

Research Article

Tephritid diversity under terai agro ecological region of West Bengal

TANGELLA MEGHANA* and NRIPENDRA LASKAR

Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar-736165, West Bengal, India

**Corresponding author E-mail:* reddymeghana444@gmail.com

ABSTRACT: A study was conducted from 2020-2021 to identify the tephritid species in fields of Pumpkin, Bottle gourd, Mango, Guava, Ber and Citrus. Through Methyl Eugenol traps, two species namely *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) were collected. Through cue-lure traps, six species namely *Zeugodacus tau* (Walker), *Zeugodacus cucurbitae* (Coq.), *Bactrocera rubigina* (Wang and Zhao), *Zeugodacus caudatus* (Fabricus)*, Bactrocera divenderi* Maneesh, Hancock and Prabhakar and *Dacus longicornis* (Wiedemann) were collected. Four species namely *Zeugodacus tau* (Walker), *Zeugodacus cucurbitae* (Coq.), *Bactrocera dorsalis* (Hendel) and *Bactrocera minax* (Enderlein) were recovered from infested fruits of pumpkin, bottle gourd, cucumber, ber and citrus. According to the Shannor-Weiner Diversity Index, species diversity was maximum in pumpkin among different fields and maximum in ber from infested fruits. Understanding the species diversity and determination in a crop ecosystem aid in strategising the management options. Clumped distribution was observed in several fields.

KEYWORDS: Tephritid, fruit flies, species diversity, *Bactrocera, Zeugodacus*

(Article chronicle: Received: 03-01-2024; Revised: 05-04-2024; Accepted: 07-04-2024)

INTRODUCTION

The flies belong to the family Tephritidae of the Insect order Diptera and are referred to as fruit flies as they infest a wide variety of vegetables and fruits in both subtropical and tropical regions of the world (Choudhary *et al*., 2014). Tephritids are the major pests of vegetables and fruits (Kapoor, 2000). Fruit flies are also referred to as "Peacock flies" due to the habit of strutting and vibrating their wings (White & Elson-Harris, 1992; Agarwal & Sueyoshi, 2005). Subfamilies Trypetinae, Dacinae, Tephritinae, and Phytalminae belonging to the family Tephritidae are recorded in India.

Tephritids draw a great deal of attention in the field of plant quarantine and biology because of their economic importance (Yujia *et al*., 2015). In tropical and subtropical regions worldwide, fruit flies cause major losses in horticultural crops (Rubabura *et al*., 2019). Among the subfamilies of tephritidae, the Dacinae causes a major threat to crops. Globally, Dacinae fruit flies of the genus *Bactrocera* Macquart are one of the most important pests of vegetables and fruits (Clarke *et al*., 2005). The genera *Bactrocera*, *Dacus*, *Anastrepha*, *Rhagolatis* and *Ceratitis* contain the most important species of economic importance on horticultural crops worldwide (White & Elson-Harris, 1992). Tephritids cause both direct and indirect damage. Direct damage by fruit dropping and making them inedible whereas indirect damage is associated with quarantine restrictions that are imposed by importing countries to prevent the entry and establishment of exotic fruit fly species (Ekesi, 2012).

Fruit fly adults and larvae have different feeding and living habits (Frias, 2008). Female fruit flies lay eggs in ripe and semi-mature fruits. It has a sharp appendage for egg laying at the tip of the abdomen, i.e., ovipositor. With the ovipositor, a female fruit fly inserts up to six eggs about 3mm under the rind of the fruit. Fruit fly eggs are white, nearly half to 1 mm in length, and banana-shaped. Maggots hatch from eggs within 2-3 days and make burrows within the fruit. Generally, maggots are creamy white, apodous, tapered anterior end and blunt posterior end. They feed on fruit pulp and go through 3 larval instars to become fully developed yellow-coloured larvae, i.e., larval period of about 7-8 days. A fully developed larva leaves the fruit by tearing the rind of the fruit with mouth hooks and falls into the soil. In the soil at a depth of 3-5 cm larva undergoes pupation. The fully developed pupa is brown coloured, barrel-shaped with pupal case and the pupal period varies from 9 days to several weeks depending on temperature. The adult emerges from the pupa and moves over the surface of the soil. Adults mate within a week. Female fruit flies lay eggs throughout their lifetime (McKenzie *et al*., 2004).

Increased populations of Tephritids (fruit flies) are brought under control by efficient management. Integrated Pest Management is one of the best methods to achieve sustainable agriculture with less environmental damage (Kogan *et al*., 1999). Integrated pest management strategies include cultural practices like soil raking, deep ploughing, field sanitation and early harvest of mature fruits (Butani, 1979; Srivastava, 1997). By bagging of fruits oviposition of fruit flies can be prevented (Godse *et al*., 2002; Srivastava, 1997). Sterile Insect Technique (SIT) is currently employed in some countries to suppress tephritid fruit flies (Hendrichs *et al*., 2005). It is successful when used with other management techniques (Gurr *et al*., 2010) like the Male Annihilation Technique (MAT) by using cue-lure and methyl eugenol (Vargas *et al*., 2012), the release of natural enemies. If the extent of infestation is maximum, then only pesticide application is required judiciously. It is a big challenge to manage fruit flies because of its biology. Therefore, eggs and larvae within fruits and pupae in soil which is difficult to control as they adapt to various regions and a wide range of hosts.

Sex attractants are used for monitoring of fruit fly population which aims at population suppression and also for identification of species collected in a trap. Identification is an important tool for tackling the management of fruit flies. Sex attractants like Methyl eugenol and Cue-lure are specific for *Bactrocera dorsalis* (Hendel) and *Zeugodacus cucurbitae* (Coq.) respectively.

MATERIALS AND METHODS

Experimental Site

The survey works were conducted during 2020 and 2021 at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India. The laboratory investigations were carried out in the Department of Entomology, UBKV, Pundibari, and Cooch Behar during the respective years of study. The Instructional Farm, where the field studies were conducted, is located at 26º 19´ N latitude and 89º 23´ E longitude at an altitude of 43 meters above the MSL (Mean Sea Level). The soil of the region is acidic in reaction and the pH of the soil ranged from 4.2-6.8. The northern tract of West Bengal is characterized by a typical humid climate with a distinct feature of high rainfall with an annual average of more than 3000mm and high Relative Humidity (R.H.) ranges between 65-95 per cent.

Collection and rearing of fruit flies

Tephritid fruit flies were collected from different fields by installing traps comprising of sex attractants such as Methyl Eugenol and cuelure. Methyl eugenol lure or cuelure is prepared in the laboratory by soaking a cotton wick in a solution containing either methyl eugenol or cuelure chemical, Cypermethrin and water. Then cotton wick is wrapped using aluminium foil and fixed in traps. The traps containing para-pheromone about four per field at a height of 1.5-2m with a distance of 20m were placed. Every day from each trap fruit flies were recorded. Fruits infested by tephritids were collected from the Instructional farm of Uttar Banga Krishi Vishwavidyalaya, Pundibari. Collected damaged fruits from pumpkin, Bottle gourd, Cucumber, Ber, and Citrus fields were placed in a glass or plastic tray $(42\times35\times7$ cm) containing a dry medium such as loose soil and covered with muslin cloth on top to prevent infestation of other flies which cause secondary infection and trays were kept for rearing in an insect rearing cage. Samples were checked every two days for puparia and fruits from which larvae emerged were discarded. After pupation, the soil was sieved to collect pupae. Again pupae were placed in a tray containing soil for adult emergence at least 3-4 weeks. The sugar solution was placed in a tray which was used by adult fruit flies as a portion of food for the development of full body and colouration which was helpful for identification.

Species identification of tephritid fruit flies

Tephritid fruit flies were collected from para-pheromone traps installed in pumpkin, bottle gourd, ber, mango, guava, and citrus fields and from rearing infested fruits of pumpkin, cucumber, bottle gourd, ber, citrus was thoroughly observed under a Stereo zoom microscope (De-winter) for identification of fruit flies by morphological characteristics such as facial spots, presence of vittae on scutum, pre scutellar setae, scutellar setae, colour of abdomen, presence of pectin in male, variation in wings based on taxonomical keys provided by White and Elson-Harris (1992) and Prabhakar *et al*. (2012).

Studies on aculeus of fruit flies

The aculeus is the tip of the egg-laying apparatus of female fruit flies i.e. Ovipositor. It is an important feature used in descriptions of and in distinguishing tephritid species (White &Elson-Harris, 1992). The aculeus varies from species to species and identifies the species based on aculeus length: small or long; Shape: blunt, pointed, sharp, trilobed, serrate or non-serrate; Presence of number or size of pre apical setae. Aculeus was dissected by ensuing a procedure provided by White and Elson-Harris (1992). For dissection fruit fly abdomen was placed in a 10% Potassium hydroxide (KOH) solution overnight at room temperature. From the KOH solution, the ovipositor was separated from the abdomen and placed in glacial acetic acid for a few minutes transferred to ethanol kept for a few minutes and again transferred to clove oil. The ovipositor was placed on a glass slide and by using dissection needles aculeus was separated from the oviscape. Canada balsam was kept over aculeus and its cover slip was kept. After drying, prepared slides were observed under a

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Stereo-zoom microscope.

Evaluation of species diversity of tephritids in different cucurbits (pumpkin, bottle gourd, cucumber) and fruits (mango, guava, ber, citrus)

Diversity indices

Shannon Weiner Index (H') (Shannon & Weiner, 1949) and Simpson dominance Index (Simpson, 1949) were used for measuring the species diversity of fruit flies collected in the course of the experiment (Magurran, 1988). Diversity indices were calculated by using the following formulae:

Shannon-Weiner Index (H') = $- \Sigma$ **Pi** \times **ln Pi**

where, $Pi =$ proportion of individuals in i th species

 $Pi = S/N$ (S is several individual species

N is the total number of individuals)

ln = Natural logarithm of a number

Simpson Dominance Index (D) =
$$
\sum_{N}^{n} \frac{n(n-1)}{N(n-1)}
$$

where, $n =$ Number of individuals in one species

 $N =$ Total number of individuals in all species

 If the value of the Simpson index is more, then the diversity is declared as less.

Distribution indices of species

Distribution indices of species were estimated to know the pattern of species distribution in that locality, i.e., Random or uniform distribution or clumped distribution (Whitford, 1949).

$$
SD = \frac{Abundance(A)}{Frequency(F)}
$$

where, Frequency was expressed in percentage and estimated by using a formula:

Frequency = $\frac{\text{Total number of fruit flies caught for one species trapped}}{100} \times 100$ **Total number of fruit flies of all species trapped**

If the value is > 0.05 then it is clumped distribution

< 0.025 then it is a uniform distribution

0.025-0.05 then it is a random distribution

RESULTS AND DISCUSSIONS

The species collected belonged to Family Tephritidae, Subfamily Dacinae, and Tribe Dacini. Two male adult fruit flies were collected from methyl eugenol traps they were *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders). Maximum occurrence of *B. dorsalis* (87.86%) was recorded followed by *B. zonata* (12.14%). Data recorded during research was presented in Table 1 and Figure 1.

Table 1. Tephritid species collected from different fields by installing methyl eugenol traps

Figure 1. Mean percentage of tephritid species collected from methyl eugenol traps**.**

Six species of male adult fruit flies were collected from cue lure traps and they were *Zeugodacus cucurbitae* (Coq.), *Zeugodacus tau* (Walker), *Zeugodacus caudatus* (Fabricus), *Bactrocera rubigina* (Wang and Zhao), *Bactrocera divenderi* (Maneesh, Hancock and Prabhakar), *Dacus longicornis* (Weidemann). Among these, *Z. tau* (49.51%) individuals were caught more compared to other fruit flies. *Z. cucurbitae* (29.57%) followed by *Z. caudatus* (9.91%), *B. rubigina* (8.17%), *B. divenderi* (2.31%) and the least individuals caught in *D. longicornis* (0.48%). Data recorded during research was presented in Table 2 and Figure 2.

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Table 2. Tephritid species collected from different fields by installing cue lure traps

Figure 2. Mean percentage of tephritid species collected from cue-lure traps.

Four species of fruit flies were recovered from infested fruits they were *Bactrocera dorsalis* (Hendel), *Zeugodacus tau* (Walker), *Zeugodacus cucurbitae* (Coq.) and *Bactrocera minax* (Enderlein). The mean percentage of fruit fly species recovered from different infested fruits was *Z. tau* (34.31%) recovered maximum followed by *Z. cucurbitae* (32.96%), *B. dorsalis* (23.86%) and the least recovered was *B. minax* (8.84%). Data recorded during research was presented in Table 3 and Figure 3.

After the collection of adult tephritid fruit flies from traps and rearing of infested fruits, morphological studies were carried out under a Stereo-zoom microscope (DEWINTER) by using taxonomical keys provided by White and Elson-Harris (1992) for identification of the recorded species.

Taxonomical characters such as Facial spots, Presence of vittae on scutum, pre-scutellar setae, scutellar setae, presence of pecten in male adults, and variation in wings were observed. From the observation, Nine species of Fruit flies were recorded as *B. dorsalis* (Hendel), *Z. cucurbitae* (Coq.), *Z. tau* (Walker), *Z. caudatus* (Fabricus), *B. zonata* (Saunders), *B. rubigina* (Wang and Zhao), *B. minax* (Enderlein), *B. divenderi* (Maneesh, Hancock and Prabhakar) and *D. longicornis* (Weidemann). Observations on morphological studies were presented in Tables 4 and 5 and Figures 4-10.

Aculeus of fruit flies

Female adult fruit fly species that emerged from different infested fruits were collected. The aculeus (tip of ovipositor) was dissected from each species under a Stereo-zoom

Figure 3. Mean percentage of tephritid species recovered from infested fruits.

Table 4. Identification of tephritids based on morphological studies

Table 5. Identification of tephritids based on morphological studies

Figure 4. Adult Tephritids: 1) B. dorsalis; 2) Z. tau; 3) Z. cucurbitae; 4) B. zonata; 5) Z. caudatus; 6) B. rubigina; 7) B. divenderi; 6) B. rubigina; 7) B. divenderi; 8) B. minax; 9) D. longicornis. 6) B. rubigina; 7) B. divenderi; 8) B. minax; 9) D. longicornis. 8) *B. minax;* 9) *D. longicornis.*

Figure 5. Head of Tephritids: 10) B. dorsalis; 11) Z. tau; 12) Z. cucurbitae; 13) B. zonata; 14) Z. caudatus; 15) B. rubigina; 16) B. riead of Tephritids: 10) *B. dorsdits*; 11) *Z. tau*; 12) *Z. cucurbitde*, 15) *B. zondid*; 14) *Z. caudatus*; 15
divenderi; 17) B. minax; 18) D. longicornis. saus; 11) Z. tau; 12) Z. cucurbitae; 15) B. zonata; 14) Z. caudatus; 15)
Jongicornis caudatus, 15).

5. Scutum of Tephritids: 19) B. dorsalis; 20) Z. tau; 21) Z. cucurbitae; 22) B. zonata; 23) Z. ca Z. caudatus;24) B. rubigina rubigina; 25) B. divenderi; 26) B. minax; 27) D. longicornis Z. caudatus;24) B. rubigina *divenderi;* 26) *B. minax;* 27) *D. longicornis.* udatus; 24) B. rubigina, **Figure 6.** Scutum of Tephritids: 19) B. dorsalis; 20) Z. tau; 21) Z. cucurbitae; 22) B. zonata; 23) Z. caudatus; 24) B. rubigina; 25) B. divenderi: 26) B. minax: 27) D. longicornis i uuuuus, 24 D.

Figure 7. Scutellum of Tephritids: 28) B. dorsalis; 29) Z. tau; 30) Z. cucurbitae; 31) B. zonata; 32) Z. caudatus; 33) B. rubigina; 34) B. 32) Z. caudatus;33) B. rubigina 32) Z. caudatus;33) B. rubigina *divenderi;* 35) *B. minax;* 36) *D. longicornis.* \mathcal{B} . \mathcal{B} . \mathcal{B} . \mathcal{B} . \mathcal{B} . \mathcal{B} . \mathcal{B} . minimax; 36) B. minimax m is.

Figure 8. Wing of Tephritids: 37) B. dorsalis; 38) Z. tau; 39) Z. cucurbitae; 40) B. zonata; 41) Z. caudatus; 42) B. rubigina; 43) B. divenderi; 44) B. minax; 45) D. longicornis. divenderi; 44) *B. minax;* 45) *D. longicornis.*

Figure 9. Abdomen of Tephritids: 46) B. dorsalis; 47) Z. tau; 48) Z. cucurbitae; 49) B. zonata; 50) Z. caudatus; 51) B. rubigina; 52) B. divenderi; 53) \overrightarrow{B} . minax; 54) D. longicornis.

Figure 10. Legs of Tephritids: 55) B. dorsalis; 56) Z. tau; 57) Z. cucurbitae; 58) B. zonata; 59) Z. caudatus; 60) B. rubigina; 61) B. divenderi; 62) B. minax; 63) D. longicornis. (a) and the state of the state of

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microscope (DEWINTER) and observed the characteristics like length, shape and presence of pre apical setae of aculeus which is an important feature for identification (White & Elson-Harris, 1992). From the observation, Four species of fruit flies were identified as *B. dorsalis* (Hendel), *Z. tau* (Walker), *Z. cucurbitae* (Coq.), *B. minax* (Enderlein). Observations on aculeus studies were presented in Figures 11-15.

Keys to Aculeus of species

5 (6). Length 3.78mm with diameter 5.84 mm *Bactrocera minax* (Enderlein). (White and Elson-Harris (1992).

Evaluation of species diversity of tephritids in different cucurbits and fruits diversity indices

Species diversity in Pumpkin, Bottle gourd, Mango, Ber, Guava, Citrus fields and Infested fruits of Ber, Cucumber, Pumpkin, Bottle gourd and Citrus have been identified by using the Shannon-Weiner Index (H') (Shannon & Weiner, 1949) and Simpson dominance Index (D) (Simpson, 1949).

Shannon-Weiner Index (H') of fruit flies collected from traps

By using H', species diversity in different fields was recognized. If the value of the Shannon-Weiner Index is more, then the diversity of fruit fly species is greater in a community. H' is directly proportional to species diversity. In pumpkin, the Shannon-Weiner Index is 1.648 followed by in citrus H' is 1.613, in ber H' is 1.544, in guava H' is 1.481, in bottle gourd H' is 1.458 and in mango H' is 1.413. Species diversity is maximum when the Shannon-Weiner Index is greater. From these, it is identified that species diversity is higher in pumpkin followed by citrus, ber, guava, bottle gourd and the least species diversity in mango. Observations are presented in Table 6 and Figure 16.

Shannon-Weiner Index (H') of fruit flies recovered from infested fruits

By using H', species diversity has been documented from infested fruits. The diversity of fruit fly species is maximum when the value of the Shannon-Weiner Index (H') is greater. In ber, the Shannon-Weiner Index is 1.095 followed by, in cucumber H' is 1.055, in bottle gourd H' is 0.839, in pumpkin H' is 0.819 and in citrus H' is 0.685. As

Example 11. The abdomen of female tephritids with ovipositor: 64) *B. dorsalis;* 65) *Z. tau;* 66) *Z. cucurbitae;* 67) *B. minax.*

Figure 12. Aculeus of tephritids: 68) B. dorsalis; 69) Z. tau; 70) Z. cucurbitae; 71) B. minax. α .

Figure 13. Pre apical setae of tephritids: 72) B. dorsalis; 73) Z. tau; 74) Z. cucurbitae; 75) B. minax. **Figure 13.** Pre apical setae of tephritids**:** 72) *B. dorsalis;* 73) *Z. tau;* 74) *Z. cucurbitae;* 75) *B. minax*.

Figure 14. Length of Aculeus: 76) B. dorsalis; 77) Z. tau; 78) Z. cucurbitae; 79) B. minax.) B. minax.) B. minax.

Figure 15. Diameter of Aculeus: : 80) B. dorsalis; 81) Z. tau;82) Z. cucurbitae; 83 \mathbf{r} α .

Table 6. Determination of species diversity by Shannon-Weiner Index in different fields

Fields taken into consideration	Shannon-Weiner Index (H')
Pumpkin	1.648
citrus	1.613
Ber	1.544
Guava	1.481
Bottle gourd	1.458
Mango	1.413

the Shannon-Weiner Index is more, then the species diversity is greater. From these, Species diversity is maximum in Ber and minimum in citrus. Observations are presented in Table 7 and Figure 17.

Simpson Dominance Index (D) of fruit fly species collected from traps

By using the Simpson Dominance Index (Simpson, 1949), species diversity of fruit flies was documented from different fields. The diversity of fruit flies is greater when the

value of the Simpson dominance index (D) is less. Simpson's dominance index is inversely proportional to diversity. In citrus, the Simpson dominance index is 0.221 followed by in ber 'D' is 0.260, in mango 'D' is 0.264, in bottle gourd 'D' is 0.273, in pumpkin 'D' is 0.306 and in guava 'D' is 0.306. As the Simpson dominance index is greater, then the species diversity is less. From these, Species diversity is maximum in citrus as 'D' is less and minimum in guava as 'D' is more. Observations were presented in Table 8 and Figure 18.

Figure 16. Shannon-Weiner Index (H') in different fields.

Figure 17. Shannon-Weiner Index (H') from infested fruits.

Table 8. Determination of species diversity by Simpson Dominance Index in different fields

Figure 18. Simpson Dominance Index (D) in different fields.

Table 9. Determination of species diversity by Simpson dominance index from recovery of infested fruits

Fruit flies infested fruits	Simpson Dominance Index (D)
Citrus	0.496
Pumpkin	0.465
Bottle gourd	0.455
Cucumber	0.351
Ber	0.323

Figure 19. Simpson Dominance Index (D) from infested fruits.

Simpson Dominance Index (D) of fruit flies recovered from infested fruits

By using the Simpson Dominance Index (Simpson 1949), species diversity of fruit flies from different infested fruits was documented. If the value of the Simpson dominance index is less, then the diversity of fruit fly species is greater. In Ber, Simpson dominance index is 0.323 followed by cucumber 'D' is 0.351, in bottle gourd 'D' is 0.455, in pumpkin 'D' is 0.465 and in citrus 'D' is 0.496. As the Simpson dominance index is greater, then the species diversity is less. From these, Species diversity is maximum in ber as 'D' is less and minimum in citrus as 'D' is more.

Distribution of species in different cucurbits and fruits

Observations were presented in Table 9 and Figure 19.

Distribution indices of species (Whiteford, 1949) in different fields were documented. If the value of the distribution is > 0.05 , 0.025-0.05, < 0.025 then the pattern of distribution is Clumped or Irregular distribution, Random distribution, Uniform or regular distribution respectively. In pumpkin, the species distribution value is 1.005 followed by guava 'SD' is 1.004, in citrus 'SD' is 1.003, in bottle gourd 'SD' is 1.002, in ber 'SD' is 1.001 and in mango 'SD' is 0.995. Here, values in pumpkin, bottle gourd, ber, mango, MEGHANA and LASKAR

Figure 20. Species Distribution (SD) in different fields.

guava and citrus are more than 0.05, which indicates that in all fields species distribution is Clumped or Irregular distribution. Observations were presented in Table 10 and Figure 20.

CONCLUSION

An effective biocontrol strategy can be employed with the thorough understanding of the pest species diversity in a given crop ecosystem. A good number of tephritid species are available in the agro-ecosystem of northern tract of West Bengal. Altogether nine species of tephritid fruit flies have been detected from Pumpkin, Bottle gourd, Cucumber, Mango, Ber, Guava and Citrus. Among them, *B. rubigina* (Wang and Zhao) and *B. divenderi* Maneesh, Hancock and Prabhakar were found to be new records. Species diversity of fruit flies in different fields and from infested fruits was maximum in Pumpkin and Citrus; and minimum in Ber as represented by Shannon-Weiner Index and Simpson Dominance Index, respectively. Species distribution in all fields is in clumped or irregular distribution.

ACKNOWLEDGEMENTS

The Authors are extremely grateful to the Head of the Department of Agricultural Entomology and Faculty of Agriculture, Uttar Banga Krishi Vishwavidyalaya.

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