International Journal of Economic Plants

https://ojs.pphouse.org/index.php/IJEP

IJEP May 2024, 11(2): 100-103

Crossref

Article IJEP5218a

Full Research

Doi: HTTPS://DOI.ORG/10.23910/2/2024.5218a

Emerging Threat of *Oxyrachis tarandus* Fabricus Infestation on Banyan Trees (*Ficus benghalensis* Linnaeus)

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Article History

Received on 14th February, 2024
Received in revised form on 24th April, 2024
Accepted in final form on 08th May, 2024

Abstract

A comprehensive survey was conducted during March 2022– October 2023 in various forest areas including Pusa Hill Forest, Alipur City Forest, and Jahanpanah City Forest, New Delhi, India to study *Oxyrachis tarandus*, cow horn bug or treehopper on *Ficus benghalensis*. This study utilized both morphological and molecular techniques for accurate species identification, revealing a significant association between *O. tarandus* and *F. benghalensis*. Morphological identification relied on established criteria, which included measurements of adult insects and the observation of distinctive features such as wings and horns. On the other hand, molecular identification involved DNA extraction from collected specimens, followed by PCR amplification targeting a fragment of the MT-COI gene. This approach facilitated precise species identification and allowed for the construction of a phenogram to analyze genetic relationships within the studied population. Phenogram construction and analysis were conducted to further validate species identification and explore genetic relationships. The treehopper's feeding behaviour, oviposition habits, and ecological interactions were thoroughly documented, highlighting its potential impact on the banyan tree's health and ecosystem dynamics. This information underscored the necessity for ongoing monitoring and development of effective pest management strategies to conserve India's national tree and forest biodiversity. The study revealed the critical need for ongoing research and conservation efforts to address pest-induced threats to iconic tree species like *F. benghalensis*, thereby ensuring the long-term health and resilience of forest ecosystems.

Keywords: *Oxyrachis tarandus*, DNA extraction, host plant association, mutualism

1. Introduction

Approximately 21.71% of India's geographical area is covered by forests, with an additional 2.9% dedicated to tree cover. Collectively, these forested areas account for about 2% of the world's total forested regions (Anonymous, 2021). Renowned for their extensive diversity, Indian forests host more than 500 tree species across 244 genera and 67 families (Dhiman, 2021). Among the numerous tree species in Indian forests, the banyan tree (*Ficus benghalensis* L.) holds the distinction of being the national tree of India (Mon et al., 2020; Chakraborty et al., 2022). This venerable tree species is revered for its cultural and religious significance, deeply rooted in Indian traditions. The Banyan tree is known for its expansive canopy, aerial prop roots, and longevity, symbolizing strength and endurance (Rajkumar et al., 2021). Its selection as the national tree reflects its integral role in India's natural and cultural heritage, embodying the country's rich biodiversity and deep-rooted traditions (Kmail et al., 2018). Accompanying this unique trait is the banyan tree's expansive canopy, comprised of large, elliptical, glossy leaves that offer substantial shade Zubair and Arshad (2021). The trunk, stout and often buttressed, showcases a smooth and grayish bark in its youth, transforming into a rough and fissured texture as the tree matures (Verma, 2016). This combination of features renders the banyan tree not only a botanical wonder but also a symbol of cultural significance (Logesh et al., 2023) and ecological prominence, embodying the richness of biodiversity in tropical and subtropical regions (Gopukumar and Praseetha, 2015). However, despite its significance, the banyan tree faces challenges from various pests (Ashalatha et al., 2023). Bark beetles, scale insects, caterpillars, aphids, and leafhoppers are among the common pests that can compromise the health of the banyan tree (Russo et al., 2023). To best of our knowledge, the present investigation is the first report of *F. benghalensis* as a new host for *Oxyrachis tarandus* Fabricus (Hemiptera: Membracidae) from Delhi province of Northern India. *O. tarandus,* commonly referred to as the cow horn bug

or treehopper, is a phytophagous insect characterized by two lateral and a median horn on the pronotum. Both nymphs and adults of this species feed on tender shoots, with the mature dark brown-to-black adults measuring approximately 7 mm in length. When disturbed, they exhibit a hopping behavior, earning them the popular name "treehoppers" (Nettimi and Iyer, 2015; Prabakaran et al., 2017). Notably, *O. tarandus* showcases diverse behavioural and life history traits, including subsociality, ant mutualism, host-plant specialization, and plant-borne vibrational communication (Wood, 1993). They exhibit diversity in behavioural and life history traits including maternal care (subsociality), ant mutualism, host-plant specialization and plant-borne vibrational communication (Wood, 1993). In this study we regularly observed all the parts of the tree especially aerial prop roots for any pest infestation or disease symptoms for two years from 2022–2023 in forest areas of National capital region, New Delhi (Mon et al., 2020). The infestation of *O. tarandus* on *F. benghalensis* represents a novel threat to one of India's most cherished tree species. This study's significance lies in its comprehensive investigation into the morphological and molecular identification of *O. tarandus*, as well as its life cycle, ecological interactions, and potential impact on banyan trees (Chakraborty et al., 2022). Furthermore, the study's focus on the forests of New Delhi underscores the broader implications for forest management and conservation efforts across India (Logesh et al., 2023). The objective of the study was to comprehensively investigate the infestation of *O. tarandus* on *F. benghalensis* in the forests of New Delhi, with a specific focus on morphological and molecular identification of the pest, its life cycle, ecological interactions, and potential impact on banyan trees. This investigation aimed to provide a deeper understanding of the threat posed by *O. tarandus* to one of India's most cherished tree species, facilitating the development of effective management and conservation strategies.

2. Materials and Methods

A systematic approach was conducted during twenty months in 2022–2023 in Pusa Hill Forest (28.6295°N, 77.107°E), Alipur City Forest (28.7938°N, 77.1307°E), Jahanpanah City Forest (28.5267°N, 77.2439°E),. The situation demanded a comprehensive strategy to address the challenges posed by the infestation. Firstly, a thorough survey of the affected regions was conducted to assess the extent of the tree hopper infestation and to identify specific areas of concern. Ten *F. benghalensis* plants were randomly selected from the single forest for insect collection. The gathered insects were carefully placed into containers with the intention of subsequently identifying the species. To ensure accurate identification, the collected specimens were meticulously compared against established reference samples and relevant literature (Ikram et al., 2023). Samples of the tree hoppers were meticulously collected and carefully preserved in a solution of 70% ethanol, maintaining a stable temperature of 20°C until DNA extraction. The DNA extraction process was executed using a modified version of the CTAB method. The extracted DNA underwent evaluation through electrophoresis on a 0.8% agarose gel infused with 0.5 g ml^{-1} of ethidium bromide. The quantified DNA samples were then subjected to further analysis via PCR. Specifically, a fragment of the MT-COI gene was selectively amplified using the universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAACTTCAGGGTGACCAAAAAATCA-3'). In a reaction mixture of 25 µl, consisting of 12.5 µl of PCR master mix (Promega M750A), 7.5 µl of nuclease-free water, 1 µl each of forward and reverse primers, and 3 µl of the DNA template, PCR amplification was meticulously carried out Gouda et al., 2024a. Subsequently, a portion (3 µl) of the PCR-amplified product was subjected to electrophoresis at 100 volts for a duration of 45 minutes on a 1.2% agarose gel in 1X TAE buffer. To ensure a thorough analysis, the purification and sequencing of the amplified PCR products were outsourced. Subsequently, a BLAST analysis was performed, utilising the National Centre for Biotechnology Information (NCBI) as a valuable resource for the identification of homologous sequences (http://ncbi.nlm.nih.gov/BLAST). The resultant sequence was submitted to the NCBI GenBank to obtain the relevant accession numbers. For the purpose of conducting homology searches, multiple alignments were conducted using the Clustal W algorithm software. Furthermore, to enhance our understanding and visualise relationships, dendrograms were generated using the MEGA11 software (Gouda et al., 2024b). Reference strain sequences, pivotal for contextualising our findings, were meticulously obtained from GenBank. This meticulous methodology was put in place to ensure the reliability and validity of the results obtained from the current study.

3. Results and Discussion

The identification process of the cow horn bug (*O. tarandus*) involved a meticulous examination of both its physical characteristics and genetic makeup. Morphological features, such as its size (4–7 mm), distinctive yellow eyes, wings, legs, and thoracic horns resembling those of a miniature cow, provided initial clues to its identity (Sharma and Pati, 2011). These features were crucial for distinguishing *O. tarandus* from other insect species and served as the foundation for further investigation (Figure 1).

To confirm the species' identity with greater precision, advanced molecular techniques were employed. The NCBI BLAST algorithm was utilized to analyze genetic sequences

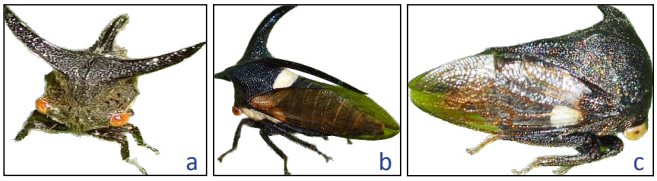

Figure 1: *Oxyrachis tarandus,* a) anterior view of horns b) lateral view of adult with extended protrusion c) nymph

obtained from specimens, resulting in an impressive 99.13% similarity match to known *O. tarandus* sequences, as evidenced by the accession number OR739577 (Figure 2). This molecular confirmation added a layer of certainty to the identification process, bolstering the accuracy of the findings.

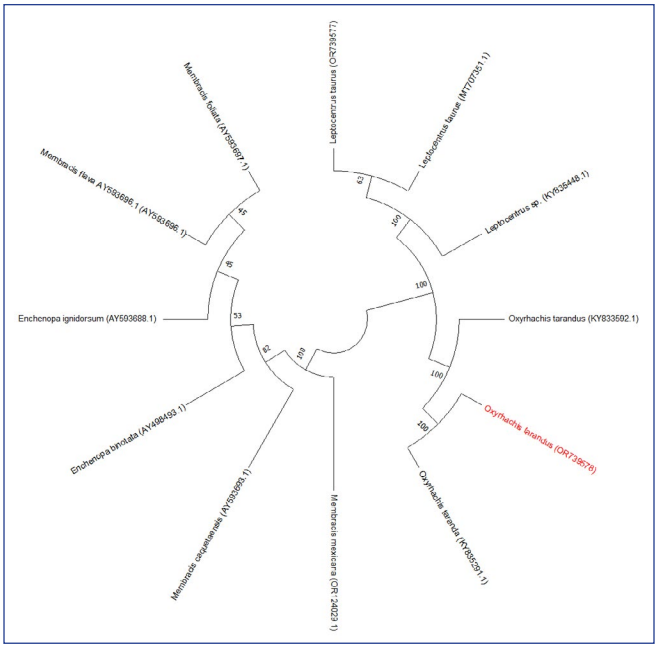

Figure 2: Molecular identifications of tree hopper. Red colour indicates sequences generated in this study

Understanding the ecological implications of *O. tarandus* is paramount due to its significant impact on various host plants. Research conducted by Garg (2015) shed light on the insect's association with a wide range of host plants, spanning multiple plant families. Among these, the Fabaceae family emerged as the most affected, underscoring the bug's broad host range and potential economic consequences.

The reproductive behavior of *O. tarandus* involves intricate interactions with its environment. Female bugs selectively choose oviposition sites on young plant tissues, where they create characteristic V-shaped slits to deposit clusters of eggs. These eggs are then protected within plant tissue, shielded by a white secretion and defended by female bugs, often with the assistance of mutualistic ants. This mutualistic relationship highlights the complexity of *O. tarandus'* ecological interactions and underscores the interdependence between different species within ecosystems.

The insect's life cycle is intricately linked with the phenology of its host plants. Nymphal stages aggregate on vigorously growing plant modules, where they feed on sap. The developmental process progresses through five distinct stages, culminating in adulthood within 2 to 2.5 months under optimal conditions. Interestingly, adult bugs may enter diapause during environmental stress, demonstrating their ability to adapt to changing conditions (Price and Carr, 2000).

The symbiotic relationship between *O. tarandus* and attendant ants further emphasizes the interconnectedness of species within ecosystems. Ants benefit from the sugary waste excreted by the bugs, known as honeydew, while providing protection against predators and parasites. This mutualistic arrangement has been observed in various ecological contexts and underscores the importance of understanding species interactions in ecological studies (Stachowicz, 2001).

The focus on the banyan tree (*F. benghalensis*) in New Delhi, India, revealed previously undocumented host associations for *O. tarandus*, expanding our knowledge of its ecological niche. This observation underscores the importance of continuous research and monitoring to better understand species distributions and ecological dynamics (Figure 3).

Figure 3: Infestation Oxyrachis tarandus on *Ficus benghalensis*. Clearly showing picture of adult, nymph and ants on aerial prop roots of banyan tree

Damage inflicted *by O. tarandus*, primarily through feeding on apical stem parts and aerial prop roots, can have significant economic implications, including reduced plant growth and vigor. Effective integrated pest management strategies are essential for mitigating these impacts and ensuring the preservation of valuable tree species like the banyan tree (*F. benghalensis*), which holds cultural and ecological significance as the national tree of India.

In conclusion, the study of *O. tarandus* provides valuable insights into species interactions, ecological dynamics, and the importance of conservation efforts in maintaining biodiversity and ecosystem health. By combining morphological,

molecular, and ecological approaches, researchers can deepen our understanding of species biology and contribute to the development of sustainable management strategies for pest control and conservation.

4. Conclusion

The information of *O. tarandus* infestation on *F. benghalensis* in the surveyed forests of New Delhi underscored the importance of monitoring and understanding the ecology of this treehopper species. Accurate identification through both morphological and molecular methods, coupled with insights into its life cycle and ecological interactions, provided a foundation for developing effective pest management strategies. Given the economic and ecological significance of the banyan tree, safeguarding it from *O. tarandus* became crucial for preserving India's national tree and maintaining the overall health of forest ecosystems.

5. Acknowledgment

We personally thanks to those helped us in performing survey during our research period

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