

**POPULATION DYNAMICS OF *DROSOPHILA SUZUKII* (DIPTERA:
DROSOPHILIDAE) IN PESHAWAR AND ITS DISTRIBUTION IN KHYBER
PAKHTUNKHWA PROVINCE OF PAKISTAN**

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ABSTRACT

The spotted wing Drosophila, *Drosophila suzukii*, native to South-East Asia, is an invasive and serious pest of fruit crops. In Pakistan, the species was first detected in 2005 and was found in cherry orchards of Khyber Pakhtunkhwa in 2014. The objective of the study was to monitor the seasonal activity of *D. suzukii* in three different locations of Peshawar and its distribution in Khyber Pakhtunkhwa province along the main commercial trading routes in different markets during, 2016/2017, with the aid of traps baited with synthetic fruits-essence. Results revealed that in Peshawar *D. suzukii* population built-up in first week of November as temperature dropped below 28°C with a peak of population in the first week of December at lowest temperatures of 20°C. Thereafter, occurred a gradual decrease in the population with another peak of January and afterwards population ended later in February when temperatures started to increase for the summer period. Further, females *D. suzukii* appeared less affected by extreme cold of December. Results of survey confirmed the presence of *D. suzukii* in most districts of the province under study. The results suggest that *D. suzukii* is present in Peshawar fruit markets, with a status of scavenger and not as pest in cold part of the year, and it is distributed in almost the whole Khyber Pakhtunkhwa province.

Key words: spotted wing Drosophila, bait trap, pest attribute, population temporal fluctuation, commercial trading routes.

Introduction

Vinegar flies include over 3000 species among which *Drosophila suzukii* Mats. (Diptera: Drosophilidae) is included in the subgenus *Sophophora* under *melanogaster* species group (Hauser, 2011). Being native to South-Eastern of Asia, *D. suzukii* was invading various locations of Japan, China, North and South Korea, and Burma around the 1930's (Cini *et al.*, 2012). Afterwards, the pest has become a global issue that directly damage fruits as female flies puncture healthy fruits prior to harvesting (Hauser, 2011).

At the start of 21st century, spotted wing *Drosophila* continued to profoundly expand its range in Asian countries chiefly by trade, to the regions with favorable environment and hosts. In Pakistan, *D. suzukii* was first detected and reported as a scavenger from Islamabad (Amin ud Din *et al.*, 2005) but had till now not shown any pest character in fruit orchards in the country. However, *D. suzukii* was found in Kalam, Swat of Khyber Pakhtunkhwa province in cherry orchards with unclear status (Khan *et al.*, 2019). The spreading of *D. suzukii* in the region was probably due to the commercial routes and exchanges between different cities of the province of Khyber Pakhtunkhwa. In parallel, the pest's characteristic in these agro-ecosystems of Pakistan might be also linked to the specific climatic ranges' conditions favorable to the female fecundity and then to the spreading capacity of *D. suzukii*.

Khyber Pakhtunkhwa (34.9526°, 72.3311°), a province of Pakistan, has diverged climatic conditions. The agroecological status of the province reflects a rich source of flora and fauna with large number of crops, vegetables and fruits that are supplied to the local markets and within the province (Khan M. S., Personal Observations). The increasing pest problems associated with fruit crops due to *D. suzukii* are possibly due to commercial transportations and exchanges. In the present study, we examined the population dynamic of *D. suzukii* in Peshawar and its distribution in various cities of the province along the main commercial routes.

Materials and Methods

Trap Preparation

Similar traps of Khan *et al.* (2019) with a bait attractant of synthetic fruit-essence solution found to be the best attractant to *D. suzukii* were used in this study. Briefly, small entry points

for flies were constructed in sides of each jar with one free side for adding and removing bait solution from the trap.

Population dynamic in Peshawar

For monitoring the seasonal activity of the spotted wing *Drosophila*, three traps were installed at each of the three marketplaces of Peshawar: Board Bazar: N 34°0'0", E 71°28'12"; Firdos Bazar: N 34°0'36", E 71°34'12"; Fruit Market Nasirpur: N 34°01'12", E 71°41'24". They are differing in terms of environment (temperature fluctuations) as well as of anthropogenic activities and hygiene conditions. The data collection started in the month of September 2016 prior to expected arrival of *D. suzukii* and continued till end of February 2017.

Traps, randomly placed, were weekly rebaited and all collected *Drosophila* specimens were properly identified. Male and female flies in each sample were separated using identification keys (Vlach, 2010; Timmeren *et al.*, 2012) and counted.

The daily temperature for Peshawar was extracted from the SMRFC-PMD dataset (<http://smrfc.pmd.gov.pk>) and subsequent weekly mean, maximum and minimum temperatures were obtained.

Spatial distribution in Khyber Pakhtunkhwa province

A survey was conducted on *D. suzukii* in 11 localities (Peshawar, Charsadda, Nowshera, Mardan, Swabi, Swat, Malakand, Jamrud, Kohat, Karak and Bannu) of Khyber Pakhtunkhwa province during 2017, with the aid of the same aforementioned traps baited with synthetic fruit-essence solution, along varying altitudes of the main commercial route.

Traps were installed in three different locations in each district to ensure fly trap, which were monitored after 24 hrs post installation and the trapped flies were collected in Eppendorf tubes for identification.

Statistical Analysis

Using weekly mean temperatures, correlation analyses were performed. The male and female flies on counting were $\sqrt{(x+0.5)}$ transformed for data normality and to homogenize the data for analyses of variance (ANOVA). Untransformed means and their interactions were compared using Tukey's HSD test. All analyses were performed using SPSS 20.0 package.

Results

Population dynamic in Peshawar

No specimens were trapped until November 2016. Thereafter, more females were trapped as compared to males (Fig. 1; results of ANOVA: $F_{1,92} = 39.59$, $P < 0.001$).

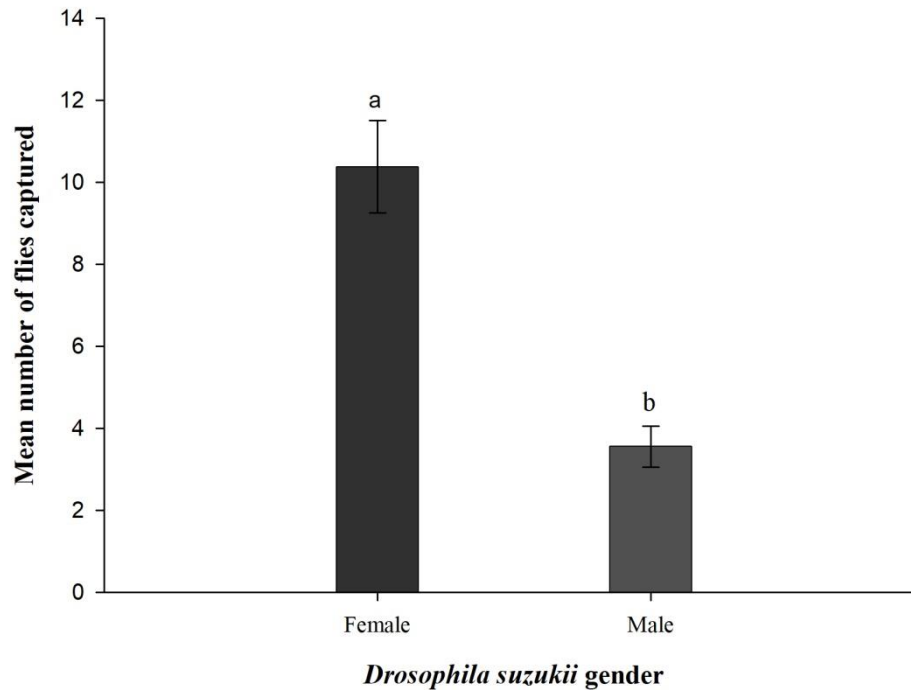


Figure 1. Overall mean (\pm SE) number of males and females of *Drosophila suzukii* captured in Peshawar throughout the winter season of 2016-17. Means with different letters differed significantly at 5% level using Tukey's HSD test.

Drosophila suzukii population appeared greatly linked to the temperature, which starts to increase when the coolest season of the year started in Peshawar and it remained active till February of the following year when the temperatures started to increase (Fig. 2). During the presence of *D. suzukii*, two peaks of populations appeared, one of 34.00 flies trap⁻¹ week⁻¹ when the mean temperature dropped to 20°C, and another one of 28.00 flies trap⁻¹ week⁻¹ when mean minimum temperature was below 10°C.

The number of flies trapped significantly correlated with mean maximum, minimum and average weekly temperatures (Table 1). A strong negative correlation of *D. suzukii* populations with decreasing temperatures was found except for males was slightly positively correlated with only mean minimum temperature. In overall, an increase in *D. suzukii* population was found in response to decreasing temperature.

Table 1. Pearson correlation analysis of *Drosophila suzukii* and their sexes with mean maximum, minimum and average weekly temperature (n=27) recorded in Peshawar during, 2016-17.

Pearson Correlation		Male	Female	Total
Temp _{max}	Correlation Coefficient	-0.625**	-0.770**	-0.746**
	Sig. (2-tailed)	0.000	0.000	0.000
Temp _{min}	Correlation Coefficient	0.564**	-0.724**	-0.695**
	Sig. (2-tailed)	0.002	0.000	0.000
Temp _{av}	Correlation Coefficient	-0.604**	-0.760**	-0.732**
	Sig. (2-tailed)	0.001	0.000	0.000

All correlations are significant at P value < 0.05.

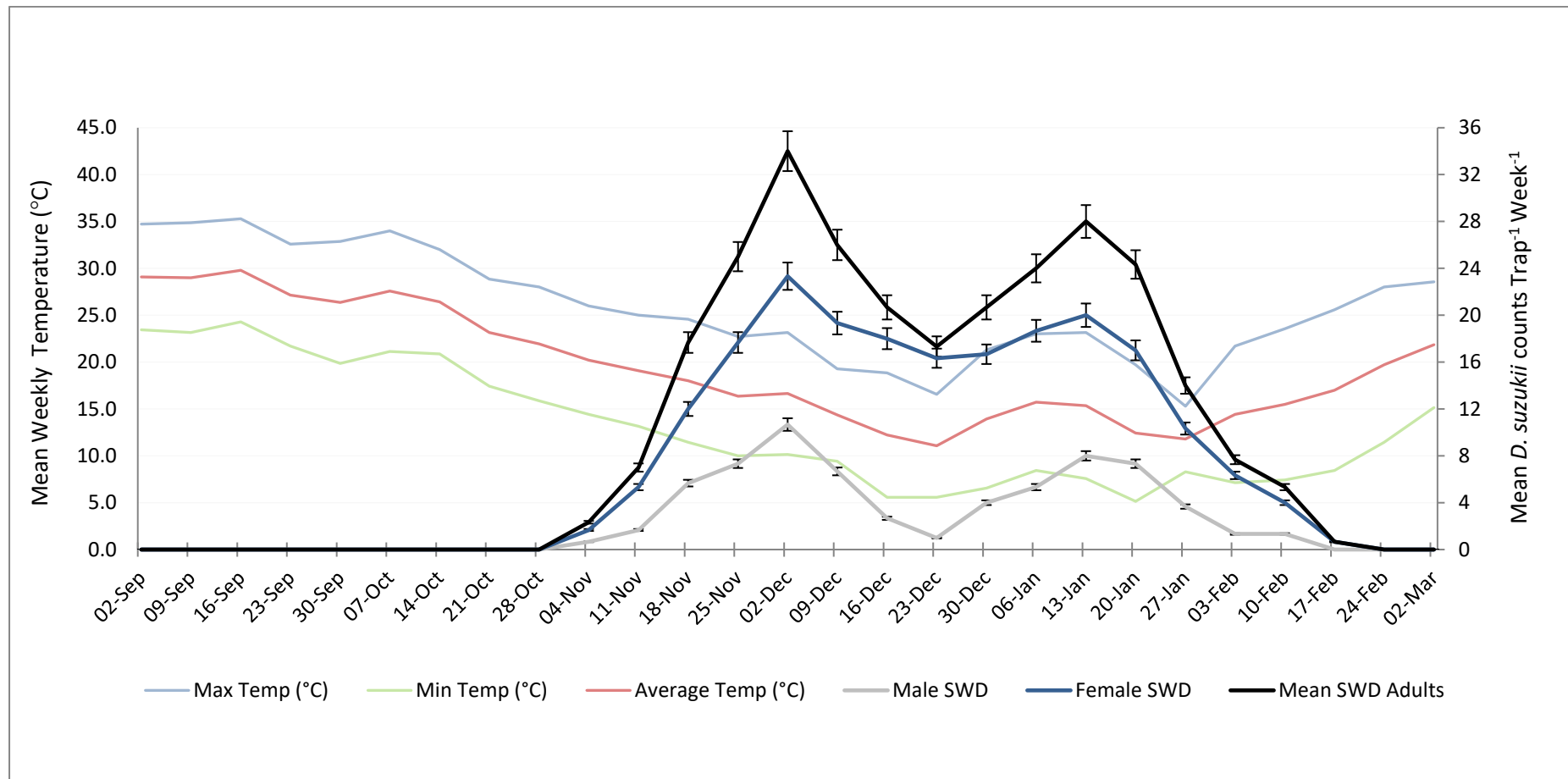


Figure 2. Weekly temperatures (average, maximal and minimal temperatures) and mean (\pm SE) numbers of *Drosophila suzukii* trapped in Peshawar throughout September 2016 and February 2017.

In addition, about three times more females were captured during the overall survey than males (Fig. 1) particularly at temperatures below 10°C (Fig. 2), suggesting a better adaptability of females than males during cold weather.

Distribution of *Drosophila suzukii* in the province of Khyber Pakhtunkhwa

The results of survey revealed that *D. suzukii* was distributed all over the province of Khyber Pakhtunkhwa (Table 2 and Fig. 3). Among the invaded parts, *D. suzukii* were found more present in Peshawar and Mardan district, however, lacking in Karak. Although in all locations more females were captured as compared to males, the number of males was significantly higher in Mardan.

Table 2. Mean (\pm SE*) numbers of *Drosophila suzukii* per trap found in different surveyed locations of Khyber Pakhtunkhwa during 2016-17.

District/ Location	Sex		
	Total	Female	Male
Swat	8.33 \pm 4.63abc	5.00 \pm 2.65A	3.33 \pm 2.03A
Malakand	5.00 \pm 2.65bc	3.00 \pm 1.73A	2.00 \pm 1.00A
Mardan	23.33 \pm 3.48a	14.33 \pm 1.76A	9.00 \pm 1.73B
Charsadda	10.67 \pm 5.36abc	7.33 \pm 3.71A	3.33 \pm 1.67A
Nowshera	15.00 \pm 2.65abc	9.67 \pm 2.03A	5.33 \pm 0.67A
Peshawar	21.67 \pm 2.96a	15.67 \pm 2.60A	6.00 \pm 0.58A
Jamrud	10.00 \pm 5.29abc	6.00 \pm 3.21A	4.00 \pm 2.08A
Kohat	5.67 \pm 3.48bc	3.67 \pm 2.33A	2.00 \pm 1.15A
Karak	00.00 \pm 0.00c	0.00 \pm 0.00	0.00 \pm 0.00
Bannu	1.67 \pm 1.67bc	1.33 \pm 1.33A	0.33 \pm 0.33A
Swabi	16.67 \pm 2.96ab	11.00 \pm 2.65A	5.67 \pm 1.20A

*Different letters (small letters for location comparisons and capital letters for sex comparisons) following mean values are significantly different at 5% level using Tukey's HSD test.

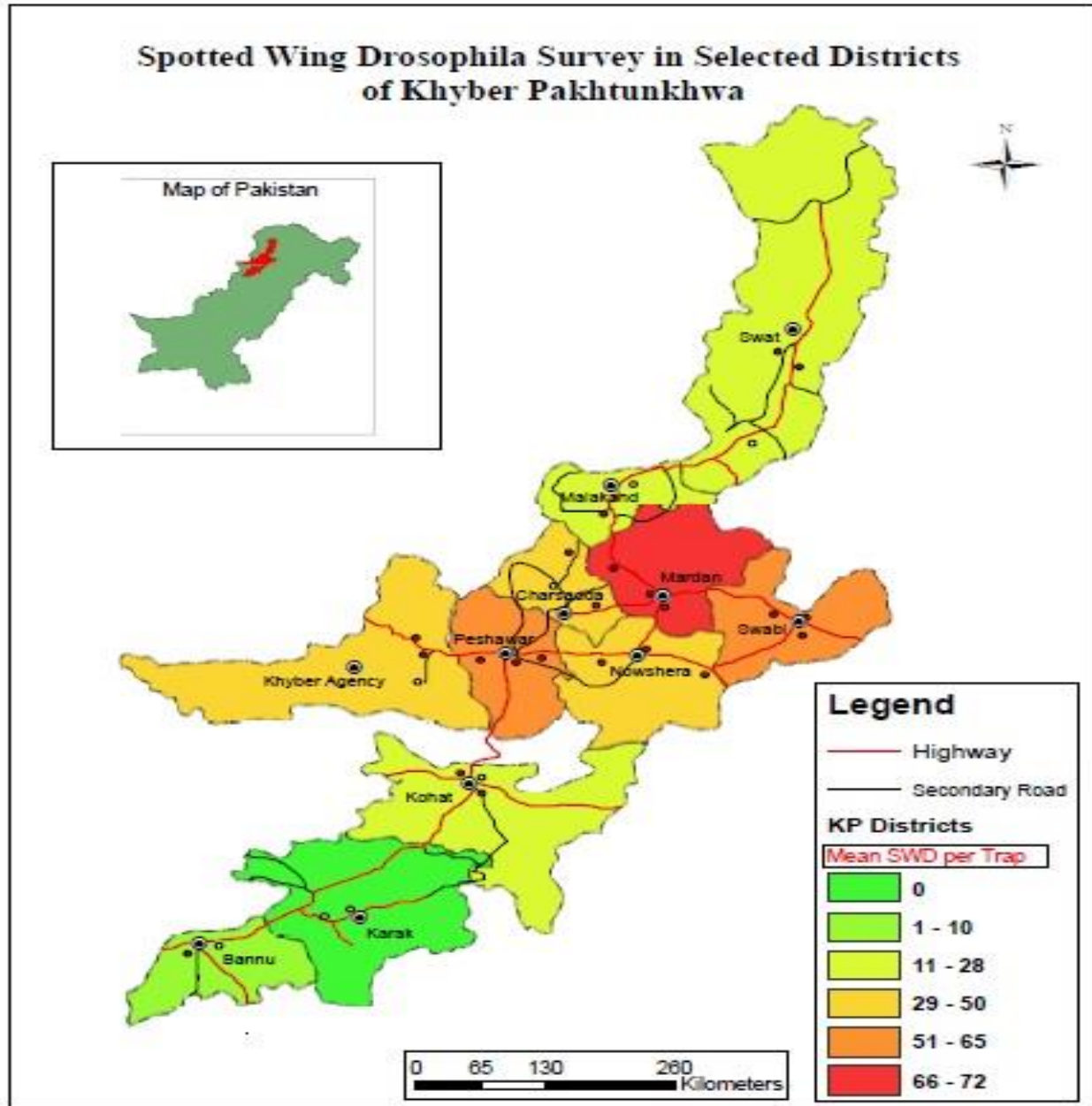


Figure 3. Map distribution of *Drosophila suzukii* in different locations along the main commercial/trading routes in Khyber Pakhtunkhwa province, using ArcGIS software.

DISCUSSION

From this study, the first incidence of *D. suzukii* was observed in November, 2016 and this is the first report of this species from Peshawar. It is assumed that possibly *D. suzukii* was present in this area before; however, not accurately monitored at the right time. In fact, Shahjehan *et al.* (2004) have not detected *D. suzukii* in Peshawar most probably because their survey was

conducted in summer months from March to September, while *D. suzukii* can only be detected at temperatures below 28°C from November onwards till February.

The temperature greatly influenced the population of *Drosophila suzukii* where increased when maximum weekly temperature dropped down to 28°C and remained steadily in winters till the mid of February the following year when again temperatures increased. Baser *et al.* (2015) found also that *D. suzukii* population initiated in October when average temperature reached to 18°C and the flies remained till March of the following year. Our study is also in agreement with Bale (2002) who acclaimed that winter temperatures determine the ability of certain invasive species, like *D. suzukii* to be established in an area. Likewise, Calibria *et al.* (2012) concluded a reduced activity and oviposition in this species when temperature exceeds 30°C.

Moreover, peak *D. suzukii* population in Peshawar was observed below 30°C and around 20°C to 25°C, during which this temperature range was reported to enhance activity and oviposition of *D. suzukii* (Dreves *et al.*, 2009; Hamby *et al.*, 2013), enhanced their fecundity (Kinjo *et al.*, 2014) and their development (Asplen *et al.*, 2015).

In addition, in our study females were more abundant than males, particularly during the coolest periods. Similarly, Ramos *et al.* (2014) found in Mexico that the population of *D. suzukii* female was twice more abundant than males. Our results exhibited that average weekly high temperatures did not adversely affect the population and sexes of *D. suzukii*, although males positively correlated only with average low temperatures. This could indicate that *D. suzukii* population increased inversely to mean temperatures; however, with a greater adaptation of females to low temperature than males. It has been already publicized that *D. suzukii* occurrence is adversely affected by an increase in weekly mean temperatures (Walsh *et al.*, 2011). Amin ud Din *et al.* (2005) suggested winter average temperatures around 18°C being favorable condition in the establishment and prevalence of *Drosophila* fauna in Pakistan.

Moreover, Ramos *et al.* (2014) revealed average weekly high temperatures as not helpful in affecting *D. suzukii* populations, but females inflict higher adaptability to fluctuations in temperatures, and influence of winter low temperatures only on males. These results are in line with the studies of Baser *et al.* (2015) explaining negative correlation of *D. suzukii* populations

with temperatures. In another trial, Kimura (2004) found that *D. suzukii* female flies are colder than their male counterparts and can thus withstand extreme temperatures.

The survey performed in Khyber Pakhtunkhwa province for *D. suzukii* along the main trading passages was a significant step in exploring knowledge on this fly distribution in the region, which confirmed its presence in almost all surveyed areas of Khyber Pakhtunkhwa province. Likewise, *D. suzukii* was previously observed in cherry orchard in Kalam (upper Swat) of Khyber Pakhtunkhwa (Khan M.S., Personal Observations in 2014); and this study confirmed the presence of this species in this region. In addition, this distribution of *D. suzukii* in Khyber Pakhtunkhwa may be due to trade and transportation of food items between districts as it is reported that spotted wing *Drosophila* are mostly carried by trade means from place to place.

CONCLUSION

Drosophila suzukii is basically a temperate insect that outbreaks as winters prevail around November in Pakistan and remain till late in February when cold season is over with peak populations in both December and mid of January of the following year. The population of *D. suzukii* is greatly influenced by the temperature which increased when the temperature dropped down below 28°C, with females colder tolerant than males. This means once established in temperate parts of Khyber Pakhtunkhwa province and specifically in Swat, the coolest place of the province during summer, *D. suzukii* would be a serious pest particularly during winter following the trading routes. Therefore, specific cares should be taken in any fruit crop growing in that region and during winter.

CONFLICT OF INTEREST STATEMENT:

The authors declare that they have no conflict of interest.

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