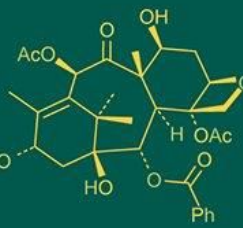
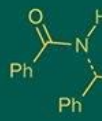


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Impact of ripening stages on host preference of *Bactrocera* sp. and post-harvest quality of guava (*Psidium guajava* L.)

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Abstract

The study investigated the host preference of *Bactrocera* sp. in relation to the ripening stages of guava (*Psidium guajava* L.) and its impact on fruit quality during the 2024 season. Results indicated a significant correlation between fruit maturity, pest susceptibility, and shelf life. The Ripen stage (T₃) exhibited the highest vulnerability, recording peak mean infestation (35.5%) and a maximum of 24.53 egg punctures per fruit. This preference was driven by higher Total Soluble Solids (TSS), peaking at 10.60 °Brix. However, T₃ also suffered the most severe Physiological Loss in Weight (PLW), reaching 10.43% by the 9th day, indicating poor shelf stability. Infestation further degraded nutritional quality, reducing Ascorbic Acid levels by up to 7.2%. In contrast, the Turning stage (T₂) demonstrated superior resilience, maintaining the highest mean Ascorbic Acid (154.50 mg/100 g) and a moderate mean PLW (7.81%) with significantly lower infestation than the ripened stage. These findings conclude that harvesting at the turning stage is the optimal strategy to maximize nutritional value and shelf life while minimizing losses to *Bactrocera* sp.

Keywords: *Bactrocera* sp., *Psidium guajava*, ripening stages, total soluble solids, ascorbic acid, ovipositional preference

Introduction

Guava (*Psidium guajava* Linn.) is an important fruit crop from the Myrtaceae family and is often referred to as the “Poor man’s apple” or the “Apple of the tropics.” Native to Central America, it was brought to India by the Portuguese in the 17th century (Siddiqui *et al.*, 2005). Its ease of cultivation, high nutritional value, and popularity in processed products have made it a significant crop in international trade and in the economies of over 50 tropical countries. Major producers of guava include India, Hawaii, Florida, South Africa, Brazil, the Dominican Republic, Haiti, Cuba, Guyana, the Philippines, and New Zealand.

Guavas are a good source of pectin, a thickening agent, with concentrations ranging from 0.1% to 1.8%. Pectin levels increase as the fruit ripens but reduces significantly in overripe fruit. The guava tree thrives in tropical and subtropical climates and can adapt to a variety of soil conditions (Gurjar *et al.*, 2018) [8]. In India, guava is cultivated on an area of 260,000 hectares, yielding approximately 3.9 million tons annually, which accounts for about 4.0 percent of the country's total fruit production. It ranks as the fifth most widely grown fruit crop in the country, with major production regions including Bihar, Andhra Pradesh, Uttar Pradesh, Maharashtra, West Bengal, Karnataka, Gujarat, and Madhya Pradesh.

However, India lags behind other nations in guava productivity due to significant insect pest issues (Vignesh, Chandrasekaran, 2020) [21]. Guava orchards worldwide are infested by almost 800 different types of insect pests, such as fruit flies, caterpillars that eat bark, and capsule moths. Fruit flies from the Dipteran order, can cause considerable damage to guava, leading to yield reductions and quality issues. The infestation of fruit fly is a major limiting factor in the production of guava (Mariadoss *et al.*, 2021) [15]. Depending on the population, location, variety, and season, the crop loss can range from a few to 100 percent (Kumar *et al.* 2011; Sharma *et al.* 2015) [13, 19]. In Uttar Pradesh, the infestation rate varies from 20 to 46 percent, resulting in losses of 16 to 40 percent, particularly during the rainy season (Verghese and Sudhadevi, 1998) [20].

Among these, fruit flies (*Bactrocera* spp.) are particularly damaging, responsible for 60 to 80% of the fruit damage (Jalaluddin *et al.*, 1999) ^[10]. They inflict direct harm by laying eggs on the fruit's skin, leading to microbial invasion and subsequent rotting of the fruit flesh. The maggots that hatch from these eggs feed on the fruit, accelerating decay and rendering it unfit for consumption (Jena and Singh, 2022) ^[12]. Severely infested fruits often fall to the ground before the maggots pupate. Key fruit fly species affecting guava in India include *Bactrocera dorsalis*, *B. zonata*, *B. correcta*, *B. caryea*, and *Zeugodacus tau*, with males attracted to methyl eugenol and Cue lure for trapping (Yashoda *et al.*, 2007 ; Rattanapun *et al.*, 2009) ^[22, 16].

Materials and Methods

The studies on the preference of fruit flies, *Bactrocera* spp. in relation to the different stages of fruit maturity and to understand the prevalence of fruit flies in the confined ecosystem the fruit fly infested fruits were collected both from tree and ground based on the ovipositional puncture marks and brought into the laboratory and incubated at ambient temperature condition for adult emergence. For the experiment, four guava trees enriched with blossoms and bearings were selected and tagged, from these trees both the intact and fallen infested fruits of three types viz., green, turning and ripening stages were taken and placed in a plastic container of dimension 10 X 8 X 15 cm (lbh), covered with the net by following the procedure of Jadhav and Patil (2013) ^[9]. The studies on the preference of fruit flies, I spp. in relation to the different stages of fruit maturity was carried out in the entomology laboratory of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli under normal room temperature 27±10°C by keeping the small, medium and large sized infested intact and fallen fruits separately in rearing cages to access the population built up in each categories of fruits. Fruit flies lay their eggs on guava fruits in partially ripened stage. On hatching, the maggots enter into endocarp portion and start to feed fruit pulp. In the intact and fallen fruits presence of tiny punctures on the surface indicates the infestation of fruit flies.

Fruit sample collection

The infested fruits were selected based on the oviposition punctures present on the host skin and it may be visible due to the exudates from the penetrated portion of the fruit. The adult females of *Bactrocera* sp oviposition their eggs in the same guava fruits in various proportions. Due to the feeding action of emerged maggots, the oviposited site become sunken with black lesions, rotten and become decayed. The infested fruits collected from the field at fortnight intervals.

Laboratory rearing of fruit flies

Initial cultures of *Bactrocera* sp were sourced from infested guava fruits. These fruits were placed on moist sand in medium-sized plastic container (23 cm x 7 cm) to facilitate pupation (see Plate 5). After 5-6 days, the pupae were separated from the sand and transferred to 10 cm diameter Petri dishes lined with moist filter paper. These Petri dishes were then placed inside a rearing cage (35 cm x 30 cm x 35 cm), which featured wire mesh on three sides, a glass top, and a single door at the front for easy access for collection and feeding. The adult flies emerging from the pupae were provided with a 5% glucose solution. This solution was

applied to cotton soaked in water and placed inside the Petri dishes. To prevent mould growth, the food source was refreshed regularly. The cultures kept under controlled conditions with a temperature of 25±2 °C and 65±5% relative humidity. The adult fruit flies were drawn from the culture for further experiments

Bioassay

Bioassays were conducted in a room with an average temperature of 25±1 °C, a relative humidity of 60±10% and a photophase of 12 h. Guava fruits were assayed during 3 stages of physiological maturity: the green, the green-yellow ripe, and the fully ripe stages. Guava Fruits were cleaned with sodium hypochlorite (1%), water-rinsed, and dried at room temperature on a paper towel before being placed in the rearing cage.

Attractiveness and infestation levels were determined in bioassays for each fruits stages separately, in free choice test. Test were conducted in a room at an average temperature of 25±1 °C, relative humidity of 60±10%. Attractiveness and infestation levels were determined in both tests and expressed as number of oviposition probes (female/fruit) and fecundity (larvae/female/fruit), respectively.

Free Choice Experiment

For the free choice experiment, fruits were placed together in a rearing cage (30 x 30 x 30 cm) with the distance of 10 cm between the fruits. Fruits were categorized into three ripening stages based on visual color and texture:

T₁ (Green stage): Hard texture, dark green pericarp.

T₂ (Turning stage): Medium-firm texture, yellowish-green pericarp (color-break).

T₃ (Ripen stage): Soft texture, fully yellow pericarp.

Ten gravid females of *Bactrocera* sp from established rearing culture was released into rearing cage for two hours. The ovipositional behaviour preference of *B. dorsalis* female was observed for 2 hours. The experiments were repeated five times and conducted under laboratory conditions (28±2 °C, 60-70% RH, 12:12 h L:D)

Biochemical analysis

Physiological Loss in Weight (%)

The loss in weight of each stages fruit due to fruit fly infestation was numbered and recorded using an electronic balance. After consequently, the weights were periodically recorded, and the following formula was used to calculate the physiological loss in weight (PLW).

$$PLW (\%) = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Total Soluble Solids (°Brix)

A digital refractometer was used to measure the total soluble solids (TSS) of the pulped healthy and infested guava fruits. Range: 0% to 32%. The values were converted to percentage TSS of guava fruits. The measurements were recorded in °Brix (°B) and were taken at room temperature (mean temperature: 28 °C).

Ascorbic Acid (mg/100 g of pulp)

The AOAC method (2001) was used to estimate ascorbic acid. 10 ml of 4% oxalic acid were used to homogenize near

half a gram of guava pulp. To obtain a clear solution, the extract was left undisturbed. After pipetting out 5 ml of the supernatant solution, the volume was made up to 10 ml with 4% oxalic acid, and the mixture was titrated against 2, 6-dichlorophenol indophenol dye. A conical flask containing two millilitres of the extract was used to titrate it against a standard dye solution. The light pink colour's end point remained for thirty seconds. A standard solution was prepared by dissolving 100 mg of analytical-grade ascorbic acid in a small quantity of 4% oxalic acid. Using the oxalic acid, 10 ml of this solution was diluted to 100 ml. Consequently; 0.1 mg of ascorbic acid per ml of solution was used as the standard ascorbic acid and was titrated against 2, 6-dichlorophenol indophenol dye. The titrated value (V2) was regarded as the standard titrate value.

$$\text{Ascorbic acid} = \frac{\text{Dye factor (0.5)} \times \text{titrate value (V2)} \times \text{Vol. made up}}{100 \text{ Sample titrate value (V1)} \times \text{Vol. taken for titration} \times \text{Weight of the sample}}$$

Results and Discussion

Incidence of fruit fly, *B.dorsalis* on guava at different fruit ripening stages

Fruit fly, *Bactocera sp* oviposition activities indicated that there were distinctive behavioural responses to guava fruit ripening stages under field conditions. There was a significant difference between stages of ripening with percent infestation and the number of oviposition puncture. Ripening Stage consistently recorded the highest infestation levels, peaking at 67.78% in late August. Turning stage showed moderate levels of infestation, reaching a low in June (2.22%) and steadily increasing to 38.89% by the end of August. Green stage was the least preferred. In most observations, infestation was 0%, with only negligible activity (1.11% to 2.22%) recorded sporadically (Table 1.).

The intensity of the fruit fly population followed an upward trend as the season progressed. Infestation was minimal in June with mean infestation rates starting at 0 and only reaching 4.81% by the end of the month. A noticeable increase was observed in July as the fruits matured, with the mean infestation rising from 5.18% to 18.15%. The peak period for *Bactrocera sp.* activity was recorded in August. The mean infestation rate climbed sharply, reaching its maximum of 35.5% by the 31st standard week.

Weekly observations indicated that ripe stage fruits were significantly more preferred than turning stage fruits. Fruit infestation remained zero in only green fruits. Overall infestation was negligible in green stage fruits, and this followed by turning stage fruits and significantly higher in ripe stage fruits (Singh and Sharma, 2013) [19].

Fruit fly preference on green fruits did not show any pattern of incidence. However, in turning and ripe stages, fruit fly infestation increased gradually (Aridasa, 2017; Fitrah *et al.*, 2025) [3, 7]. Female flies made significantly more egg punctures into ripe fruits than turning or green fruits. However, fruit firmness act as limiting factor in egg laying by the females. In ripen fruits as decline in such fruit firmness takes place it enables the females to lay more eggs (Vignesh & Chandrasekaran, 2020) [21].

Incidence of fruit fly, *B.dorsalis* on guava at different fruit parts (Top, Bottom and Middle) (Table2)

The number of egg punctures made by female flies into the three fruit portions between different ripening stages of

guava, it indicated a significant difference in their preference to different portions of the fruits. Female flies made very low numbers of egg punctures in all three fruit portions of the green guavas (1.3 No. of egg puncture/fruit) and the pattern of distribution of punctures was uneven with no differentiation between fruit parts.

Oviposition counts on top, middle and lower parts of green fruit were found to be 0.85, 0.1, 0.475 No. of. egg puncture/fruit, respectively. In turning stage fruits, significantly more oviposition attempts were made into the top portion of fruits (24.3 egg punctures) followed by bottom (4.7 egg punctures/fruit) and middle part of fruit with 2.4 no.of egg punctures. From the number of such punctures made on ripe fruits it could be observed that top portion of the fruit consistently recorded the highest number of egg punctures, with a peak of 24.53 punctures/fruit in the ripened stage. This is likely due to the top portion (near the stem) ripening earlier or having a softer texture. and bottom portion of the fruit almost had more number of oviposition marks showed moderate activity, significantly lower than the top but higher than the middle, with a maximum of 4.73 punctures/fruit in the ripened stage (Table 2). Middle portion was least preferred by the fruit fly, with less than 1 puncture/fruit even at the fully ripened stage. The results indicated that female *Bactocera spp* preferred to oviposit on the top part followed by bottom over the middle part of ripe and turning guavas, even though such preference did not exist in green (unripe) fruits of guava (Table 2).

Impact of Ripening Stages

The number of egg punctures increases significantly as the fruit matures from green to ripened green stage had minimal egg-laying activity (max 0.85 at the top), as the hard pericarp of unripe fruit acts as a physical barrier to the fly's ovipositor. In the turning stage, a noticeable increase in punctures (up to 4.03 at the top), indicating the fly begins targeting fruit as it starts to soften (Devi and Jha, 2017) [6]. The repining stage is the most susceptible stage with a mean of 24.53 punctures at the top, representing more than a 600% increase from the turning stage.

In the healthy fruits the TSS generally increased as the fruit matured and aged. The highest individual TSS was recorded in Healthy T₃ (Ripen) on the 9th day (11.4 °Brix), with an overall mean of 10.6 °Brix. While TSS in the Infested Fruits also increased with ripeness in infested fruits, the values were generally lower than their healthy counterparts. The mean TSS for infested T₃ was 10.35 °Brix, compared to 10.6 °Brix in healthy fruits, suggesting that larval feeding may deplete the fruit's sugar reserves (Jang and Light, 1999; Reddy and Vasugi, 2008) [11, 17].

Impact of Ripening Stages on fruit fly infestation

T₁ (Green) stage recorded the lowest TSS levels. Interestingly, healthy green fruits showed a sharp decline on the 9th day (5.98 °Brix), whereas infested green fruits maintained a higher mean (8.82 °Brix), likely due to induced stress-ripening caused by the infestation. T₂ (Turning) showed intermediate TSS levels, with healthy fruits averaging 10.1 °Brix and infested fruits slightly lower at 9.95 °Brix. Consistently T₃ (Ripen): maintained the highest TSS across all observation days, confirming that *Bactrocera sp.* infestation is most prevalent in fruits with higher sugar concentrations. In healthy turning and ripening stages, there was a steady upward trend in TSS

over time. In infested fruits, the TSS levels showed less stability toward the 9th day (e.g., T₂-Turning dropped from 10.22 on day 6 to 10.12 on day 9), indicating fruit degradation and nutrient consumption by the larvae.

There is a direct, positive correlation between the sweetness of the fruit and the mean egg punctures per fruit escalate from less than 1 to over 24. The high sugar content in ripened fruits acts as a chemical attractant, signaling a high-quality substrate for larval development (Aarti *et al.*, 2023) [1]. In the turning and ripening stages, healthy fruits consistently maintain a higher TSS than infested ones. This suggests that as larvae develop, they consume the free sugars (fructose, glucose, and sucrose) within the fruit pulp, leading to a reduction in the overall Brix value (Devi and Jha, 2017; Afroz *et al.* 2021) [6, 2].

Spatial Preference and Susceptibility

The data indicates that the top portion of the fruit is the primary site for egg-laying, especially in the ripened stage (punctures). This is likely because the stem-end often ripens

and softens first, providing both the chemical signal (high TSS) and the physical ease of penetration required for successful oviposition (Cortés-Martínez *et al.*, 2021) [4]. Nutritionally, the decline in ascorbic acid across all treatments confirms that *Bactrocera* sp. infestation accelerates the degradation of Vitamin C, likely through increased oxidative stress and enzymatic breakdown (Louzeiro *et al.* 2020) [14]. The Turning stage is the critical "resilience window," offering the best balance of high antioxidant content (154.50 mg/100 g) and relatively low infestation. From a management perspective, this supports the practice of harvesting at the color-break (turning) stage to bypass the exponential increase in pest activity associated with fully ripened fruits (Deepthi *et al.*, 2016) [5]. The transition from the "Turning" to "Ripen" stage marks the onset of peak susceptibility. Protection measures, such as fruit bagging or targeted insecticide applications, should be intensified during this window to prevent the exponential increase in egg-laying activity seen in the ripened stage.

Table 1: Preference of *Bactrocera* sp in relation to fruit ripening stages of guava

Month (2024)	Standard Meteorological week	Percentage of fruit fly infestation at different ripening stages			Mean
		Green	Turning stage	ripening stages	
June	22	0	-	-	0
	23	0	2.22	5.56	2.55
	24	2.22	3.33	8.89	4.81
July	25	0	5.56	10	5.18
	26	0	11.11	14.44	8.51
	27	0	18.89	35.56	18.15
August	28	0	26.67	47.78	24.81
	29	0	23.33	53.33	25.55
	30	1.11	30	64.44	31.8
	31	0	38.89	67.78	35.5

*Mean of 10 observations

Table 2: Oviposition punctures by *B. dorsalis* on guava at different ripening stages

Fruit Portion	Egg punctures count at different ripening stages (No. of egg puncture/fruit)		
	Green	Turning	Ripen
Top	0.85	4.03	24.53
Middle	0.1	0.26	0.73
Bottom	0.47	1.01	4.73
SE(d)	0.01	0.05	0.04
CD(P = 0.05)	0.03	0.02	0.03

*Mean of 10 observations

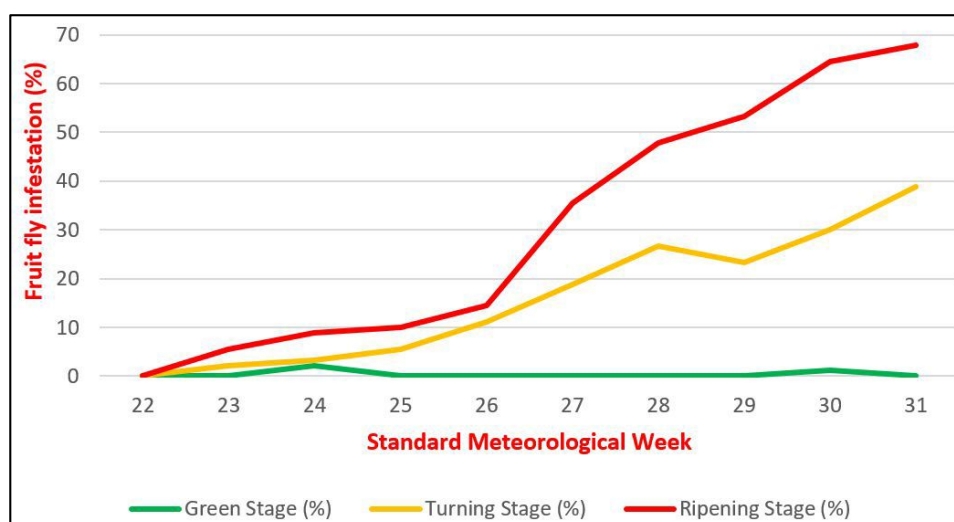


Fig 1: Temporal distribution of *Bactrocera* sp. infestation (%) across different guava ripening stages

Table 3: Total Soluble Solids in healthy and fruit flies infested guava fruits

Treatments	TSS(Brix) of healthy fruits				Mean	TSS (Brix ^o) of infested guava fruits				Mean
	1 st day	3 rd day	6 th day	9 th day		1 st day	3 rd day	6 th day	9 th day	
T ₁ -Green	8.52	8.78	9.10	5.98	8.09	8.43	8.67	9.06	9.10	8.82
T ₂ -Turning	9.57	10.36	10.06	10.76	10.1	9.5	9.86	10.22	10.12	9.95
T ₃ -Ripen	10.25	9.57	11.3	11.4	10.6	10.1	10.33	10.55	10.38	10.35
SE(d)	0.07	0.02	0.04	0.01	0.06	0.23	0.07	0.15	0.05	0.12
CD(P = 0.05)	0.15	0.05	0.1	0.03	0.02	0.48	0.16	0.31	0.10	0.26

*Mean of 10 observations

Table 4: Ascorbic acid content in healthy and fruit flies infested guava fruits

Treatments	Ascorbic acid content of healthy guava fruits (mg/100 of pulp)				Mean	Ascorbic acid content of infested guava fruits (mg/100 g of pulp)				Mean
	1 st day	3 rd day	6 th day	9 th day		1 st day	3 rd day	6 th day	9 th day	
T ₁ -Green	176.1	159.5	158.0	0	123.42	175.6	158.0	124.4	0	114.5
T ₂ -Turning	181.7	166.3	149.7	120.2	154.5	182.2	165.0	144.4	118.4	152.5
T ₃ -Ripen	184.4	151.3	138.7	118.2	148.23	168.1	146.9	137.9	115.2	142.0
SE(d)	0.67	1.2	1.0	0.8	0.9	0.3	0.5	0.4	0.3	0.47
CD(P = 0.05)	1.40	2.5	2.2	1.7	1.95	0.6	1.1	1.0	0.7	0.85

*Mean of 10 observations

Table 5: Physiological weight loss in guava fruits

Physiological loss in weight (%)					
Treatments	1 st day	3 rd day	6 th day	9 th day	Mean
T ₁ -Green	5.58	6.56	7.33	8.40	6.97
T ₂ -Turning	6.65	6.94	8.34	9.32	7.81
T ₃ -Ripe	7.67	8.34	9.36	10.43	8.95
SE(d)	0.3	0.3	0.6	0.4	0.7
CD(P = 0.05)	0.6	2.0	0.7	0.3	0.5

*Mean of 10 observations

Conclusion

This study confirms that the ripening stage of guava is the primary determinant of both *Bactrocera* sp. infestation levels and post-harvest quality. The Ripen stage (T₃) is highly susceptible to fruit fly attack, with a dramatic peak in egg punctures (24.53/fruit) driven by high sugar content (10.60 °brix) and this stage also suffers from the highest physiological weight loss (8.95%), rendering it unsuitable for extended storage. Conversely, the Turning stage (T₂) offers a "nutritional peak" with maximum Ascorbic Acid (154.54 mg/100 g) and significantly lower pest pressure. Therefore, harvesting at the turning stage is recommended as a sustainable agricultural practice to reduce yield loss and maintain fruit integrity.

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