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# Genomic insights and bionomics of *Bactrocera dorsalis* on *Ziziphus jujuba*: Strategies for pest management

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**Abstract**
Extensive surveys were conducted to assess the ecological status, biology, and molecular taxonomy of the fruit fly *Bactrocera dorsalis*, which is associated with Ber (*Ziziphus jujube* Mill) plantations in the Solapur district of Maharashtra. The survey encompassed 20 different Ber plantation fields across various regions of Solapur district and was carried out from October 2023 to February 2024. Most observations were made during the winter season, coinciding with the fruiting period. *Bactrocera dorsalis*, known as the Oriental fruit fly, has been identified as a highly destructive and economically significant invasive polyphagous pest in agricultural fields. To mitigate potential losses for farmers, it is crucial to implement rapid surveys, accurate species identification, and immediate control measures. Failure to address these issues could result in substantial crop damage. In light of this, our study aims to provide accurate identification and a comprehensive understanding of the ecology and biology of *Bactrocera dorsalis*.

**Keywords:** Molecular, taxonomy, *Bactrocera dorsalis*, ber, destructive

## Introduction
The ber (Ziziphus mauritiana Lamarck), commonly known as desert apple, jujube, or Chinese apple, is a tropical fruit tree species belonging to the family Rhamnaceae. It is also referred to by various names in different languages, including Badari in Sanskrit, Kul or Boroi in Hindi, Dongs, Bor, Beri, and Indian plum. The ber is notable for its resilience in arid environments and is cultivated in many tropical and subtropical regions around the world. Its fruits are often enjoyed for their sweet flavor and nutritional benefits [1]. In India, the ber occupies a significant area of approximately 22,000 hectares and is a popular fruit crop suited for arid and semi-arid regions. Most of the cultivated areas are concentrated in the states of Rajasthan, Haryana, Punjab, Gujarat, Maharashtra, and Uttar Pradesh (Jamandar *et al.*, 2009). Its adaptability to dry conditions makes it an important agricultural resource in these regions [2]. Jujube fruit exhibits neuroprotective properties, including the ability to protect neuronal cells from neurotoxic stress. It stimulates neuronal differentiation, enhances the expression of neurotrophic factors, and supports memory and learning processes. These benefits make jujube a valuable fruit for promoting brain health [3]. Several insect pests attack ber (jujube) fruit, but fruit flies are among the most significant threats to the quality of these fruits. The damage caused by fruit flies is considerable, with an estimated annual loss of fruits and vegetables amounting to approximately 144.4 million US dollars. Their impact not only affects the yield but also significantly reduces the market value of the produce [4]. Fruit flies (Diptera: Tephritidae) are among the most significant quarantine pests worldwide, occupying a prominent position due to their destructive nature. They are highly damaging to fruits and vegetables, making them a major concern in quarantine regulations and agricultural trade. Their infestations can lead to severe economic losses and pose a serious threat to both crop production and market access [5]. More than 800 species of tephritid fruit flies belong to the subfamily Dacinae, primarily categorized under the genera *Bactrocera* and *Dacus*. Of these, approximately 60 species are known to be present in India, where they mainly infest fruits and vegetables, causing significant yield losses [6, 7, 8]. *Bactrocera dorsalis*, commonly known as the oriental fruit fly, is a major polyphagous pest that affects a wide range of fruit crops globally. In the Solapur district, this fruit fly causes substantial damage to ber fruits during the fruiting season, making it difficult to control its population effectively. The life cycle of *B. dorsalis*, which includes egg-laying, larval development within fruits, pupation, and adult emergence, showcases its adaptability to various environmental conditions. Factors such as temperature, humidity, and food availability significantly influence its growth and reproduction, contributing to its status as a highly destructive pest across different agricultural systems. A deeper understanding of its biology is essential for devising effective management strategies to control its population and mitigate the damage caused to fruit crops. *Bactrocera dorsalis* is a polyphagous pest that inflicts significant damage on ripening ber fruits and is notoriously difficult to control. This study was conducted to explore various aspects of this highly destructive pest, including its genomic history, biology, and ecology, in order to better understand its behavior and develop more effective management strategies.

## Material and Methods
### Method of Rearing of *Bactrocera*
The study was conducted at various field sites in the Solapur district during the fruiting season, which extends from October to February. Infected fruits were collected from multiple farm plots in the district. Infested fruits were then placed in rearing cages (20 cm in height and 14 cm in width) containing a 4-5 cm thick layer of sterilized sand to facilitate pupation. The larval stage was studied separately, with observations of three distinct instars, each with a specific developmental period. Fully grown larvae (maggots) pupated within the sand, so a plastic container

with a 4-5 cm layer of sterilized sand was provided for this purpose. The container was monitored daily for signs of pupation. Once pupation occurred, all pupae were collected and transferred to large screened cages, where they later developed into adults. The adult flies were observed to be highly active and strong fliers. To monitor adult longevity, cotton balls soaked in honey were provided as a food source, and the cages were maintained at room temperature (30-35°C). Water was also added to maintain consistent humidity levels.

**Method of DNA Barcoding**

Sample received: From specimen stored in 70% ethanol. Number of samples: 1specimon

**Protocol**

1. Genomic DNA isolation was performed using the CTAB method.
2. The sample was purified through lithium chloride treatment.
3. PCR optimization was conducted for 16S rRNA sequencing, using the following primers:

- **Forward Primer:** 8F
- **Reverse Primer:** 907R.

| Reagents | Amount (IN µL) |
|---|---|
| MgCl2 | 5 |
| Taq assay Buffer(10X) | 5 |
| dNTPs mix | 4 |
| Forward Primer | 2.5 |
| Reverse Primer | 2.5 |
| Taq DNA Polymerase | 1 |
| Template DNA | 2 |
| Deionized Water | 28 |
| TOTAL | 50 |

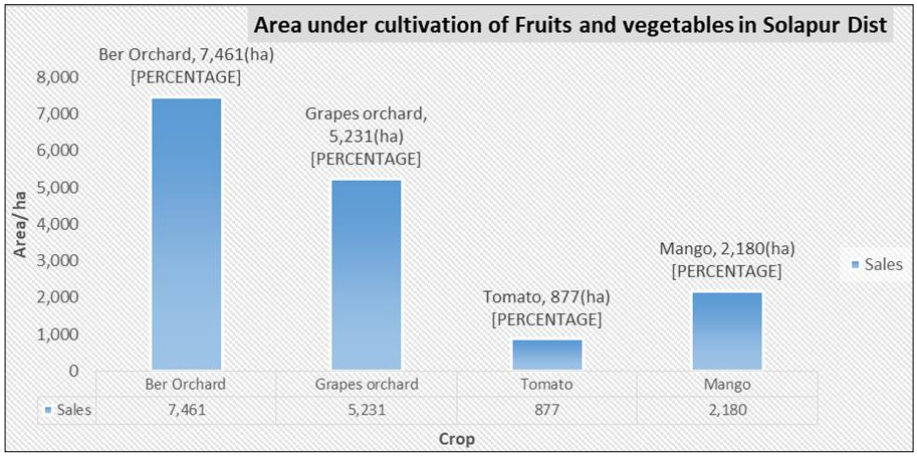
| Temperature | 96˚C | 95˚C | 58˚C | 72˚C | 72˚C |
|---|---|---|---|---|---|
| Time | 5mins | 1min | 1min | 1min | 10mins |

**Reaction Conditions**

- **Reaction volume:** 50 µL
- **Number of cycles:** 35
- Upon completion of the cycles, the samples were stored at 4°C.

1. Gel excision and purification of the PCR product were performed using a gel extraction kit.
2. The gel-purified product was sequenced, and the raw sequence was aligned using Multalin software.
3. The final sequence was submitted to the BLAST tool on the NCBI website to identify the possible organism.

Geographically, Solapur is situated between 17.10 to 18.32 degrees north latitude and 74.42 to 76.15 degrees east longitude. The district lies on the southeastern fringe of Maharashtra State and is bordered by several districts: to the north by Ahmednagar and Osmanabad, to the east by Osmanabad and Gulbarga (in Karnataka State), to the south by Sangli and Bijapur (also in Karnataka State), and to the west by Satara and Pune. Covering an area of 150,000 hectares, Solapur experiences an average annual rainfall of less than 750 mm and has a bimodal climate, with maximum temperatures reaching up to 40.1°C and minimum temperatures dropping to 16.1°C. The district features two primary canals: the Bhima Right Bank Canal and the Neera and Man Left Bank Canal. The cultivation of irrigated fruit and vegetable crops in the district is rapidly increasing, with ber, pomegranate, and grape dominating the market. Common vegetables grown in the area include onion, chili, brinjal, and tomato. The graph above illustrates the maximum land area cultivated with ber orchards in Solapur District. Due to the significant damage caused by fruit flies to ber crops, it is crucial to study their life cycle and conduct molecular identification of the species. This research is essential for developing effective strategies to control fruit fly populations. (Data on fruit and vegetable crops cultivated in Solapur District was obtained from the Krishi Vigyan Kendra, Solapur.)




**Result**

During the fruiting season, female fruit flies lay their eggs in small clusters within the mesocarp of ripe fruits. While puncturing the fruit, the fly introduces bacteria from the skin into the flesh, which promotes fruit rot and creates a substrate for the larvae to feed on (Drew and Lloyd, 1989; Fletcher, 1987). Female fruit flies show a preference for laying their eggs in ripe fruits. The eggs of *Bactrocera dorsalis* measure approximately 0.9 mm in length and 0.3 mm in breadth, with a color ranging from white to creamy. Temperature and humidity significantly influence egg hatching, with eggs typically hatching within two days under room temperature and relative humidity conditions. Infested fruits exhibit reddish decaying marks and eventually fall from the tree. After hatching, the larvae feed on the pulp, which appears normal from the outside. There are three larval stages, known as instars. The fruit fly larvae are elongated, apodous, and cylindrical in shape, resembling maggots with a narrowed anterior end and a broader caudal end. They are creamy white in color.

**First Instar:** The larvae that hatch from the egg are tiny but active. The first instar measures approximately 4 mm in length and 1 mm in width. The anterior section of the body is white, transitioning to a dull yellow. After 4 to 5 days, the first instar larva develops into the second instar.

**Second Instar:** The second instar larva measures approximately 5 mm in length and 1-2 mm in width. During this stage, the body color changes to creamy. The maggot continuously feeds on the soft pulp and, after 4 to 5 days, transitions into the third instar.

**Third Instar (Prepupa):** Third instar larvae are relatively stout and sluggish, measuring approximately 7 mm in length and 2-2.5 mm in width. The first and second instar larvae feed on the pulp of rotten fruit. Once fully grown, the third instar larvae stop feeding, exit the fruit, and fall into the soil to pupate. The entire larval stage is completed in 12 to 14 days.

**Pupa:** After completing larval development, the fully developed larva emerges from the fruit and is placed into moistened sand. After a short period, the larva burrows into the sand, its body shortens, and it becomes immobile. The cuticle then hardens, transitioning from tan to brown and eventually becoming brittle, forming a pupa that resembles a seed and ranges in length from 5 to 8 mm.
The newly formed pupae are tan-colored, while older pupae appear dark brown. The adult fly develops inside a hard, brown, barrel-like shell formed from the larva's epidermis. The pupation period lasts for 11 to 12 days.

**Adult:** The flies emerge from the puparium and come to the surface of the sand. The transition from egg to adult takes approximately 25 to 26 days. Upon emerging, the adult fly begins to seek food, mate, and deposit eggs. The body length of the adult is around 6 to 8 mm, with wings measuring approximately 7.5 mm long, primarily hyaline in color. The leading edge of the hyaline wings is blackish. The coloration of the fly can vary significantly, but the thorax typically features distinct yellow and dark brown to black patterns. A black T-shaped band is visible on the abdomen. The female fly has a pointed, thin ovipositor that allows her to deposit eggs beneath the epidermis of the host fruit. The total life cycle of *Bactrocera dorsalis* from egg to adult spans 25 to 27 days.

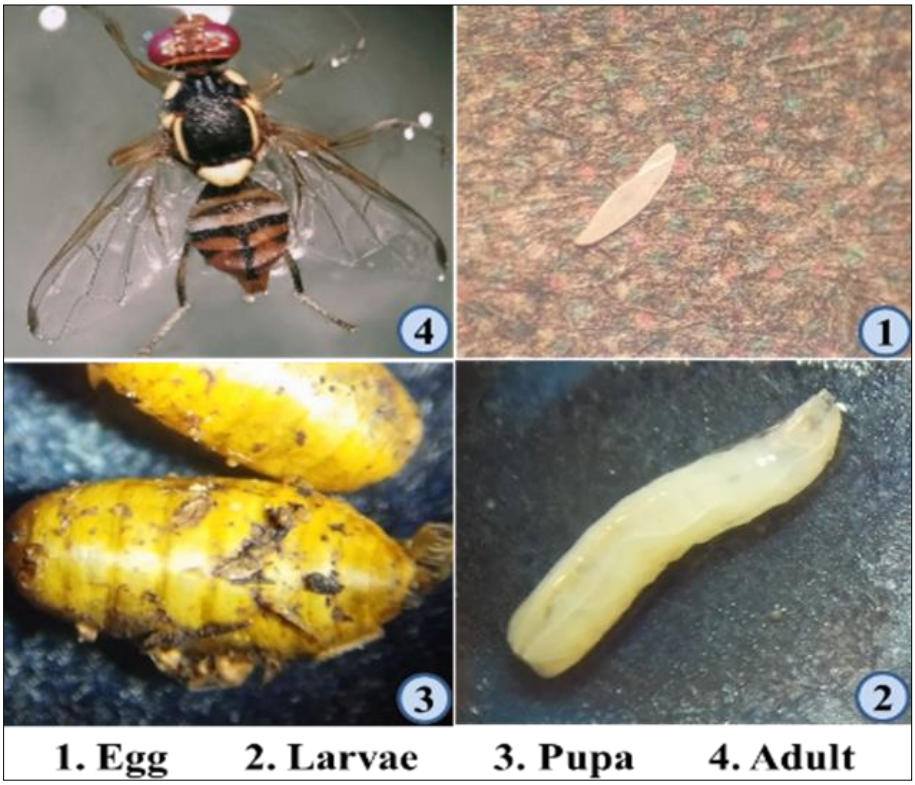



**Fig:** Life stages of *Bactrocera dorsalis*

**Table:** Morphometric measurement of different developmental stages of *B. dorsalis*

| Development stages | Length (mm) | | Width(mm) | |
|---|---|---|---|---|
| | Range | | Range | |
| | Minimum | Maximum | Minimum | Maximum |
| Egg | 0.4 | 0.5 | 0.2 | 0.3 |
| 1st instar | 1 | 2 | 0.2 | 0.4 |
| 2st instar | 4 | 5 | 2 | 3 |
| 3rd instar | 6 | 7 | 4 | 6 |
| Pre- pupae | 2 | 3 | 2 | 4 |
| Pupa | 5 | 6 | 2 | 3 |
| Male ♂ | 7 | 8 | 8 | 10 |
| Female ♀ | 8 | 9 | 11 | 12 |

**Molecular Identification by DNA Barcoding**

Morphological identification is often limited, requiring adult specimens and expert analysis. Therefore, molecular identification was performed using DNA barcoding. The genomic sequence was aligned using Multalin software, and the final sequence was submitted to the NCBI BLAST tool to identify the organism. The sequence matched the genomic sequence of *Bactrocera dorsalis*, confirming the identified fruit fly species as *Bactrocera dorsali.*


**Fig.** Genomic sequence of *B. dorsalis*

**Discussion**

Butani (1979) [1, 10] recorded 80 insect species feeding on ber trees in India [9]. Fruit flies are among the most serious agricultural pests globally, causing significant yield losses. Tephritid flies of the genus *Bactrocera* are of particular concern, especially across Asia and Australia (Kim *et al*., 1999) [11], where they pose a major threat to fruit and vegetable production [10]. Lakra and Zile Singh (1983) [2, 12] observed that the commercial cultivation of ber in northern India has become increasingly difficult due to attacks by over 100 species of insect pests. These pests pose a significant challenge to growers, leading to reduced yields and economic losses [11]. Morphological identification of all 4 species of *Bactrocera, viz., B. tau, B. zonata, B. correcta,* and *B. dorsalis*, was carried out by White (1992) [13] and Drew (1994) [12, 13, 14]. The identification of specimens within species complexes is highly challenging due to morphological convergence and overlapping characteristics. Furthermore, taxonomic uncertainties complicate the accurate classification and differentiation of these species [14, 15].

DNA barcoding has emerged as a revolutionary tool in entomology, enabling precise species identification through the use of short, standardized genetic sequences. This technique is particularly valuable for distinguishing *Bactrocera dorsalis* from closely related species, especially in regions where multiple fruit fly species coexist. DNA barcoding enhances traditional morphological methods, which can be challenging due to the high variability both within and between species. This genetic approach provides a more accurate and reliable means of species identification, improving pest management efforts. Therefore, present research *B. dorsalis* studied with molecular taxonomy.

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