highly frequent), f (frequent), pf (infrequent); C = constancy: w (constant), y (accessory), z (accidental) (Marsaro Júnior et al. 2012).

Table 3. Diversity indices of fruit flies population from different agro-ecological zones of Khyber Pakhtunkhwa.

Agro-ecological Zone	District	Localities	H′[a]	Me[b]	E[c]	D (1/D)[d]	d (1/d)[e]	Number of specimens	Number of species	flies per trap per d[f]
A	Swat	Madyan	0.97	0.88	0.43	0.50 (2)	0.68 (1.47)	94	5	2.7
		Sakhra	0.40	0.57	0.29	0.82 (1.22)	0.90 (1.11)	194	4	6.9
	Lower Dir	Rabaat	1.22	0.63	0.54	0.32 (3.12)	0.47 (2.13)	557	5	13.2
		Munjai	0.54	0.72	0.24	0.76 (1.32)	0.86 (1.16)	266	5	9.5
B	Abbottabad	Havelian	1.57	0.80	0.70	0.20 (5)	0.28 (3.57)	143	5	5.1
C	Swabi	Sarra Cheena	0.80	0.53	0.73	0.54 (1.85)	0.71 (1.4)	42	3	1.4
		Kalu Khan	1.27	0.64	0.92	0.30 (3.33)	0.41 (2.43)	110	4	3.9
	Nowshera	Rashakai	0.97	0.80	0.43	0.53 (1.89)	0.71 (1.4)	145	5	0.5
	Peshawar	Agri-farms	0.66	0.84	0.34	0.70 (1.43)	0.83 (1.2)	1,273	7	5.5
		Forest nursery	1.09	0.87	0.52	0.42 (2.38)	0.60 (1.66)	3,234	8	1.8
D	Kohat	Behzadi	0.39	0.15	0.56	0.77 (1.3)	0.87 (1.15)	682	2	24.3
		Kurd	0.57	0.16	0.82	0.61 (1.64)	0.75 (1.33)	481	2	17.2
	Lakki Marwat	Zhangi Khel	0.45	0.40	0.41	0.78 (1.3)	0.88 (1.14)	140	3	5.0
	Bannu	Torka	0.76	0.30	0.69	0.51 (1.96)	0.63 (1.59)	804	3	9.6

[a]Shannon-Weaver diversity index (Shannon & Weaver 1949); [b]Margalefs Richness Index (Margalef 1958); [c]Pielou's Evenness index (Pielou 1975); [d]Simpson's Index (Simpson 1949); [e]Berger Parker dominance index (Berger & Parker 1970); [f]Fruit flies per trap per d (IAEA 2003).

of fruit fly species revealed significant similarity of fruit fly pests among hosts of the families Oleaceae, Moraceae, and Musaceae (Fig. 3). A second clade was comprised of 2 sub-groups, 1 group including Rosaceae and Rutaceae, and the other group including Myrtaceae, Anacardiaceae, Solanaceae, and Cucurbitaceae. Seven species of fruit flies were collected in traps in citrus orchards and 6 species from guava and peach orchards. *Bactrocera nigrofemoralis* was sampled only from mango orchards. *Bactrocera signata* and *B. cucurbitae* showed significant association with the type of host plant family ($P = 0.00$) (Supplementary Table 2).

SEASONAL DYNAMICS OF FRUIT FLIES IN DISTRICT PESHAWAR

Overall, 8 species (3,234 fruit fly specimens) of *Bactrocera* fruit flies were observed in the annual collection conducted in Peshawar during Jan to Dec 2019, namely *B. dorsalis*, *B. zonata*, *B. correcta*, *B. nigrofemoralis*, *B. cucurbitae*, *B. tau*, *B. zahadi*, and *B. signata*. Seasonal population dynamics of fruit flies studied in Peshawar showed a peak abundance of fruit flies during warmer mo of the yr with the highest number ($N = 1,584$; flies per trap per d = 10.2) reported in May. The population declined during the winter mo with the lowest number of flies sampled in Nov ($N = 74$, FTD =0.49) and Dec ($N = 73$; flies per trap per d = 0.47) (Fig. 4a, b). The maximum number of species were reported in Jan, Mar, and Apr ($N = 7$) (Fig. 4c). Abundance of *B. dorsalis* and *B. zonata* increased with increasing temperature towards May but declined from Jun onwards (Fig. 4d, e). *Bactrocera cucurbitae* numbers peaked in Mar, coinciding with maximum rainfall, then decreased towards Aug but peaked again in Sep (Fig. 4f). *Bactrocera zahadi* and *B. signata* numbers were significantly positively correlated with rainfall whereas *B. nigrofemoralis* were significantly negatively correlated with relative humidity. Significant Peasrson's correlation coefficient values ($P < 0.05$) are represented (Table 4).

INFESTATION INDICES

The results of *Bactrocera* infestation rate in local guava sampled from agricultural farms of Peshawar revealed the level of infestation as 397.1 pupae per kg of fruit. The number of adults emerged from these samples was 375 (179 adults per kg of fruit) with an emergence rate of 45%. Two species were recovered from guava: *B zonata* and *B. dorsalis*; the former species was the most abundant species ($N = 353$; 94%) represented by 173 (46%) male and 180 (48%) female specimens. Weight and numbers of fruit collected correlated positively with the number of pupae whereas negative association was observed with emergence of adult flies (Table 5).

EFFICACY OF FRUIT FLY TRAPS AND ATTRACTIVENESS OF DIFFERENT TRAPPING CHARACTERISTICS

Among the 9 *Bactrocera* species collected through different collection methods, 4 species (*B. dorsalis*, *B. zonata*, *B. correcta*, and *B. nigrofemoralis*) were trapped in methyl eugenol-baited traps whereas 5 species (*B. signata*, *B. tau*, *B. cucurbitae*, *B. scutellaris*, and *B. zahadi*) were collected in raspberry ketone-baited traps. The species caught in pheromone traps were represented by only 1 sex (male), whereas both males and females were captured in food-baited traps and the adults reared from infested fruits. Methyl eugenol-baited traps were attractive to *B. zonata* (the most dominant species), *B. dorsalis*, *B. correcta*, and *B. nigrofemoralis*. Raspberry extract-baited traps were preferred by *B. cucurbitae* (the dominant species), *B. tau*, *B. scutellaris*, *B. zahadi*, and *B. signata*. Food-baited traps were attractive to *B. dorsalis*, *B. cucurbitae*, *B. zonata*, and *B. tau* (Table 6). *Bactrocera dorsalis*, *B. zonata*, and *B. tau* numbers showed significant variation ($P \leq 0.05$) among different localities for methyl eugenol and raspberry extract traps (Supplementary Table 3).

Sampling of fruit flies through different food-baited traps in 3 treatments revealed that traps baited with peach and guava pulp attracted the highest number of flies (flies per trap per d = 2.36), followed by combination traps of banana + sugar + yeast (flies per trap per d = 1.71**)**. The number of fruit flies showed significant association with baits used in treatment 1 ($P \leq 0.001$) and 3 ($P = 0.0074$) (Table 7).

Comparison of the relative number of fruit flies trapped in single methyl eugenol traps, raspberry extract traps, mixture traps (methyl eugenol + raspberry extract) as well as methyl eugenol and raspberry extract traps (placed side-by-side) showed that more fruit flies were attracted to mixture (methyl eugenol + raspberry extract) traps compared to single pheromone-baited traps when these traps were placed side-by-side (Fig. 5). The number of *Bactrocera* species showed significant association with the trap type (1-way ANOVA; $P \leq 0.001$).

Trapping characteristics including effect of trap color and direction were assessed for methyl eugenol and raspberry extract traps. South

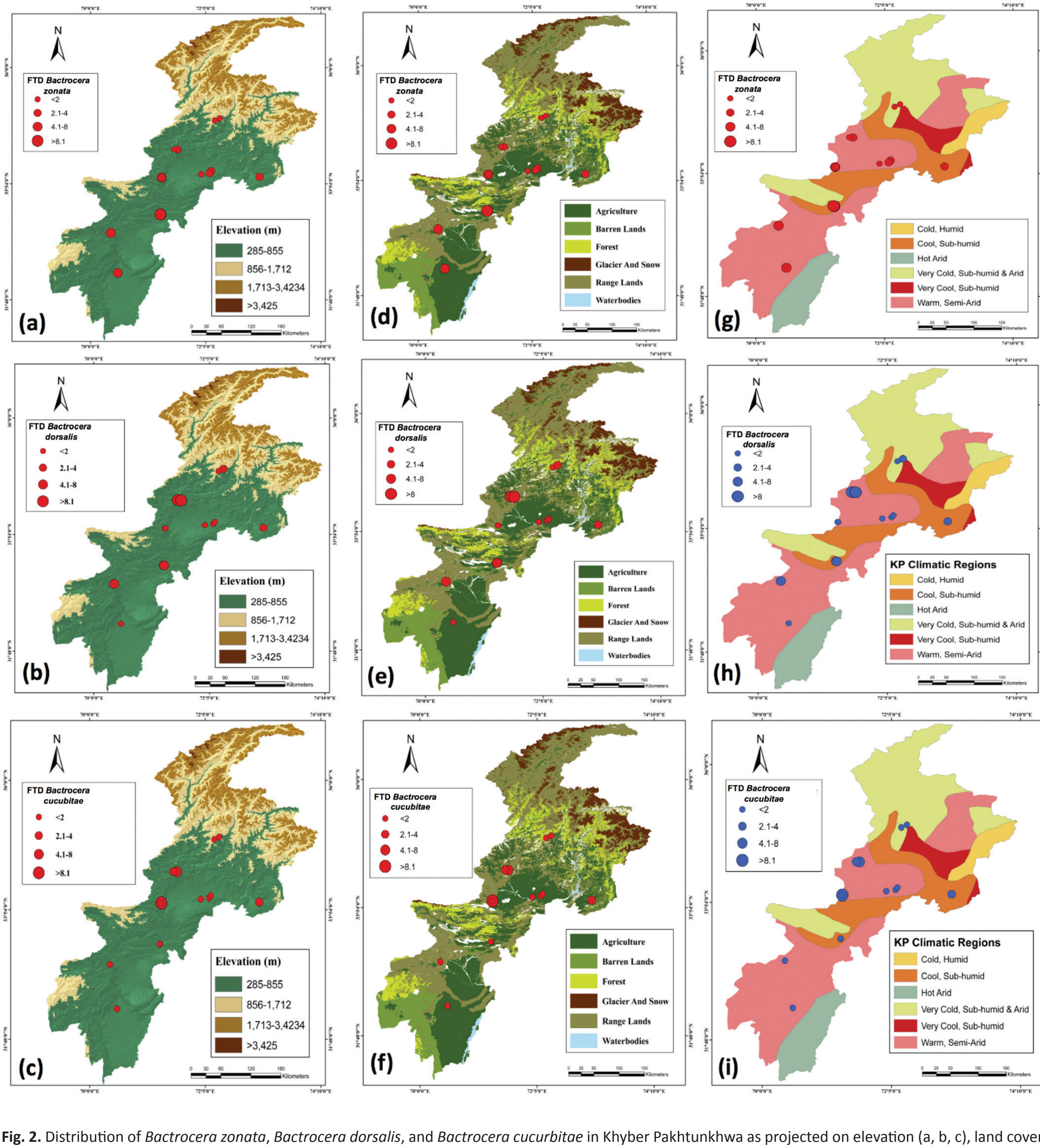

Fig. 2. Distribution of *Bactrocera zonata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* in Khyber Pakhtunkhwa as projected on elevation (a, b, c), land cover (d, e, f), and climatic zones (g, h, i) maps.

facing traps caught the highest number of flies (N = 522) followed by north (N = 362), east (N = 198), and west (N = 191) facing traps. The maximum number of flies was collected during the third sampling wk. Green color was the most attractive to fruit flies. Higher numbers of *B. zonata* and *B. dorsalis* were caught on green and yellow colored traps whereas higher numbers of *B. cucurbitae* were collected from green and white traps (Table 8). Two-way ANOVA revealed a significant association of the attractant traps with the number of fruit flies and species ($P \leq 0.001$).

Discussion

Our study confirms the presence of 9 species of *Bactrocera* fruit flies in 14 localities of 9 districts selected in distinct agro-ecological zones of Khyber Pakhtunkhwa, Pakistan. The results are in accordance with previous literature concerning the occurrence of these species from various regions of Pakistan (Ahmad et al. 2019; Zubair et al. 2019; Zain-Ul-Aabdin Abro et al. 2020). *Bactrocera nigrofemoralis*, *B. signa-*

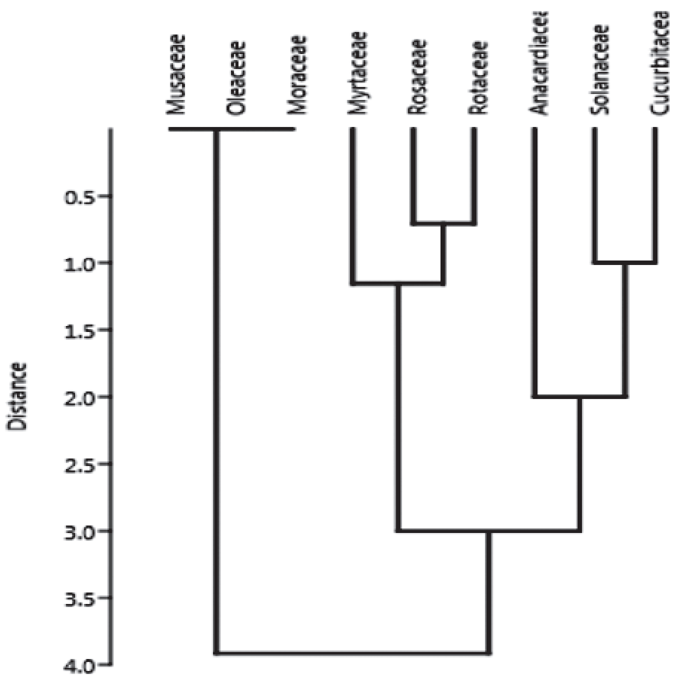

Fig. 3. Dendrogram of host plant families based on presence or absence of fruit fly species pests using Ward's distance matrix.

ta, *B. scutellaris*, and *B. zahadi* have not been reported previously in similar past studies from Khyber Pakhtunkhwa. However, they have been reported from different regions elsewhere in Pakistan including Islamabad (Gillani et al. 2002), Faisalabad (Ahmad et al. 2019), Azad Jammu and Kashmir (Zubair et al. 2019), Sindh (Zain-Ul-Aabdin Abro et al. 2020, Khan et al. 2021), and neighboring countries like India (Drew & Raghu 2002), Indonesia (Hasyim et al. 2008), and Bangladesh (Khan et al. 2015).

The presence of *B. zonata*, *B. dorsalis*, and *B. cucurbitae* as dominant and constant species during the present study is in corroboration with previous records in the country (Javaid et al. 2020; Khan et al. 2021); this indicates that none of the other minor species are invasive. It is demonstrated for fruit fly species, including *Bactrocera*, that when areas previously occupied by polyphagous fruit flies are invaded by other *Bactrocera* species, the ensuing interspecific competition may result in population decline or niche differentiation in the already-established species (Duyck et al. 2004). Faunistic diversity indices for *Bactrocera* species varied among different localities in the selected districts of the study, thus reflecting the crucial role of environmental or climatic variations and host availability in determining the distribution and abundance of species in a specific locality (Hill et al. 2016). Differences in environmental and geographical factors, cropping pattern in localities, cultivation methods, and host availability and abundance with variable susceptibility levels to fruit flies may be possible factors shaping variations in species richness and diversity of fruit flies in the region (Qureshi et al. 2000). Similarly, dominance of a certain species in ecosystems may be associated with climatic preferences and presence of hosts favorable for establishment of a population (Papadopoulos 2014).

In order to make better pest management decisions, it is essential to understand spatio-temporal variability of fruit flies. Spatial distribution of *B. dorsalis*, *B. zonata*, and *B. cucurbitae* showed that these pests occur in the highest numbers at elevations ranging from 285 to 855 masl. Climatic factors, particularly temperature, could be a determining factor driving these altitudinal variations (Duyck et al. 2004). For instance, a study by Liu and Ye (2009) in Yunnan, China, where annual average temperature remains consistently around 15 °C, found

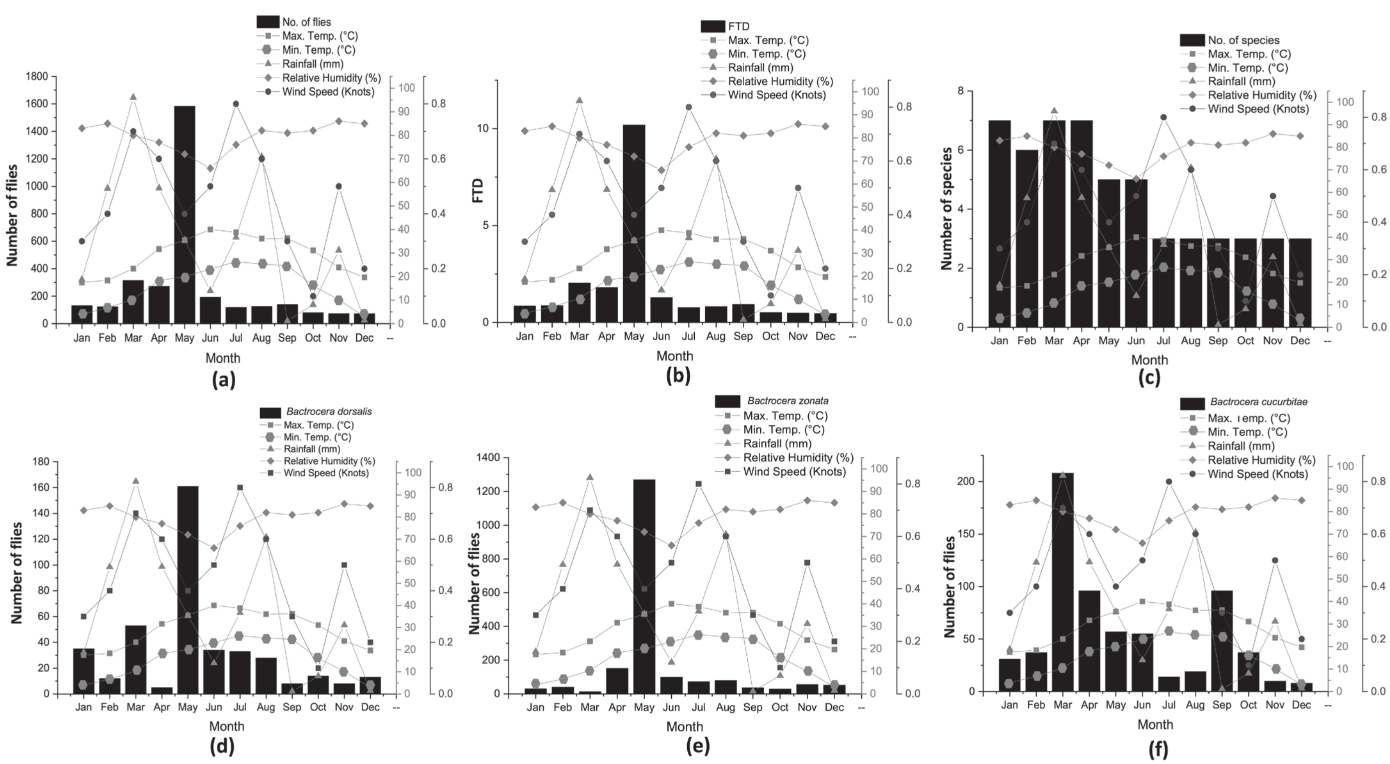

Fig. 4. Annual population dynamics of (a) number of fruit flies, (b) flies per trap per d, (c) number of species, (d) number of *Bactrocera dorsalis*, (e) number of *Bactrocera zonata*, (f) number of *Bactrocera cucurbitae* flies in relation to climatic factors in Peshawar District.

Table 4. Correlation of species-wise seasonal abundance of *Bactrocera* with climatic variables.

Species	Max. temp. (°C)	Min. temp. (°C)	Rainfall (mm)	Relative humidity (%)	Wind speed (knots)
B. dorsalis	0.2332	0.1631	0.1476	–0.4929	0.0886
B. zonata	0.2816	0.2036	0.0069	–0.4515	–0.0341
B. correcta	0.0632	0.0075	0.0589	–0.3405	–0.0876
B. signata	–0.4576	–0.4017	0.6406*	0.1213	0.3110
B. cucurbitae	–0.0005	0.0322	0.5257	–0.1912	0.3009
B. tau	–0.5217	–0.4592	0.5592	0.0865	0.1282
B. zahadi	–0.2682	-0.2336	0.6502*	0.0273	0.3843
B. nigrofemoralis	0.4031	0.2759	–0.2303	–0.7213*	0.0762
No. of flies	0.2498	0.1800	0.1120	–0.4716	0.0224
Flies per trap per d	0.2499	0.1804	0.1131	–0.4736	0.0226
No. of species	–0.3757	–0.3622	0.4837	–0.1814	0.2098

*Significant Pearson's correlation coefficient values ($P < 0.05$).

Table 5. Infestation indices of *Bactrocera* species reared from guava fruits.

Fruit weight	Pupae per kg	Adult flies per kg	% Emergence per pupae viability
0.5 kg	456	160	35%
0.495 kg	368	181	49.4%
0.503 kg	388	200	51.3%
0.542 kg	415	194	46.7%
2.09 kg	397.1	179	45.6%
Pearson's Correlation r (*P*-value)*	0.9911 (0.0089)	–0.1939 (0.8061)	

*Significant P value < 0. 05.

B. correcta only at low altitudes. Altitudinal spatial patterns also have been known to be dynamic over a certain time of the yr for some species. The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), density, for example, has been shown to vary from 200 to 4,000 masl over the monitoring season where their highest density shifted to lower altitudes in autumn from high altitudes in summer (Castrignanò et al. 2012). In Khyber Pakhtunkhwa, these flies occurred at the highest numbers in warm, semi-arid, and sub-humid zones. *Bactrocera dorsalis* has been observed previously to dominate warmer lowland habitats (Odanga et al. 2018).

Seasonal population dynamics of fruit flies and its relationship with environmental factors was studied in Peshawar during Jan to Dec 2019 and showed that the peak abundance of flies occurred during the warmer mo of the yr (May), whereas the population started declining during the winter mo (Nov and Dec). Higher fruit fly populations in warmer mo of the yr (Jun to Aug) have been reported earlier in Pakistan (Gillani et al. 2002; Ahmad et al. 2003; Mahmood & Mishkatullah 2007; Uddin et al. 2016). The peak or increasing trend observed can be linked to favorable weather conditions and fruit availability (Win et al. 2014; Khan & Naveed 2017). The decline in flies per trap per d recorded in the colder mo could be credited to the onset of harsh environmental conditions in winter that negatively impact larval development or the unavailability of hosts in these mo (Mahmood & Mishkatullah 2007; Darwish et al. 2014; Win et al. 2014; Khan et al. 2021). Studies suggest that temperatures around 30 °C support the maximum densities of flies, whereas the number declines with a decrease in temperature (Rai et al. 2008). Several studies have established that species like *B. zonata* are non-diapausing and multivoltine species, and in Pakistan the adults are known to be present throughout the yr with the exception of Jan and Feb (Qureshi et al. 1992).

Our results indicated a significant positive correlation of fruit fly abundance and flies per trap per d with temperature (minimum and

Table 6. Abundance and sex ratio of *Bactrocera* fruit fly species collected through different collection methods.

	Parapheromone traps *N**				
Species	Methyl eugenol	Raspberry extract	Food-baited traps *N* (male: female)	Fruit rearing *N* (male: female)	Total *N* (male: female)
B. dorsalis	1,734	0	50 (23: 27)	22 (5: 17)	1,806 (1,762: 44)
B. zonata	7,803	0	17 (11: 6)	353 (173: 180)	8,173 (7,987: 186)
B. correcta	289	0	0	0	289 (289: 0)
B. signata	28	28	28 (28: 0)	0	28 (28: 0)
B. cucurbitae	1,258	1,258	25 (14: 11)	0	1,283 (1,272: 11)
B. tau	245	245	7 (3: 4)	0	252 (248: 4)
B. zahadi	38	38	0	0	38 (38: 0)
B. scutellaris	187	187	0	0	187 (187: 0)
B. nigrofemoralis	2	0	0	0	2 (2: 0)
Total	11,584 (96%)		99 (0.82%)	375 (3.2%)	12,058

*Flies collected were male individuals only.

Table 7. Comparative efficacy of different combinations of food-baited traps.

Treatment	Food baits	*B. dorsalis N* (male: female)	*B. zonata N* (male: female)	*B. cucurbitae N* (male: female)	*B. tau N* (male: female)	*P*-value	Total	Flies per trap per d
Treatment 1	Sugar solution	0	0	0	0	0.0074	0	0
	Banana extract + sugar + insecticide	7 (3: 4)	0	0	0		7	0.5
	Peach extract + sugar + insecticide	10 (4: 6)	2 (2: 0)	0	0		12	2.36
	Guava extract + sugar + insecticide	12 (6: 6)	0	0	0		12	2.36
	Total	29 (13: 16)	2 (2: 0)	0	0		31	
Treatment 2	Yeast only	4 (2: 2)	0	0	0	0.2642	4	0.57
	Sugar only	5 (2: 3)	0	0	0		5	0.71
	Sugar +yeast	1 (1: 0)	5 (2: 03)	0	0		6	0.86
	Grapes extract + sugar + yeast	0	2 (2: 0)	0	0		2	0.28
	Banana extract + sugar + yeast	2 (0: 2)	8 (5: 3)	0	0		10	1.43
	Total	12 (5: 7)	15 (9: 6)	0	0		27	
Treatment 3	Sugar only	0	0	2 (1: 1)	0	0.0000	2	0.29
	Yeast only	0	0	0	0		0	0
	Sugar + yeast	0	0	0	0		0	0
	Banana extract + sugar + yeast	2 (2: 0)	0	15 (9: 6)	7 (3: 4)		24	1.71
	Guava extract + sugar + yeast	7 (3: 04)	0	8 (4: 4)	0		15	1.07
	Total	9 (5: 04)	0	25 (14: 11)	7 (3: 4)		41	

**P*-value calculated for 2-way ANOVA.

maximum) as reported by others (Khan & Naveed 2017; Zain-Ul-Aabdin Abro et al. 2020; Khan et al. 2021). Species number also seemed to decrease with increasing temperature (minimum and maximum) and relative humidity, whereas it increased with increase in rainfall and wind speed (Chen & Ye 2007; Abbas et al. 2018). The apparent differences in correlation with climatic factors might exist due to the fact that fruit fly abundance is not only influenced by the climatic factors but by other confounding factors such as host availability, ripening, or harvesting stage of fruit crops (Rwomushana & Tanga 2016).

Broad host range and invasive power of some *Bactrocera* species has made the genus a serious threat to fruit crops (White & Elson-Harris 1994; Clarke et al. 2005). We collected *Bactrocera* species from traps installed in host plants of the families Rosaceae, Rutaceae, Anacardiaceae, Myrtaceae, Solanaceae, Cucurbitaceae, Oleaceae, Moraceae, and Musaceae. Several *Bactrocera* species have been reported to be polyphagus (Bomfim et al. 2014; Hafsi et al. 2016). Host-specificity to native fruits is likely to change upon the native fruit's unavailability (Robacker & Ivich 2002). Host availability is regarded as a main driver of seasonal abundance and population build-up or fluctuations in *Bactrocera* fruit flies (Hossain et al. 2019). Population of fruit fly species tends to increase with fruit ripening (Mwatawala et al. 2006) and tends to decline when fruit is harvested (Drew & Hooper 1983; Khan & Naveed 2017). Different volatiles or odors emitted by a fruiting tree lead a fruit fly to a particular habitat of host plants (Fletcher & Prokopy 1991; Aluja & Prokopy 1993; Light & Jang 2020). In addition, other characteristics like plant architecture and surface texture, properties of leaf, fruit size, shape, and color play a significant role in host searching and use (Prokopy & Owens 1983; Diehl et al. 1986; Aluja & Prokopy 1993). Yellow to orange hues in traps (particularly plastic traps) have been assumed to be visually attractive to these flies (Thomas et al. 2001).

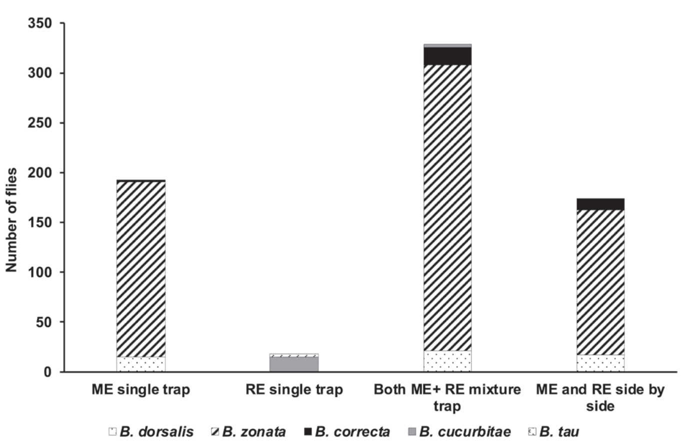

Fig. 5. Fruit fly species response to methyl eugenol single trap, raspberry extract single trap, methyl eugenol + raspberry extract mixture traps, methyl eugenol and raspberry extract traps placed side-by-side.

It is known that different species of tephritids respond variably to different traps and lures (Aluja et al. 1989). It was thus important that we conduct our study in different habitats, multiple locations, and with different species of fruit flies. The survey showed that pheromone traps collected higher numbers of several species of *Bactrocera*, which indicates that such traps are suitable tools for monitoring population trends of fruit flies (Zain-Ul-Aabdin Abro et al. 2020). Food-baited traps reflected the feeding activity of fruit flies because adult tephritid flies actively search for food sources for their survival and reproduction (Prokopy & Roitberg 1992). Our findings revealed that peach and guava pulp-baited food traps collected significantly higher numbers of both male and female fruit flies compared with the other food baits tested. The addition of sugar and yeast significantly increased the number of fruit flies trapped. Protein and yeast hydrolysate baits likely were more attractive to fruit flies because adult flies require protein for gonad maturation, and hence seek both carbohydrates and proteins after emergence (Fontellas-Brandalha & Zucoloto 2004). Such food attractants can thus be useful in detecting and monitoring both sexes of fruit flies (Thakur & Gupta 2013).

Conclusions

This survey is an important contribution towards exploring bionomics and complex ecological status of fruit flies in Khyber Pakhtunkhwa, Pakistan. The study provides baseline knowledge regarding fruit flies in the region. Our results clearly show the variability of *Bactrocera* flies in different

Table 8. *Bactrocera* species collected in different colored traps installed in different directions.

Trapping factor	Treatment	*B. dorsalis* N	*B. zonata* N	*B. correcta* N	*B. cucurbitae* N	*B. tau* N	*B. zahadi* N	*B. signata* N	Total
Wk	1st	1	87	9	31	4	3	0	135
	2nd	8	233	12	30	2	0	0	285
	3rd	41	476	11	23	0	0	0	551
	4th	16	264	8	11	2	0	1	302
Attractant	Methyl eugenol	66	1,060	40	0	0	0	0	1,166
	Raspberry extract	0	0	0	95	8	3	1	107
Color	White	6	124	5	27	2	0	0	164
	Red	11	189	5	19	0	0	1	225
	Green	28	376	10	27	2	1	0	444
	Yellow	21	371	20	22	4	2	0	440
Direction	North	15	319	9	18	1	0	0	362
	South	26	430	20	41	3	1	1	522
	East	22	166	5	4	1	0	0	198
	West	3	145	6	32	3	2	0	191

regions because their relative abundance and dominance varied across sites and among mo of the yr. Our findings suggest that different factors (biotic and abiotic) can influence the bionomics and distribution of fruit flies. Species of fruit flies also seemed to display a level of host specificity. Possible limitations of this study could include the trapping of only male flies of certain species by pheromone traps. However, using a combination of different sampling and trapping methods may have overcome this bias to an extent. Comparison of trapping characteristics in the present study can be of interest and significance in fruit fly monitoring and surveillance. Findings from this study can be useful in time-effective control and prevention of fruit fly infestations. However, there is a need for extensive studies on the biodiversity of fruit flies followed by determining the pest status of different species in various regions of the country. Further studies can provide a broader insight of activity patterns or biological rhythms of these economically important pests because information regarding abundance and dynamics under natural conditions is required to understand the pest status of a species. Fruit fly population forecasting for management purposes can be improved by using new analytical tools for weather estimation. Monitoring programs can thus be established corresponding with the biological dynamics of these flies.

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