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Report on an accidental termite infestation on lemongrass (*Cymbopogon citratus*)

MN Rudra Gouda[1]; JS Pavan[1]; G Pradeep Raja[1]; M Komal Priya[2]; Ashwini LMB[3]; Yogananda M[4]*

[1]Division of Entomology, Indian Agricultural Research Institute, New Delhi- 110012, India.

[2]Division of Entomology, Indian Agricultural Research Institute, Jharkhand- 825405, India.

[3]Division of genetic and plant breeding, University of agricultural sciences, Dharwad-580005, India.

*[4]*Division of vegetable sciences, Indian Agricultural Research Institute, New Delhi- 110012, India.*

***Corresponding Author(s): Yogananda M**

Division of vegetable sciences, Indian Agricultural Research Institute, New Delhi- 110012, India

Email: yogananda.m95@gmail.com

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Abstract

Objective: The research aimed to examine the infestation of insects on lemongrass (*Cymbopogon citratus*) plants in Ranchi, Jharkhand. The study focused on providing detailed insights into the taxonomy and behavior of termite populations that infest lemongrass

Methods: Field surveys were conducted to identify termite-infested lemongrass plants, followed by insect collection and morphological identification of the termites. Molecular techniques, including DNA barcoding, were employed for species identification. Samples of the infesting termites were collected and preserved for DNA extraction, followed by PCR amplification of the mtCOI gene fragment and subsequent sequencing. BLAST analysis and phylogenetic reconstruction were performed to elucidate genetic relationships among termite populations infesting lemongrass.

Results: Field surveys revealed termite infestation on lemongrass plants in multiple locations within the study site. Morphological examination and molecular analysis identified the infesting termites primarily as belonging to the genus *Odontotermes*. The mtCOI gene fragment sequencing and phylogenetic reconstruction provided insights into the genetic diversity and relationships among termite populations infesting lemongrass.

Conclusion: The study confirmed termite infestation on lemongrass plants, primarily by *Odontotermes* species, highlighting the adaptability of termites to multifunctional plants. Despite being the documented report of termite infestation on lemongrass, the findings underscored the necessity for further investigation into natural insecticidal properties for effective pest management strategies. This research contributes to understanding termite ecology and emphasizes the importance of integrated pest management approaches in agricultural contexts.

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Introduction

Termites are highly adaptable pests known for their destructive impact on a wide range of agricultural, horticultural, agroforestry, and plantation crops [1,2]. They are ubiquitous, found across tropical, subtropical, and temperate regions, and are particularly problematic in areas with water stress conditions, posing a serious threat to various crops [3]. Termites exhibit diverse life styles, with species ranging from small colonies to highly organized societies with complex nest systems. Depending on their habits and habitats, termites can be classified into wood dwellers and ground dwellers. Wood dwellers inhabit both damp and dry woods, while ground dwellers include subterranean species and mound builders [4].

Economically significant mound-building species include *Odontotermes obesus* (Rambur), *O. redemanni* (Wasmann), and *O. wallonensis* (Wasmann), while major subterranean species include *Heterotermes indicola* (Wasmann), *Coptotermes ceylonicus* (Vietnam), *C. heimi* (Wasmann), *Odontotermes homi* (Wasmann), *Microtermes obesi* Holmgren, *Trinervitermes biformis* (Wasmann), and *Microcerotermes beesoni* Snyder [5].

Odontotermes obesus is particularly destructive, targeting developing and maturing stages of various crops [6]. The majority of termite species belong to the family Termitidae, accounting for about 70% of all species [7]. While termites play a crucial role in recycling woody and plant material, they become economic pests when they begin attacking crops [8]. Termites typically prefer dead plant material, but they can shift to living plants when their preferred food sources are depleted. They tunnel through plant stems and consume cellulose-rich roots, leading to stunted growth and weakened plants that are easily uprooted from the soil [9]. Lemongrass, *Cymbopogon citratus* stands out as a multifunctional plant with culinary, medicinal, and insecticidal properties. Essential oils extracted from lemongrass contain bioactive compounds, including terpenes like citral, known for their insect-repellent and insecticidal effects. The insecticidal properties of lemongrass essential oil have been extensively studied and exploited for pest management in various agricultural contexts. However, despite its reputation as a natural insect repellent, there have been reports of termites infesting lemongrass plants under certain conditions. This unexpected phenomenon raises questions about the efficacy of lemongrass essential oil as a deterrent against termite infestation and highlights the need for further investigation into the interaction between termites and lemongrass.

Materials and Methods

Study Site: The study was conducted in Ranchi, Jharkhand, where lemongrass (*Cymbopogon citratus*) is commonly cultivated.

Collection of Samples: Lemongrass plants showing signs of termite infestation were identified and sampled from multiple locations within the study site.

Identification of Termites: Termites found infesting the lemongrass plants were collected for identification. Morphological characteristics such as body shape, size, coloration, and antennal features were observed under a stereomicroscope. Additionally, molecular techniques such as DNA barcoding may have been employed for accurate species identification. In response to the termite infestation in various farmers field from Ranchi, a systematic approach was initiated. 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 termite infestation and to identify specific areas of concern. Ten *C. citratus* plants were randomly selected from the single farm 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 [10]. For the purpose of molecular identification, samples of the termites were meticulously collected and carefully preserved in a solution of 70% ethanol, maintaining a stable temperature of 20 ºC 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 of ethidium bromide. The quantified DNA samples were then subjected to further analysis via PCR. Specifically, a fragment of the mtCOI 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 [11]. 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. 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.

Results and Discussion

The comprehensive study conducted in farm field of Ranchi, Jharkhand aimed to investigate the occurrence of termite infestation on lemongrass (*Cymbopogon citratus*) plants and assess the effectiveness of lemongrass essential oil as a natural insecticide against termites. The research utilized a systematic approach combining field surveys, insect collection, morphological identification and molecular techniques to address the research objectives.

Field surveys revealed termite infestation on lemongrass plants in multiple locations within the study site. Morphological characteristics of the collected termites were observed under a stereomicroscope, providing initial insights into their taxonomy and behavior. Molecular techniques, including DNA barcoding, were employed for accurate species identification. The mtCOI gene fragment was selectively amplified and sequenced, followed by BLAST analysis and comparison with reference sequences in the NCBI GenBank. The termite species infesting lemongrass plants were identified as primarily belonging to the genus *Odontotermes*, with multiple economically significant

species identified, consistent with previous literature [10].

Subsequent molecular analysis and phylogenetic reconstruction using dendrograms provided further insights into the genetic diversity and relationships among the termite populations infesting lemongrass (Figure 1).

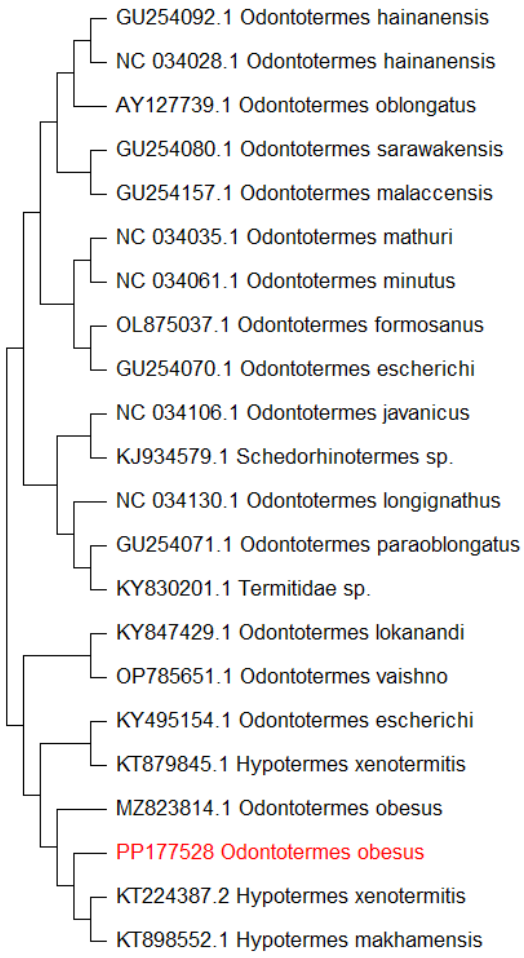

Figure 1: Molecular identifications of *Odontotermes obesus.*

Figure 2: Infestation of termite *Odontotermes obesus* on lemon grass.

Damage on grass caused by termites typically presents as thinning and yellowing of the turf, often accompanied by wilting and stunted growth due to root consumption. Infested areas may display patchiness or irregular growth patterns, with the soil feeling hollow or spongy underfoot as termites tunnel through it (Figure 2). Visible termite activity, such as mud tubes or crawling insects, may be observed, while affected grass may fail to respond to watering efforts, remaining dry and parched. Additionally, increased lemon grass growth in damaged areas further indicates compromised grass health. Early detection and intervention are vital to mitigate termite damage and preserve the vitality and appearance of the crop.

Conclusions

In conclusion, the study condcted in Ranchi, Jharkhand, revealed termite infestation on lemongrass (*Cymbopogon citratus*) plants, primarily by *Odontotermes* species. Through meticulous field surveys, morphological examinations, and molecular analyses including DNA barcoding, the research provided comprehensive insights into the taxonomy, behavior, and genetic diversity of the infesting termites. Despite being the documented report of termite infestation on lemongrass, the findings underscored the adaptability of termites to multifunctional plants and highlighted the necessity for further investigation into natural insecticidal properties for effective pest management strategies. This study contributes significantly to understanding termite ecology and emphasizes the importance of integrated pest management approaches in agricultural contexts.

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