

Identification of Cucurbit Fruit Flies and Their Relative Attractiveness to Different Cues and Releasers

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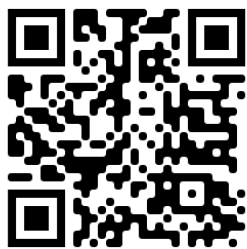
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ABSTRACT

Tephritids are the major challenges for fruit and fleshy vegetable growers of the tropics and sub-tropics. Nepal also incurs huge losses of fruits and vegetables to the tephritids. This study was designed to identify the tephritid flies in Malepatan and Lumle of Gandaki Province. Bucket traps baited with methyl eugenol (ME) in Malepatan, and ME, cue lure (CL) and a mixture of ME and CL (ME+CL) with different releasers; tube, cotton and paper were installed at Lumle in 2020 summer. Three species of the fly (*Bactrocera dorsalis*, *Bactrocera zonata* and *Bactrocera correcta*) were recorded from Malepatan, dominated by *B. dorsalis*. The *B. correcta* count was negligible. While in Lumle, *B. dorsalis* was the most dominant, followed by *B. zonata* till July, after which, till August, *B. tau* followed the *B. dorsalis*. Seven species of the tephritids (*B. zonata*, *B. dorsalis*, *B. tau*, *B. diversa*, *B. scutellaris*, *B. correcta* and *B. cucurbitae*) were recorded from this region. Among the releasers, paper releaser was found more effective as compared to cotton and tube. However, the lures should be changed in a short interval, by two weeks, to acquire a satisfactory result. This study unveils that the study site already inhabits seven species of fruit flies. So, the monitoring with different cues could be an effective tool for species actuation. Additionally, the paper releasers could be recommended for further use in the traps for monitoring and mass trapping purposes.

Keywords: Fruit fly, Cue lure, Methyl eugenol, Tephritids, Releaser effectiveness

1. INTRODUCTION

Tephritids are a group of economically important insects with a sub-cosmopolitan distribution. Doorenweerd *et al.* (2018) have reported a total of 1864 tephritid species worldwide. The genus *Bactrocera* only consists of more than 650 described species most of which are predominantly found in the Asia Pacific (Drew & Hancock 1994, Drew & Romig 2016). Among all, *Bactrocera dorsalis* (Hendel) is the most invasive one (Clarke *et al.* 2005) and has already colonized Africa and the Americas (Lux *et al.* 2003). The flies cause direct damage to fruits and vegetables, leading to 90-100% yield loss, depending on fruit fly population, locality, variety and season (Plant Health Australia 2018). Adult female fruit flies have a needle-like ovipositor as a weapon to puncture the soft skin of fruits into the flesh for egg laying. The activities of its maggots in the pulp cause the fruits to rot internally, leading to premature fruit drop.

Nepal is important for the study of population dynamics and species diversity and also for the planning of management strategy. Seventeen species of fruit flies are reported in Nepal and it is shown that the seasonal variations greatly influence the fruit fly population (Adhikari *et al.* 2019, 2020). Post-winter temperature warm up and continuous availability of host plants trigger the changes in diversity, abundance, and spatial-temporal variation and accompanied damage of and by the fruit fly species. Monitoring and identifying the weather-density relation of the fruit fly species is crucial before actuating the species-specific management strategy. Many lure agents are deployed into the traps to attract the *Bactrocera* species. However, in the context of the Nepalese farming system, Agri-entrepreneurs treat all to be similar resulting in the heavy loss of the crop despite the huge catches of a single species.

Phenyl propanoids are attractive to numerous species of *Dacinae* fruit flies (Diptera: Tephritidae), including species members of the genera *Bactrocera* and *Dacus*. Methyl eugenol (ME) (4-allyl-1, 2-dimethoxybenzene-carboxylate), cue-lure (CL) (4-(p-acetoxyphenyl)-2-butanone), and raspberry ketone (RK) (4-(p-hydroxyphenyl)-2-butanone) are very powerful male-specific lures.

These lures are used in current control programs of the fruit flies for the detection and monitoring of populations and for Male Annihilation Technique through mass trapping (MA-MT) (Vargas *et al.* 2010). The ME is believed to be an effective chemical cue for species like *Bactrocera correcta* (Bezzi), *B. dorsalis*, *Bactrocera zonata* (Saunders), *Bactrocera invadens* Drew, Tsuru & White, *Bactrocera carambolae* Drew & Hancock, *Bactrocera caryeae* (Kapoor). At the same time, Cue-lure is effective for *Bactrocera scutellaris* (Bezzi), *Bactrocera cucurbitae* (Coquillett), *Bactrocera tau* Walker, *Bactrocera diversa* (Coquillett), etc. (Minhibo *et al.* 2018). However, farmers are ratifying the conventional management techniques without specifying the flies. Nevertheless the identification of available species in a region is mandatory to effectively manage the pest. On the other hand, farmers are fully dependent on local suppliers like agro-vets for the fruit fly traps, in which the chemical constituents of the lures specific to the fruit fly species are not indicated. So, farmers are confused that the traps are ineffective means of fruit fly management programs.

Though sparsely reported, cotton swabs, either made locally or available in the market, are the pre-dominant releasers mostly used in the fruit fly traps. Easiness of use and availability are the governing reasons for the common use of the swabs. This study, with the use of different cues and releasers, intends to identify the fruit fly species inhabiting the study area, and to unveil the effectiveness of different cues and releasers in attracting different fruit fly species.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in cucumber fields of Horticulture Research Station at Malepatan (HRS-Malepatan) (28° 21' N, 83° 98' E, 895 m above sea level) and Directorate of Agricultural Research at Lumle (DoAR-Lumle) (28° 37' N, 83° 83' E 1750 m above sea level). The climatic parameters of the HRS-Malepatan were acquired through an e-mail request to the Provincial Meteorological Office of Gandaki, and that of DoAR-Lumle was obtained from its in-built meteorological station. The weather parameters of the study period are summarized in Fig. 1.

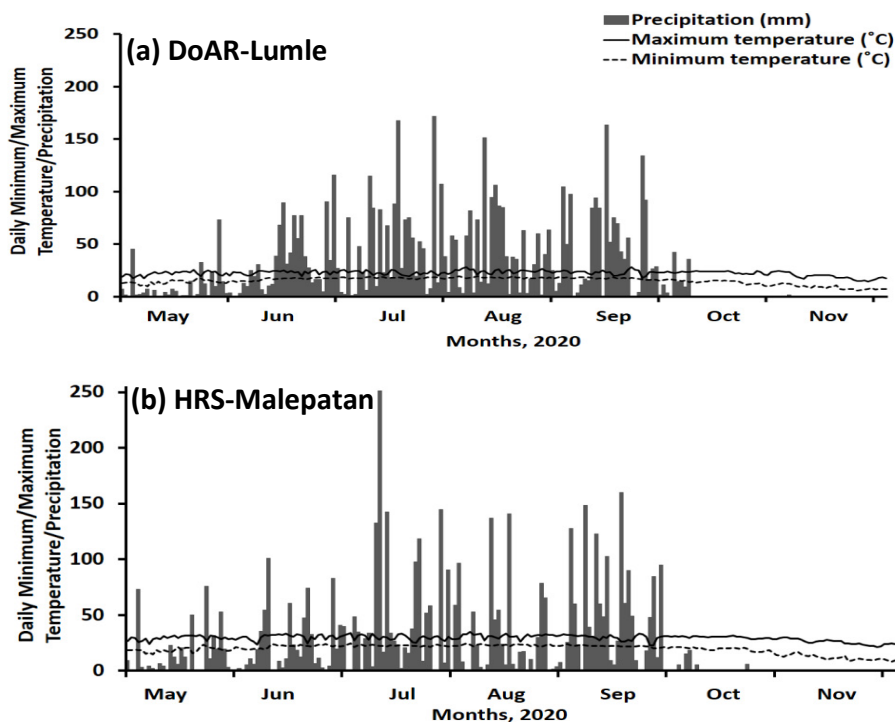


Fig. 1. Climatic records of DoAR-Lumle (a) and HRS-Malepatan (b) during the study period of *Bactrocera* species attraction.

2.2 Trap Preparation and Installation

2.2.1 Experiment at Malepatan

In Malepatan (geographical location as described above), a transparent black lidded bucket trap (upper $\text{\O} = 8.8$ cm, base $\text{\O} = 6.5$ cm, total height = 20 cm, cap height = 7 cm, transparent bucket height = 13 cm, releasers' height = 9 cm) baited with ME was used. In the bucket trap as in Fig. 2 (middle), a cotton swab ($\text{\O} = 2$ cm) was soaked with ME and hung in an inbuilt structure. The trap was then hung above one and a half meters from the ground either on the stakes or on the tree branches maintaining a trap distance of at least 10 m from one another. The fruit flies caught in the traps were collected and identified fortnightly, and the cotton releasers were again recharged with the cues and the trapping period extended from July to November 2020.

2.2.2 Experiment at Lumle

In Lumle condition, along with the study of species trapped, efficacy of different releasers and cues was also evaluated.

2.2.3 Species identification

In Lumle condition, four different cues; methyl eugenol (ME), cue lure (CL), ME+CL and company formula) were used. The traps were established as in the Malepatan experiment. The trapping period again started in July (during the fruit set of the cucumber plantation) through November 2020.

The fruit fly species caught in the bucket traps were collected fortnightly which were then carried to the entomology laboratory of DoAR-Lumle and identified to the species level following the pictorial keys given by Prabhakar *et al.* (2012), Adhikari and Joshi (2018). After trap collection, the cues were recharged again for another fifteen days.

2.2.3 Effectiveness of Cues and Releasers

Different cues were tested in the Lumle condition. The cues used were ME, CL, ME+CL, company formula and control. Among the cues, company formula (1 mL) was used either in tubes (Fig. 2a) or in cotton swabs ($\text{\O} \sim 2$ cm) (Fig. 2b) or papers ($\text{\O} \sim 3.7$ cm) in a layer of two with circular artifact (Fig. 2c).

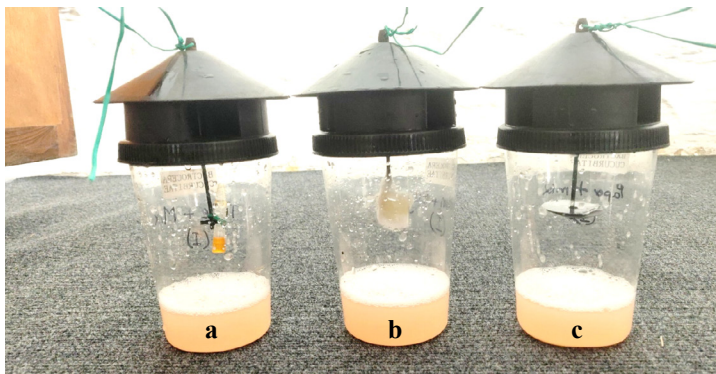


Fig. 2. Different kinds of releasers (a) tube, (b) cotton and (c) paper used in bucket traps for the study of relative efficacy of the cues in Lumle, 2020.

3. STATISTICAL ANALYSES

The effectiveness of the cues and releasers are presented in percentages. In case of the fortnightly comparison between Malepatan and Lumle catches, the Chi-square statistic was calculated using GraphPad Prism version 9.1.0.

4. RESULTS

4.1 Identification of Species

In total, three cucurbit fruit fly species at Malepatan and seven at Lumle were identified

(Fig. 3). The Malepatan species were common to that Lumle species of fruit flies. The identified seven species were *B. tau*, *B. cucurbitae*, *B. dorsalis*, *B. correcta*, *B. zonata*, *B. scutellaris* and *B. diversa*. Among these, *B. dorsalis*, *B. zonata* and *B. correcta* were recorded from Malepatan and from Lumle, all the seven species were recorded. The identification keys of the recorded species were compared with those of Prabhakar *et al.* (2012), Adhikari and Joshi (2018), and Choudhary *et al.* (2014).

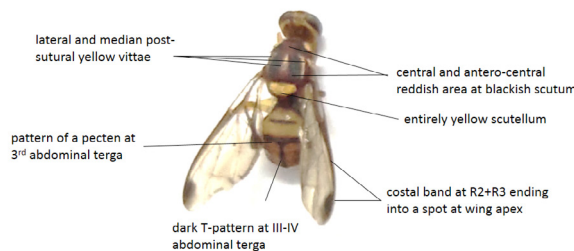


Fig. 3. Photographs of the specimen of identified *Bactrocera* species attracted to different lures at Malepatan and Lumle (continuously from left to right: *B. tau*, *B. cucurbitae*, *B. dorsalis*, *B. scutellaris*, *B. zonata*, *B. correcta*, and *B. diversa*).

B. tau

Scutum had both lateral and medial post-sutural vitae. The scutellum was entirely yellow. Abdominal tergite was not fused; a transverse band was present in tergite III and medial

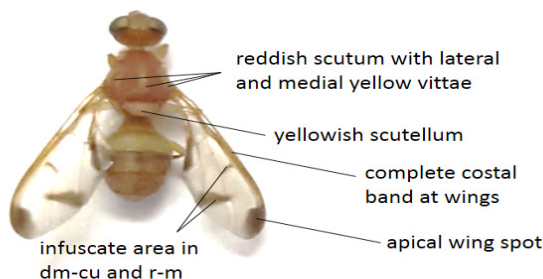
longitudinal stripe on Terga (III-V), which formed a black T pattern. Wings had a narrow dark fuscous complete costal band overlapping vein R_{2+3} and expanding into a distinct apical spot.



B. cucurbitae

Distinctive characteristics of these flies were; the wing pattern and dorsum of the thorax, reddish-yellow without black markings. Scutum had both lateral and medial post-sutural vitae. The

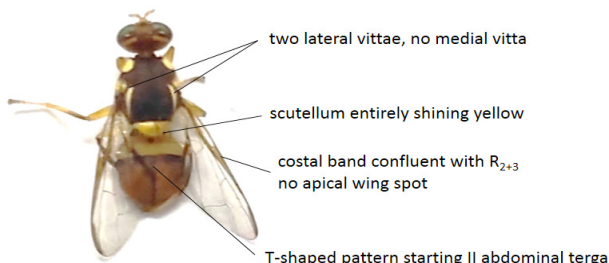
Transverse band across tergum III was in the abdomen and medial longitudinal stripe on terga III-V. Wing with a wide coastal band, which was expanded into a spot at apex; cross-vein dm-cu and r-m covered by infusate area, the broad fuscous cubital streak was present.



B. dorsalis

The colour of the flies varied, but there were prominent yellow and black margins on the thorax. The abdomen had two horizontal black stripes and

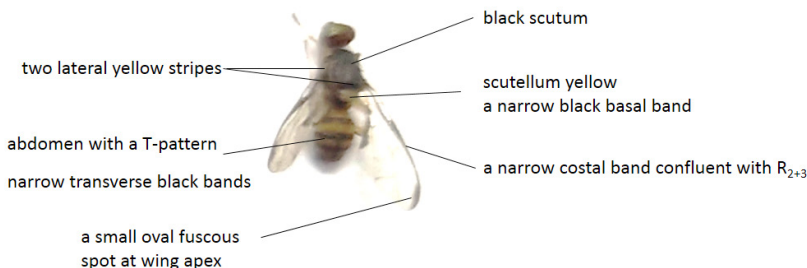
alongitudinal median stripe extending from the third segment's base to the abdomen's apex, forming a T-shaped pattern. The body length was about 8 mm. The costal band was confluent with R_{2+3} , not expanding into a small spot in the wing apex.



B. correcta

These flies were generally distinguished by the thorax color, predominantly black with lateral yellow stripes. The scutellum was yellow with a narrow black basal band. The abdomen forms

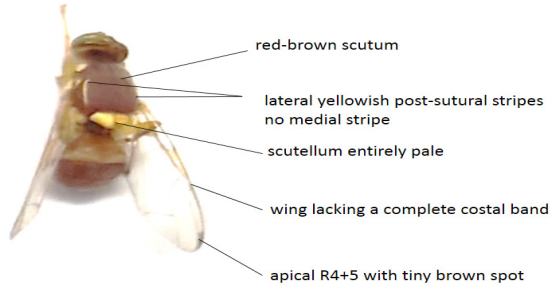
a T pattern consisting of narrow transverse black bands and wings, a narrow pale fuscous costal band confluent with R_{2+3} , and a small oval fuscous spot was found at the end of the apex.



B. zonata

Scutum was entirely red-brown, with lateral yellow or orange post post-sutural stripes; the scutellum was entirely pale coloured. A pair of dark marks were found on the tergum

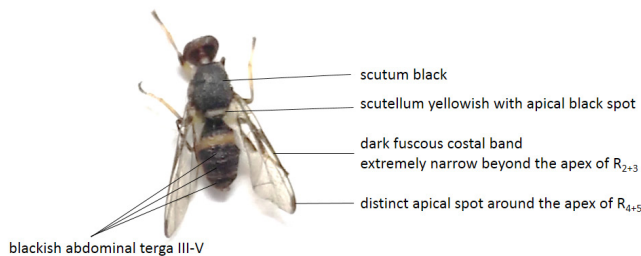
III of the abdomen, and no medial dark line except on tergum V. The body length of the flies was about 6 mm on average. Wing lacked a complete costal band and apical R_{4+5} with brown spot.



B. scutellaris

Scutum was shining black with narrow lateral and median post-sutural yellow vittae; Scutellum was yellow with an apical black spot. Abdominal terga III-V were found mostly

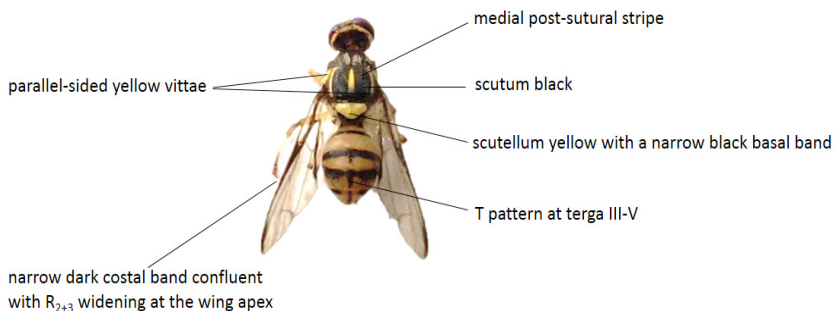
dark fuscous to black. It had wings with a narrow dark fuscous complete costal band which was extremely narrow beyond the apex of vein R_{2+3} and expanding into a distinct apical spot around the apex of R_{4+5} .



B. diversa

Scutum was entirely black, with broad parallel-sided lateral yellow stripes and a narrow medial longitudinal post-sutural stripe. The scutellum was yellow with a narrow black basal band.

Red-brown with a black T pattern was found on the abdominal terga III-V. A wing with a narrow dark costal band confluent with R_{2+3} and widening across the apex of the wing was found.



4.2 Relative Abundance of Species at Malepatan and Lumle

Bactrocera dorsalis: The species was the most dominant among the trapped ones. During the first ($\chi^2 = 104.89, P = 0.0001$) and third week of July 2020 ($\chi^2 = 47.68, P = 0.0001$); and the first week of August 2020 ($\chi^2 = 10.80, P = 0.0001$) the number of male *B. dorsalis* trapped in Malepatan condition was significantly higher in comparison to Lumle. After those dates, the traps were insignificant (August 3rd week: $\chi^2 = 2.00, P = 0.16$; September 1st week: $\chi^2 = 2.78, P = 0.096$; and September 3rd week: $\chi^2 = 0.00, P = 1.00$) except on the October 1st week ($\chi^2 = 6.40, P = 0.011$).

Bactrocera zonata: This species was also significantly higher in Malepatan than in Lumle during two regular weeks (July 1st week: $\chi^2 = 5.95, P = 0.015$ and July 3rd week: $\chi^2 = 5.44, P = 0.019$). During the rest of the observation dates, the species were nil in both-locations.

Bactrocera correcta: This species was found in a significant number in Malepatan during the 1st week of July 2020 ($\chi^2 = 16.20, P = 0.0001$). During the rest of the times, the trap catches were negligible, with zero capture on most of the dates.

B. tau, B. cucurbitae, B. scutellaris and *Bactrocera diversa* were found only in Lumle condition but the counts were very negligible except for *B. tau*, which was the second most dominating one.

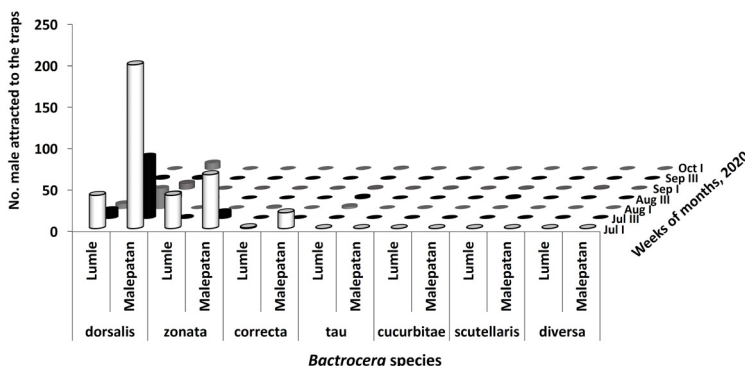


Fig. 4. Relative abundance of *Bactrocera* species under Lumle and Malepatan condition.

4.3 Relative Attractiveness of Fruit Fly Species to Different Cues

Among the different cues used (ME, CL and ME + CL), attractiveness to the CL was higher (43%), and to the ME was only 24%. There was an inhibitory effect of using CL and ME together as the attractiveness was only 33%, less

than the sum of the separate use of the two cues (i.e. 67%). However, the attractiveness seems to be species-specific. The dominant species, *B. dorsalis* was attractive to both the lures, ME and CL, and the attractiveness was much higher to the lures with ME+CL. The rest of the species trapped were attracted to the CL lures (Fig. 5).

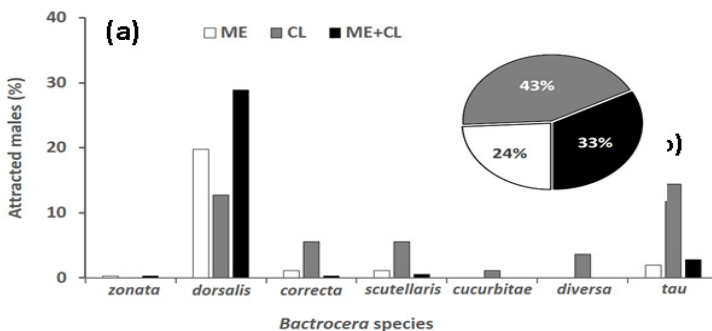


Fig. 5. Attractiveness (%) of male *Bactrocera* species to the different sources of cues (a) and total male attractiveness (%) to the cues (b)

4.4 Attractiveness of Fruit Fly Species to Different Releasers

The cue-releasers were also found species-specific (Fig. 6). The most dominant species, *B. dorsalis* was highly attractive to the paper releasers compared to the other trapped species. The other

species were captured in very negligible numbers, and the attractiveness was still different for the different species. The cumulative attractiveness to all the species was higher for paper (56.28%) followed by tube (22.29%) and cotton (21.43%) releasers.

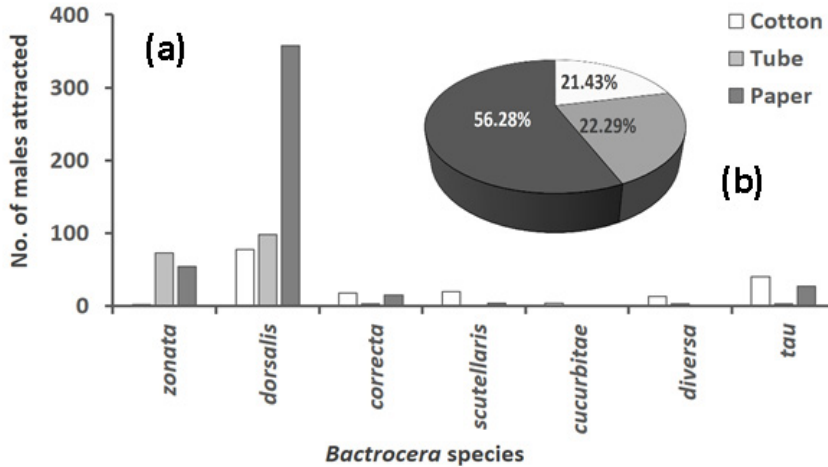


Fig. 6. Attractiveness (No) of male *Bactrocera* species to the different releasers (a) total male attractiveness (%) to the releasers (b)

4.5 Attractiveness influenced by the temperature

As shown in Fig. 7, the total number of fruit flies caught in the traps were highest in June, when the

experiment was started, which continued to the last week of August. After this, the trap catches were drastically reduced and touched the X-axis by November.

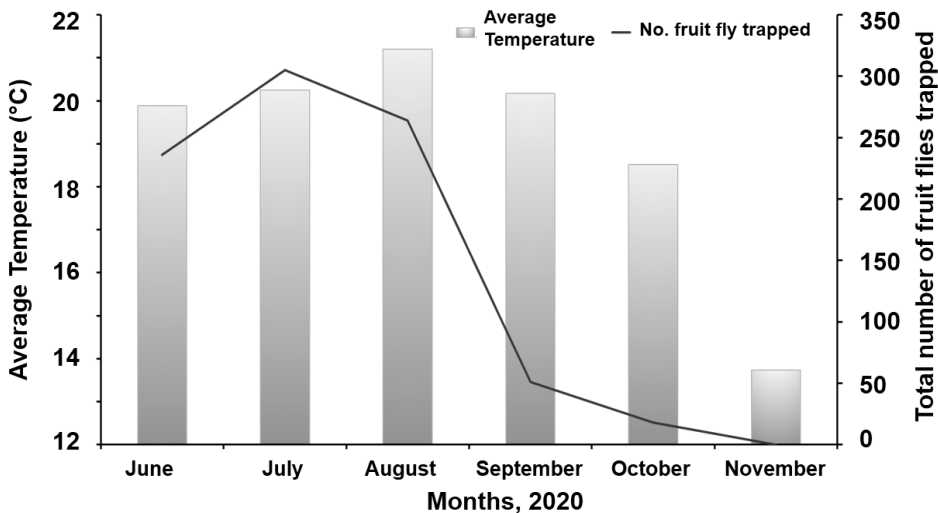


Fig. 7 Trend of fruit flies trapped and its relation with temperature pattern of the DoAR, Lumle.

5. DISCUSSION

Nepalese farms, especially the cucurbit fields, are becoming the hot spots of *B. species* mainly due to two reasons; one, the farmers are not aware of about the presence of different species of the fly and second, the relative attractiveness of different species to the different cues were not evaluated so far. This study unveils that Lumle and Malepatan of Gandaki Province inhabit seven species (*B. tau*, *B. cucurbitae*, *B. dorsalis*, *B. correcta*, *B. zonata*, *B. scutellaris*, and *B. diversa*) of the flies. Shrestha (2006) identified six different fruit fly species (*B. cucurbitae*, *B. dorsalis*, *B. zonata*, *B. tau*, *B. scutellaris* and *Bactrocera yashimotoi*) from Kathmandu and Kabhrepalanchowk during the study period of 1994-1996. A review report by Sharma *et al.* (2015) mentioned the record of six reference fruit fly species preserved at the Entomology Laboratory of Fruit Development Directorate, Kirtipur and also mentioned the record of nine fruit fly species from across Nepal preserved at National Entomology Research Center, Khumaltar. Comparing the previous literature, it could be the first report on identification of seven fruit fly species at a time from a narrow geographical range. Although the species diversity was higher in Lumle, the abundances of the three recorded species were higher in the Malepatan condition, most probably due to the warmer weather conditions as shown in Fig 1 and of course, the density proportionally relates with the temperature regimes. A similar trend in fruit fly catches was reported by Sharma *et al.* (2015) while experimenting from May to December 2014 using cue lure and methyl eugenol baited traps where the catches peaked during July- August. Acharya and Adhikari (2019) also reported the highest number of fruit flies trapped in the methyl eugenol and protein hydrolyxate baited traps at various altitudes of the Sindhuli district of Nepal. While studying the seasonal phenology of *B. minax* in western Bhutan, Dorji *et al.* (2006) also recorded the peak catches during May-July of two study years 2000 and 2002. In China, Hou *et al.* (2018) also reported the highest peaks of *B.*

minax attraction to the eight attractant traps during the warmer months, June-July.

Nepalese extension personnel commonly refer to the fruit fly the *B. cucurbitae*. Irrespective to this, the present study documented a higher population of *B. dorsalis*. Two major reasons may lie behind this fact. *B. cucurbitae* dominated the population during the surveillance or cue lure was only the major source of attraction to the flies. The second and most important fact could be the highest rate of invasion of the *B. dorsalis* as reviewed by David *et al.* (2017). In any case, this demands a separate comparative study of the population dynamism of the flies.

The diffusion of the pheromone into the surroundings may affect the effective attraction radius (EAR) (Byers 2007), the distance from the point of the pheromone source. This could be why the attractiveness was different for the different releasers (paper, tube and cotton) tested in this experiment, and the attractiveness was most probably due to the differences in the population size of the species. *B. dorsalis* was dominant and highly attracted to the releasers and cues. This is the reason why *B. dorsalis* is the most invasive among the tephritids (Moquet *et al.* 2020), and is colonizing in the recently invaded African continent. Mutamiswa *et al.* (2021) reported on its East to West Africa colonization from 2003 to 2014.

Regarding relative attractiveness to the cues, *B. dorsalis* seems to have some degree of attraction to cue lure, which is not the general tendency in its ecology. However, this cross-attraction was probably due to the mishandling of the treatments during the study. The similar, non-specific attraction was reported by Acharya and Adhikari (2019), where *B. cucurbitae*, *B. tau* and *B. scutellaris* were cross-attracted to methyl eugenol traps, which generally respond to the cue lures. Similarly, the fruit flies (*B. dorsalis* and *B. zonata*) responsive to methyl eugenol were also attracted to cue lures (Sharma *et al.* 2015).

The second most dominant species was *B. tau*. An interesting result was achieved in a trial (data not presented) where the damaged

cucumber fruits were cultured inside a cage to study the emerging adults. Only the *B. tau* emerged in the late season of the cucumber plantation, which corroborated closely with the study of Singh *et al.* (2010). Moreover, in most cases, the damage was due to the *B. tau*. Despite its second-highest abundances in this study, the damage was much more than that of other species, which demands a separate study to evaluate the intensity of cucurbits losses to the *Bactrocera* species. It is mainly due to two reasons; one, it has been identified to infest multiple plant hosts from different families like cucurbits, tomatoes and other fleshy vegetables (Singh *et al.* 2010), and second, the optimal developmental temperature (25 - 31° C) of the pest (Zhou *et al.* 1994) prevails during the cucurbit season of mid-high hills of Nepal. In a short-term high-temperature exposure treatment by Yuyu *et al.* (2020), the most temperature-tolerant *B. tau* stage, the pupa showed an LT_{50} value of 42.06° C. So, what would be the *B. tau* population in Nepal Terai is, of course, a matter of further investigation covering a wide regime of agro-ecology.

6. CONCLUSION

This study has shown that the cucurbit field of the study sites inhabited seven fruit fly species which emphasizes the use of different cues for their monitoring and the future directives for mass trapping and annihilation. The other fact is that the paper releasers are more effective in catching the most dominant species, *B. dorsalis*. Moreover, it has demanded a couple of future investigation priorities; damage intensities by the different fruit fly species on cucurbitaceous crops, study on the niche-specific specificity on the dominance of the fruit flies, and of course, the temperature effect on the survivorships of the flies.

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