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Exploring The Pest Behaviour of Drosophilids On The Basis Of Their Presence In Fruit Belt Regions Of Uttarakhand Along With Observation Of Their Ovipositor

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Abstract: Uttarakhand is an alpine state which has many districts relying on fruit crops for economic purposes. This study has been conducted in three districts of Uttarakhand namely, Chamoli, Uttarkashi and Tehri where the soft-skinned fruit orchards were targeted for collection of drosophilids during different stages of ripening fruit. One of the sites sampled was taken as control site where fruit orchards were not present but had only few fruiting trees with mixed vegetation. With this study, total of ten species has been collected extensively and there seems to be a pattern in the presence of these drosophilids. At the ripening stage of fruits, the species that were present in the sites were *Drosophila suzukii, Drosophila subpulchrella, Drosophila punjabiensis*. It indicates that these species are attracted toward ripening fruits and later their ovipositor was also observed and were confirmed as serrated ones. On the contrary, the drosophilids present at ripen stage were *Drosophila nepalensis, Zaprionus indianus* and *Drosophila takahashii* and these species were also present in control site. Interestingly, *Drosophila melanogaster* were only present when the fruits were plucked and there were only piles of rotten fruits. The control site, on the other hand, had a few additional species that were not found in the fruit orchards. Later study of their ovipositors also suggested that these species might not harm the ripening fruits as they may not act as primary pests.

Keywords: Drosophilids, Fruit orchards, Ovipositor, Pest behaviour, Uttarakhand

Introduction

Uttarakhand is a hilly state nestled in the Himalayan foothills. Here, horticulture practice is rapidly increasing and is becoming an important sector for state's economic development. This industry has the potential to make the state shift away from conventional agriculture and toward horticulture, resulting in higher yields. Crops like apple, pears, apricot, plum, citrus fruits etc. are grown for economic purposes in the hilly regions of state. Uttarakhand ranks first in the country in production of Pear (0.788 lakh MT), Peach (0.579 lakh MT), Plum (0.362 lakh MT) and Apricot (0.282 lakh MT). While it ranks third in the country in production of Apple (0.62 lakh MT. (*STATE PROFILE: Department of Horticulture, Government Of Uttarakhand, India*, n.d.). Fruits account for 73.12 percent of the total area under horticulture crops, but yield share is only 53.61 percent of the production (Tuteja, 2013). Fruit crops must be undamaged and fully grown to yield the highest profit. Fertilizers, herbicides, protective netting, pollinators, and other methods are employed to meet these requirements, along with suitable environment. All these elements work together to ensure that the yield is as high as possible. However, predators and pests continue to damage fruit crops. Many insects feed and breed on various portions of the crop, particularly fruits, causing damage to the crop. Agricultural losses owing to the introduction of exotic pests as a result of globalization and climate change are estimated to be over 1.4 trillion dollars per year (Pimentel et al., 2001).

Few of such pests belong to family Drosophilidae, of which many fruit flies act as primary or secondary pests. Generally, Drosophilids are not considered pests as they deposit their eggs on decaying fruits, vegetables, and wild mushrooms (Deprá et al., 2014). However, *Drosophila suzukii* (Spotted Wing *Drosophila* = SWD) has been confirmed to harm soft-skinned and ripening fruits over the world, resulting in huge economic losses (Asplen et al., 2015; Deprá et al., 2014; Farnsworth et al., 2017; Ferronato et al., 2019; Hauser, 2011; Schetelig et al., 2018; Spotted-Wing *Drosophila - D. suzukii (Matsumura)*, n.d.; Walsh et al., 2011). *D. suzukii* is native to southeast Asia and has its natural span cover in China, India, Korea, Myanmar, Russia, and Thailand (Hauser, 2011; Spotted-Wing *Drosophila - Drosophila suzukii (Matsumura)*, n.d.). Matsumura firstly described the species in 1931. Because of its preference for ripening (not merely overripe) soft skinned or stone fruits for laying eggs via its serrated ovipositor, this species is being studied and monitored as a pest all over the world (Ahmed et al., 2019; Asplen et al., 2015; Calabria et al., 2012; Cini et al., 2012; Deprá et al., 2014; Lee et al., 2011; Mitsui et al., 2006). The *Zaprionus indianus*, has also been collected and confirmed as pest drosophilid (Bernardi et al., 2016; Fartyal et al., 2014). The mechanism behind these fruit flies being arduous pest is the fact that these pests have ovipositor which is serrated and saw like. The ovipositor structure aids drosophilids in laying their eggs. With help of it, the pest drosophilids can insert their eggs within the soft skinned fruits by puncturing it. This serration causes spots that leads to reduction of its aesthetic value, and it also increases the vulnerability of fruit to be attacked by other drosophilid species and further bacterial and fungal growth (Walsh et al., 2011). Some of the drosophilids may also act as secondary pest making further damage and economic losses. These secondary pests drosophilids rely on other pests to cause initial punctures and then use it for further damage.

Drosophilid's pest behaviour, particularly in the case of *D. suzukii* and *Z. indianus*, are being investigated extensively around the world due to the amount of losses it is generating in temperate zones (Asplen et al., 2015; Bernardi et al., 2016; Cini et al., 2012; Deprá et al., 2014; Fartyal et al., 2014; Hauser, 2011; Walsh et al., 2011). However, research on drosophilid's pest behaviour is scarce in India even though hilly states like Uttarakhand, Jammu & Kashmir, and Himachal Pradesh rely on horticulture as a cash crop for economic upliftment and here the chances of attacks of these pest drosophilids is also high. Drosophilids are not even considered as pest in India's existing major integrated pest management systems (CHERRY, n.d.). This research paper attempts to draw attention toward exploration of different drosophilid species which may act as pest drosophilids based on their presence in fruit orchards along with possession of slight to heavy serrated ovipositor.

Materials and Methods

Collection Sites / Study area:

Temperate zone within the Garhwal region of Uttarakhand, where fruit farming has been fulfilling economic upliftment of locals were selected after pre collection exploration of the Garhwal region. Three different districts namely Chamoli, Uttarkashi and Tehri were selected for this study. In these districts there are many fruit orchards cultivating stone and soft skinned fruits including, Strawberry, Plums, Apricot, Peach, Pears, and Apple. Within all the sites, the Apple had dominancy regarding number of trees. Jangal Chatti, Gopeshwer, Harshil and Kanatal were selected within upper mentioned districts (Figure. 1). Out of all these sites, the site Gopeshwer was chosen as control (scattered fruit trees) as there are not much of fruit orchards. Here, the sampling was done in fields with mixed vegetation and few

fruit trees only. Collection of drosophilids within the sampling sites was conducted around the fruiting season in year 2020 and 2021. Sampling was done thoroughly and repeatedly for collection of drosophilids.

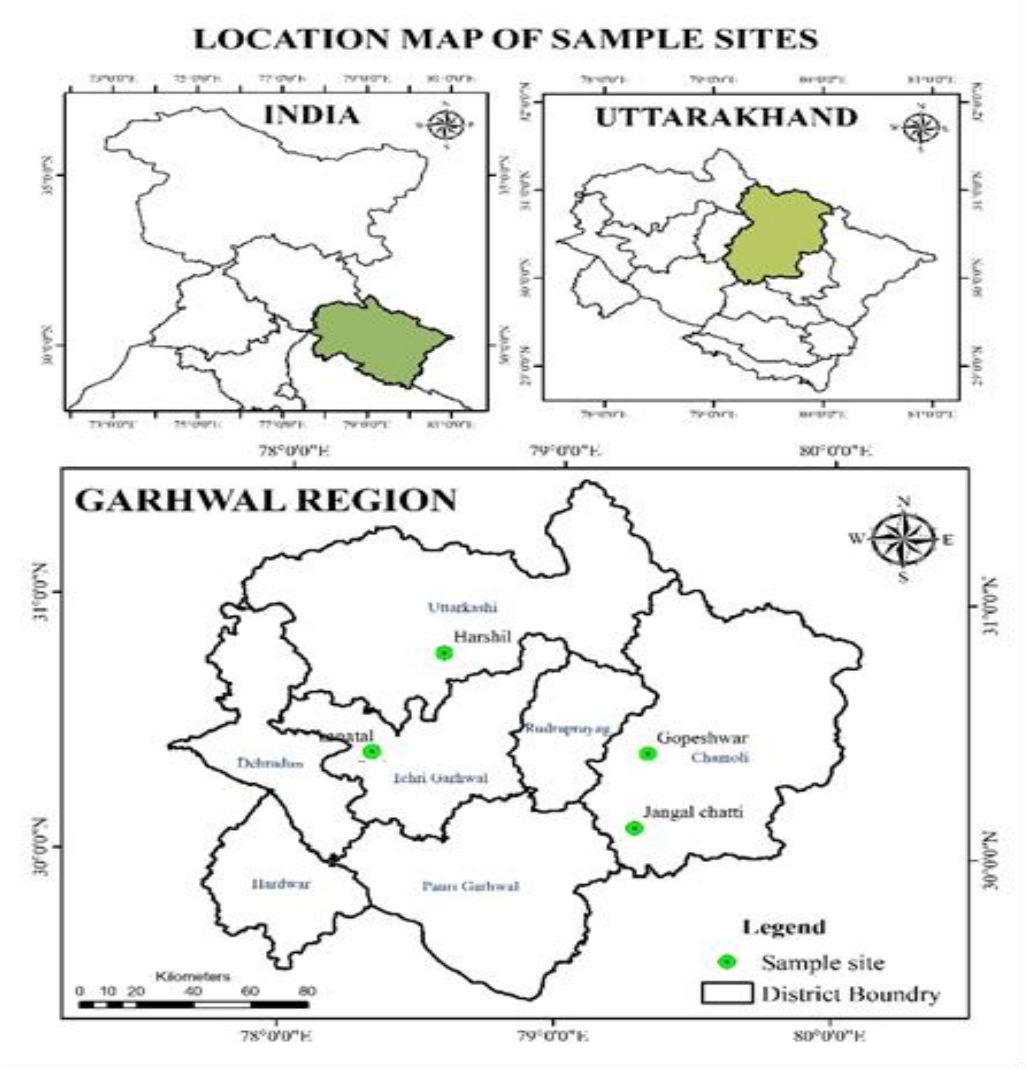

Figure 1: Location of sampling sites within Uttarakhand

Methods

The methods used to collect the drosophilids were following:

(a) Collection with the Aspirator: This approach is widely used to collect flies that are performing courtship or resting over damaged and undamaged fruits in fruit orchards.

(b) Net sweeping method: Fruiting trees and natural feeding places such as rotting fruits and leaves were swept using a modified insect net (Pipkin, 1965 Okada, 1956). Net sweeping is used to catch flies that are hovering around fruiting trees and aren't attracted to the trap.

(c) Trap-Bait method: For trap bait, 500 mL bottles were employed. Various fruits were blended with yeast and vinegar as bait. In the

orchard area, the bait traps were hung in various niches of fruiting trees. The traps were collected at dawn in order to catch more flies.

Most of the flies were successfully collected via net sweeping directly from plant leaves and fruits. Absolute ethanol was used to preserve the specimens. The drosophilids were etherized, classified, and identified using a stereo zoom microscope after collection. The organs were detached from the body to analyse the precise structure of the head, male legs, male terminalia, and female terminalia. They were cleaned by boiling for several minutes in a 10% KOH solution at 100°C, then studied under a compound light microscope while adding a droplet of glycerol. Individual females were given separate vials containing routine laboratory food medium to breed on. The offspring of such solitary gravid females were used to identify the species, especially the male ones. Standard taxonomic protocols (Sturtevant, 1927; Thorckmorton, 1962; Gupta, 2005) and online literature and identification sites (Japan *Drosophila* Database [JDD] http://www.*Drosophila*.jp/jdd/index_en.html, Biological Classification System [BCIS] https://evolgen.biol.se.tmu.ac.jp/Classification/index.jsp and Fly Base https://flybase.org/) were used to identify species. For observation of ovipositor the female drosophilids were selected and their ovipositor were dissected and boiled in 10% KOH solution at 100°C for several minutes. Their bristles were then observed under compound light microscope for better visual.

Results

During different ripening phases of fruits, the drosophilids that were collected in bulk from all of the collection sites have been presented within the table - 1 and pictures of drosophilids along with their ovipositor have also been provided.

Table – 1

S.No.	Name of species	Collection site/s	Fruit stage at the time of collection
1.	*Drosophila suzukii* Matsumura,1931	Jangal chatti, Harshil and Kanatal	Ripening
2.	*Drosophila subpulchrella* Takamori & Watabe, 2006	Harshil and Kanatal	Ripening
3.	*Drosophila nepalensis* Okada, 1955a	Jangal chatti, Gopeshwer, Harshil and Kanatal	Fully ripen or plucked
4.	*Drosophila punjabiensis* Parshad & Paika, 1964	Harshil, Jangal chatti and Kanatal	Ripening
5.	*Drosophila takahashii* Sturtevant, 1927	Jangal chatti, Gopeshwer, Harshil and Kanatal	Fully ripen or plucked
6.	*Drosophila ananassae* Doleschall, 1858	Gopeshwer	In presence of mixed vegetation
7.	*Drosophila parabipectinata* Bock, 1971	Gopeshwer	In presence of mixed vegetation
8.	*Drosophila sulfurigaster* Duda, 1923	Gopeshwer	In presence of mixed vegetation
9.	*Zaprionus indianus* Gupta,1970	Jangal chatti, Gopeshwer, Harshil and Kanatal	Fully ripen or plucked
10.	*Drosophila melanogaster* Meigen, 1830	Harshil, Kanatal	Plucked

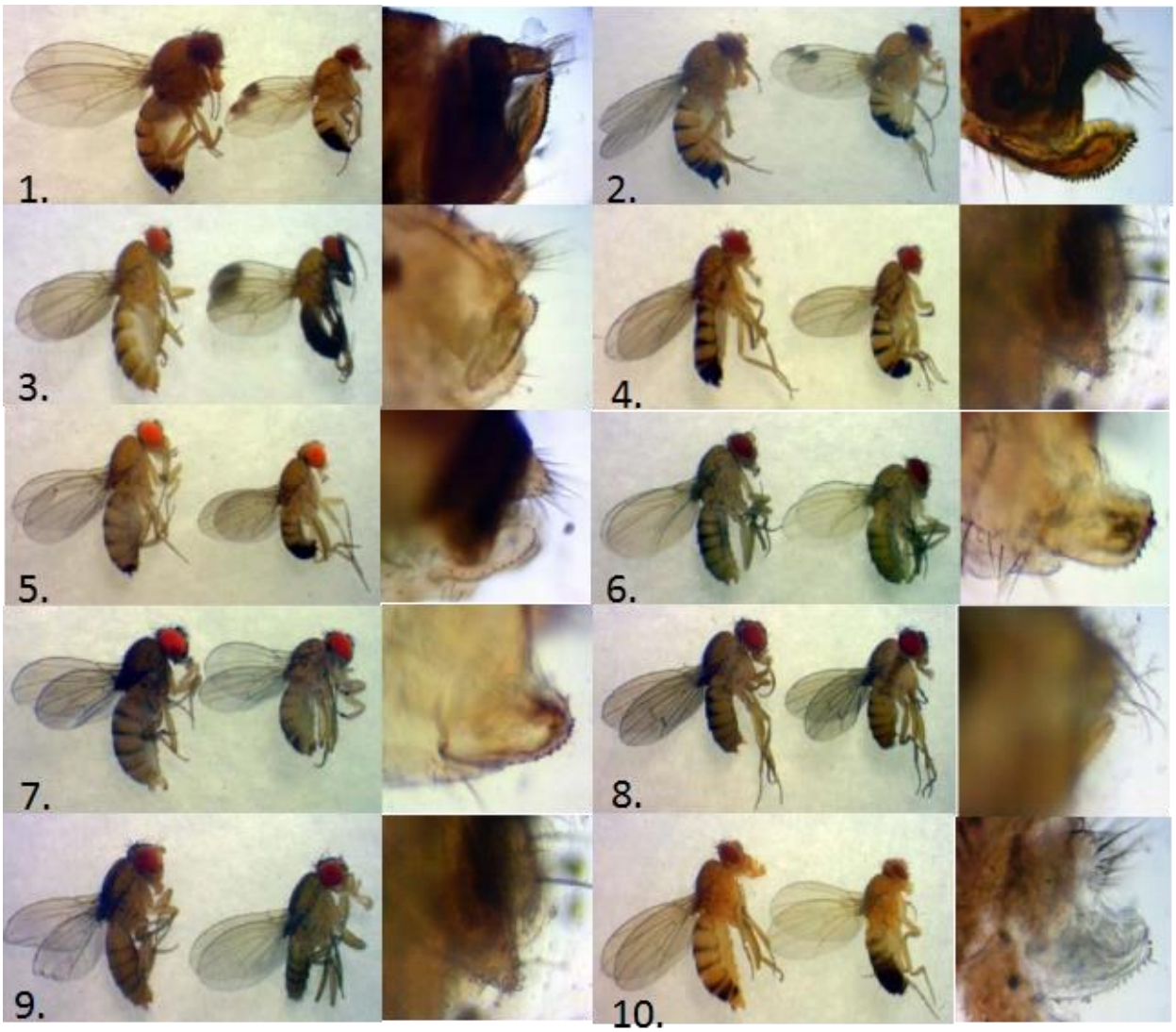

Figure 2: 1. *D. suzukii* 2. *D. subpulchrella* 3. *D. nepalensis* 4. *D. punjabiensis* 5. *D. takahashii* 6. *D. anannasae* 7. *D. parabipectinata* 8. *D. sulfurigaster* 9. *Z. indianus* 10. *D.melanogaster*

Discussion

The pest behaviour of drosophilids significantly stresses the farming of soft skinned fruit crops, that are already perishable and require extra precautions. The drosophilid species collected at different stages of fruit's ripening from the same area were different. It indicates that different species of drosophilids were attracted differently and those species having attracted to ripening fruits are of concern. As the data regarding presence of drosophilids was collected and studied, it was observed that *D. suzukii, D. subpulchrella, D. punjabiensis* are the species that were only collected when the fruits were at ripening stage and the climatic conditions were moderate humid and moist. With collected drosophilids it was also observed that *D. subpulchrella* was collected in very high number from Harshil and Kanatal in the ripening stage of fruits and their number in collection got decreased as the fruits started turning into ripen

ones. Although there are mixed views regarding it being a pest and preferring ripening fruit than ripen fruit (Atallah et al., 2014; Crava et al., 2020; Durkin et al., 2021). However, its ovipositor has serrated modified bristles arranged along its outer margin. Further study is needed to be done regarding its behavioural ecology. The presence of *D. punjabiensis* in the ripening fruit stage is also needed to be looked upon. Although its ovipositor is not much heavily serrated but its presence at the ripening stage gives us prospect to explore its ecological and behavioural patterns regarding preference for ripening fruit. Within study it was also observed that *D. nepalensis, Z. indianus* and *D. takahashii* were present in all the sites including the control site. It was also of great interest that these three species were more prominently present during the fully ripen stages of fruits. Their ovipositors bear only few small bristles and it clearly indicates that these species can't acts as primary pest. However, they may act as secondary pest if fruits are already damaged and are only attracted toward the ripen ones. *D. melanogaster* was only present in the sites at the time when plucking of fruits was almost done and there were piles of rotten and damaged fruit in the fields. Their ovipositor also has few small bristles. Other than *D. nepalensis, Z. indianus* and *D. takahashii* the control site had *D. ananassae, D. parabipectinata and D. sulfurigaster*. It depicts that these species are simply attracted towards the bait and fruit orchards are not their obligatory niche. The study of their ovipositor also supports the fact that they may not harm much of the ripening fruits as these flies has not much serrated modified bristles arranged along its outer margin of ovipositor.

All this indicates that different species captured from fruit orchards were attracted because of the odor of ripening and ripen fruits. During ripening and development of fruits, the composition of volatiles produced and discharged changes dramatically, perhaps offering distinct cues for different species (Krause Pham & Ray, 2015). With this study this it was again verified that the volatiles do acts as cues for different species.

Throughout the ripening phase, fruits undergo increased rates of respiration and produce a higher level of CO2 which must be avoided by drosophilids preferring ripen fruits (Krause Pham & Ray, 2015). This must be acting as cue in case of drosophilids which were collected during the ripen phase. However, CO2 emissions drop as the fruit ripens, whereas yeast-derived volatiles increases (Krause Pham & Ray, 2015). While working on drosophilids it has been widely accepted that yeast volatiles do attract frugivorous drosophilids and this is one of the reasons why yeast along with fruits are used as attractants in trap bait.

According to experiment performed on *Drosophila* flies it was observed that when vinegar and CO2 were tested independently, both males and females were drawn to the vinegar-baited traps and avoided the CO2-baited traps (Faucher et al., 2013). It further strengthens the fact that *Drosophila* flies generally avoid the CO2 that is released from ripening fruits. However, (Krause Pham & Ray, 2015) concluded with experiments that *D. suzukii* don't avoid the increased concentration of CO2. This point assists with the fact that this species has been acting as prominent pest as it is attracted toward the ripening fruits. *D. subpulchrella* and *D. punjabiensis* too are also following the same patterns and the study of their ovipositor also favors the fact that they may harm the soft skinned and stone fruits. Further similar studies can be conducted for other species to predict their pest status and behaviour.

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