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Blister Beetle *Mylabris Pustulata* (Thunberg), Incidence on Ornamental *Plant Ruellia Tuberosa*: A New Report from New Delhi, India

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Abstract

Objective: This study presents a novel observation of *Mylabris pustulata*, a blister beetle species, infesting *Ruellia tuberosa* plants in the New Delhi region of India.

Methods: The identification of the insect was established using both morphological characteristics and confirmed through subsequent molecular analysis. *M. pustulata* is a widely distributed species with a known history of causing economic harm to various crops globally. While direct economic impacts on *R. tuberosa* have not been explicitly documented, this infestation raises concerns about its potential to develop into a substantial threat in the future.

Results: The period of infestation occurred between June and August, during which distinct symptoms were noted, including consumption of flower components and the premature shedding of petals and flowers. Although immediate economic losses have not been evident in relation to *R. tuberosa*, the findings underscore the necessity for comprehensive investigations to gauge the true extent of damage inflicted by *M. pustulata*. It is postulated that the beetle's adaptability to new environments, polyphagous nature, and potential alteration of cropping patterns could be contributing factors to this infestation. This report serves as a preliminary insight into the behavior of this insect and its interaction with *R. tuberosa* in the New Delhi context. The significance of continuous monitoring and the implementation of effective pest management strategies are emphasized to safeguard the cultivation of *R. tuberosa* in Northern India."

Conclusion: The occurrence of *Mylabris pustulata* infestation on *Ruellia tuberosa* in New Delhi, India, is being reported for the first time. This infestation significantly harms the plant and results in a decrease in productivity. It is crucial for farmers to be aware of this threat, prompting the need for further research to develop integrated pest management (IPM) strategies.

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Introduction

A study was executed within the confines of the National Capital Region (NCR) of New Delhi with the core objective of unravelling the intricate dynamics associated with insect pests, encompassing their spectrum of diversity, dispersal mechanisms, and patterns of invasion within the crop system. The primary goal of this comprehensive survey was to attain a profound comprehension of the fluid and ever-evolving scenarios involving insect pests within the intricate fabric of this agricultural system.

Of particular interest within this research was the Meloidae family, colloquially recognized as blister beetles and classified under the esteemed Coleoptera order. This family boasts an impressive diversity, comprising an estimated tally of 3000 species dispersed among an intricate network of 120 genera [1]. These blister beetles exhibit an innate propensity to inhabit an array of habitats and have garnered notoriety for inflicting substantial economic ramifications within the realm of major field crops [2].

Among the notable inhabitants of the blister beetle clan, *M. pustulata*, bearing the common moniker of the banded blister beetle, emerges as the most frequently encountered member. This species has demonstrated a marked affinity for an eclectic array of plant families, encompassing the likes of Amaranthaceae, Asteraceae, Fabaceae, and Solanaceae [3]. The feeding habits of this beetle have been unequivocally linked to the infliction of damage upon a multitude of essential crops, inclusive of cucurbits, okra, red gram, mungbean, cotton, and brinjal [4,5]. The beetle's feeding proclivities extend across a spectrum of plant components, encompassing buds, blossoms, nascent pods, and tender foliage [6], which collectively culminate in the deleterious consequence of diminished crop yield.

An intriguing facet that deserves attention stems from the prior observations conducted by Siddiqi [7], which unveiled the presence of adult *M. pustulata* on ornamental plants such as *Russelia equisetiformis*, *Hamelia patens*, *Caesalpinia pulcherrima, Jatrophapandurifolia*, and *Tecoma grandiflora*. This revelation imparts an added layer of complexity to the ecological role played by these insects. Notably, up until the present juncture, no documented instances of *M. pustulata* infestation on *Ruellia tuberosa* have been documented.

To bridge this knowledge gap, a meticulous and systematic survey was conducted across diverse locations ensconced within the expanses of the NCR of New Delhi. The principal aim of this survey encompassed the illumination of both the prevalence and behavioral nuances exhibited by insect pests, with a special emphasis placed upon *M. pustulata*, within the intricate backdrop of the *Ruellia tuberosa* crop ecosystem. Through the rigorous monitoring of diversity, discerning dispersal patterns, and foreseeing potential incursions orchestrated by insect pests, the survey aimed to bestow invaluable insights pivotal for the formulation of effective and pragmatic pest management strategies. The ramifications of such insights extend beyond the realm of *Ruellia tuberosa,* potentially benefiting other susceptible crops within the geographical confines of the region.

Materials and Methods

In a 100-square meter plot infested with blister beetles, a systematic approach was undertaken. Ten *Ruellia tuberosa* plants were randomly selected from the plot 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.

For the purpose of molecular identification, samples of the beetle were meticulously collected and carefully preserved in a solution of 70% ethanol, maintaining a stable temperature of 20 degrees Celsius 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 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 [8]. 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, utilizing 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 visualize relationships, dendrograms were generated using the MEGA11 software. Reference strain sequences, pivotal for contextualizing our findings, were meticulously obtained from GenBank [9]. This meticulous methodology was put in place to ensure the reliability and validity of the results obtained from the current study.

Results and discussion

During the survey conducted on July 1, 2022, in the geographical coordinates of 28.6156 latitude and 77.1984 longitude in New Delhi, India, a significant discovery was made concerning the presence of *Mylabris pustulata* on *Ruellia tuberosa*. Employing well-established morphological criteria [10], the blister beetle species responsible for the flower damage on *R. tuberosa* was accurately determined to be *M. pustulata*. The identification process relied on specific features, including the distinct presence of eleven segmented moniliform antennae **(Figure 1a)**, a triangular and deflexed head **(Figure 1b),** along with an anteriorly impunctate clypeus and a slightly sinuate transverse labrum.

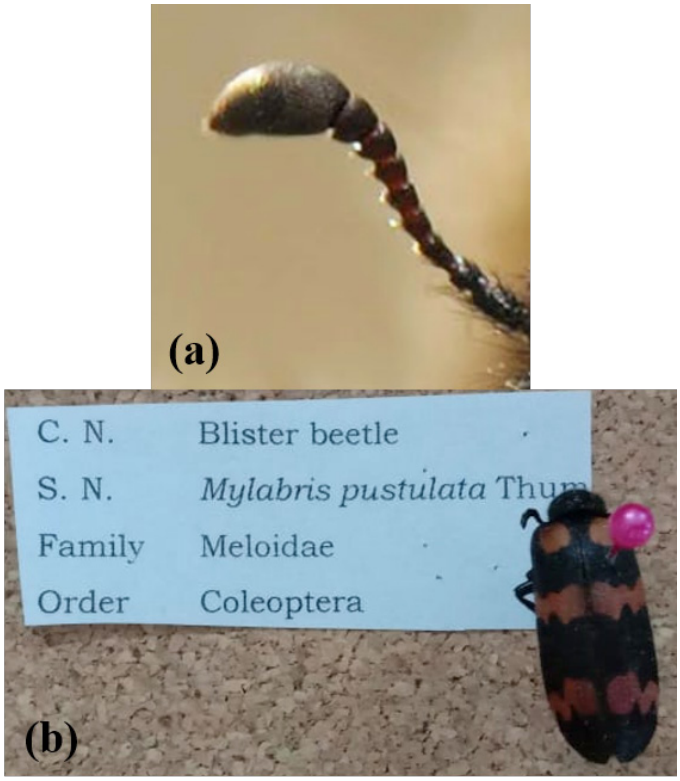

Figure 1: Image of blister beetle (*M. pustulata* Thunb.) collected from New Delhi: **a**: antenna; **b**: dorsal view.

To solidify the species identity beyond doubt, advanced molecular identification techniques were harnessed, utilizing the NCBI BLAST algorithm. The outcome of this analysis revealed an impressive 99.13% similarity match with *M. pustulata*, leading to the successful assignment of the obtained sequence with the accession number OR272191 **(Figure 2).** Subsequently, a comprehensive phenogram construction unveiled the formation of two distinct clades. Notably, OR272191.1 clustered harmoniously with the reference sequences attributed to M. pustulata, while the outgroup aligned itself alongside *M. phalerata*. This insightful molecular scrutiny further fortified the case for the accurate identification of the beetle as *M. pustulata.*

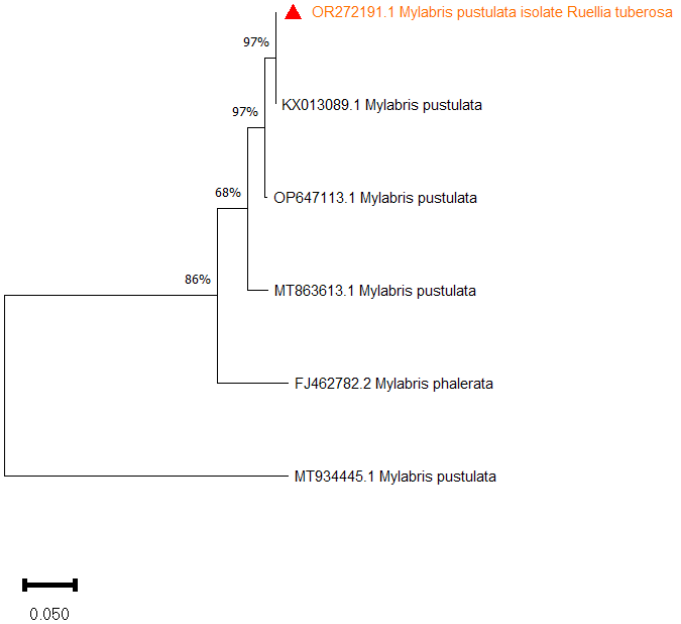

Figure 2: Molecular identifications of Mylabris pustulata.

This study marks a significant milestone as it documents the inaugural occurrence of *M. pustulata infesting R. tuberosa* crops within India's field conditions. A cosmopolitan species, *M. pustulata* thrives on both cultivated and non-cultivated plants across the expanse of India [11]. Its reputation as an agent of economic detriment spans a diverse array of crops, including red gram, cucurbits, hibiscus, bean, pea, potato, turnips, and various other vegetables and fruits [12]. Despite its notoriety for inflicting severe damage on numerous field-grown crops, no direct economic harm to the *R. tuberosa* crop has been ascertained so far.

This study stands as foundational groundwork, delivering invaluable insights into the *M. pustulata* beetle and its conspicuous infestation on *R. tuberosa* crops specifically within the geographic context of Delhi state, nestled within the northern frontiers of India. Parallel occurrences have been documented by Patra [13] and Joshi [14] from diverse hilly regions of India. The timeline of blister beetle infestation recorded in the last week of June and the first week of July aligns seamlessly with earlier findings reported by Ahad [15] and Sharma [16], who also identified blister beetle infestations during the month of June in the northern realms of India. This synchrony might be attributed to shared geographic conditions.

Notably, blister beetle adults exhibited a strong attraction to flowers, with an initial manifestation seen as petal consumption in flowers **(Figure 3a)**. Their mouthparts displayed a precise adaptation to flower and tender shoot consumption **(Figure 3b)**, resulting in remarkable damage within a brief timeframe, consistent with the feeding pattern elucidated by Raju [17]. The affliction of *R. tuberosa* crops by blister beetles may be a consequence of evolving cropping patterns, the surge in monoculture practices, the beetle's polyphagous tendencies, and its facile acclimatization to new environments. Although current instances of pest infestation appear sporadic, the potential for this pest to ascend to a significant menace in the northern sphere looms on the horizon [18].

Figure 3: Infestation of blister beetle *(M. pustulata Thunb.)*: **(a)** eating of beetle on flower of *R. tuberosa* **(b)** blister beetle feeding young shoots.

Consequently, this report serves as an initial dossier chronicling *the M. pustulata* beetle's infestation of *R. tuberosa* crops in the New Delhi territory. However, the urgency of undertaking further investigations to comprehensively gauge the extent of damage inflicted by these blister beetles cannot be understated. This knowledge is pivotal for the formulation of robust pest management strategies, ultimately safeguarding the vitality of *R. tuberosa* cultivation.

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