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Research Paper

Genetic diversity and host-specific genetic distinctness exhibited by *Bemisia tabaci* (Gennadius) populations infesting pulse crops in India

Vishal Singh[1,2], Velu Mani Selvaraj[1,3], Sujayanand GK[1], DebJyoti Sen Gupta[1], Anup Chandra[1], Aravind Kumar Konda[1] and GP Dixit[1]

[1]*ICAR-Indian Institute of Pulses Research, Kalyanpur, Kanpur - 208 024, Uttar Pradesh, India*
[2]*Rani Lakshmi Bai Central Agricultural University Jhansi - 284 003, Uttar Pradesh, India*
[3]*Chhatrapati Shahu Ji Maharaj University, Kalyanpur, Kanpur - 208 024, Uttar Pradesh, India*

***Corresponding author email:** aravindkumark.ars@gmail.com*

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ABSTRACT

The present study investigates the genetic variation in populations of whitefly (*B. tabaci*) infesting green gram and black gram across various agro-climatic zones of India. Phylogenetic analyses identified three distinct genetic groups: Asia I, Asia II-1 and Asia II-8 with the sequence analysis of specifically a 658 bp region of the mitochondrial cytochrome oxidase I (mtCOI) gene. Black gram populations were exclusively associated with the Asia II-1 group, suggesting host-specific adaptations, while green gram populations exhibited greater genetic diversity spanning all three groups. These findings highlight potential drivers of genetic differentiation, including environmental factors, cropping practices, and agro-ecological conditions. Region-specific associations between genetic groups and agro-climatic zones further underscore the adaptability of *B. tabaci* populations. The results have significant implications for pest management strategies, emphasizing the need for targeted interventions tailored to specific genetic groups and crops. By linking genetic diversity to host preferences and environmental factors, the study contributes critical insights for sustainable whitefly management in pulse crops.

***Key words*:** Black gram, Green gram, mtCOI gene, Phylogenetic analysis, Whitefly

INTRODUCTION

Bemisia tabaci (Gennadius), a highly polyphagous and globally invasive whitefly species, infests over 600 plant hosts (Mound and Halsey 1978, Secker *et al*. 1998) and is recognized by the International Union for Conservation of Nature and Natural Resources (IUCN) as one of the world's most invasive alien species. In addition to direct damage caused by sap-feeding on the abaxial leaf surface, *B. tabaci* serves as a vector for over 300 economically significant plant viruses, severely impairing plant growth and yield (Navas-Castillo *et al*. 2011, Ghosh *et al*. 2015, Mugerwa *et al*. 2021, Polston *et al*. 2014). Among crops, pulses such as green gram and black gram host a diverse range of insect-pests (Bhargav *et al*. 2024, Gupta *et al*. 2022) and are particularly vulnerable, with yield losses attributed to Yellow Mosaic Disease a viral infection transmitted by *B. tabaci* ranging from 5% to 85% (Ambika *et al*. 2022, Heu *et al*. 2020). Genotypic screening efforts have highlighted natural resistance against YMD and its vector in select mungbean cultivars (Sujayanand *et al*. 2021), reaffirming the need for region-specific host-pest profiling. Despite the critical role of pulse crops in sustainable agriculture, including their nutritional value and contributions to soil fertility through nitrogen fixation, their productivity is severely threatened by *B. tabaci* infestations. Current control strategies heavily rely on chemical insecticides, leading to environmental concerns, health risks and the emergence of resistant whitefly biotypes (Palumbo *et al*. 2001).

B. tabaci is considered a species complex exhibiting little morphological differences but revealing distinct genetic differences, environmental distinctness, and host specificity. The biological and genetic variability within *B. tabaci* further complicates management efforts, as biotypes exhibit considerable differences in host preference, insecticide resistance, pathogen transmission and reproductive capacity (Bedford *et al*. 1994, Costa and Brown 1991, De Barro *et al*. 2000). Therefore, accurate genotype identification is essential for the development of sustainable and effective whitefly management strategies. Advances in molecular techniques, such as mitochondrial cytochrome oxidase I (mtCOI) gene sequencing, have enabled the classification of 46 cryptic species worldwide,

with 13 of them reported in India, i.e., Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-6, Asia II-7, Asia II-8, Asia II-11, Asia II-13, China 3, China 7, MEAM-1 and MEAM-K (Ellango *et al*. 2015, Kanakala and Ghanim, 2019, Ramesh *et al*. 2025, Rehman *et al*. 2021, Roopa *et al*. 2015). Compared to green gram, black gram is more preferred by whitefly (Chandra *et al.* 2021). But the genetic diversity of *B. tabaci* populations infesting key pulse crops like green gram and black gram remains underexplored. The present study investigates the genetic diversity of *B. tabaci* populations associated with green gram and black gram across diverse agro-climatic regions in India, addressing a critical knowledge gap in pest management and sustainable agriculture.

MATERIALS AND METHODS

Sample collection

Adult *B.tabaci* individuals were collected using an aspirator during early morning hours from green gram and black gram fields at 10 different locations spread across various agro-climatic zones of India. Each sample was stored in a 1.5 ml microcentrifuge tube containing 70% ethanol, and details such as latitude, longitude, location name, agro-climatic zone, and date of collection were recorded. In total,13 samples were obtained: one from the Trans-Gangetic Plains Region, two from the Central Plateau and Hills Region, three from the Middle Gangetic Plains Region, one from the East Coast Plains and Hills Region, one from the Eastern Himalayan Region, one from the Western Himalayan Region, two from the Eastern Plateau and Hills Region, and two from the Southern Plateau and Hills Region. Samples were stored at –20 °C freezer until DNA extraction.

Genomic DNA extraction and PCR amplification

Two adult whiteflies were randomly selected from each population for genomic DNA extraction. Individual whiteflies were briefly desiccated on clean paper towels and then homogenized using an autoclaved micropestle and liquid nitrogen in a 1.5 ml microcentrifuge tube. Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. DNA concentrations were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific), with absorbance values recorded at 260 nm and 280 nm.

PCR amplification of the mt COI barcode region (~850 bp) was performed using the isolated DNA. Annealing temperature parameters were optimized via gradient PCR. The 20 µL PCR reaction mixture consisted of 2 µL 10X *Taq* buffer (Genei™), 1.5 µL each of forward (2 pmol/µL) and reverse primers (2 pmol/µL), 1.5 µL dNTPs (10 mM; Genei™), 0.5 µL *Taq* Polymerase (3 U/µL; Genei™), 6 µL $MgCl_2$ (10 mM), 5 µL sterile water, and 2 µL DNA template. Amplification was carried out using primers C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and L2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Frohlich *et al*. 1999) in a Veriti Pro 96-well thermocycler (Thermo Fisher Scientific). Thermal cycling conditions included initial denaturation performed for 4-5 min at 94°C, subsequently at 94°C for 30 sec, 48°C for 45 sec, and 72°C for 1 min for 40 cycles, with a final extension at 72 °C for 10 min.

Visualization and purification of PCR products

The PCR products were visualized using 1.2% agarose gel electrophoresis in 1X TAE buffer stained with ethidium bromide (EtBr). Visualization was performed using a gel documentation system (Cleaver Scientific Limited, U.K.). The amplified products were excised from the gel and purified as per the manufacturer's instructions mentioned in the Favor Prep GEL/PCR purification mini kit.

COI Sequence generation

PCR products from positive samples, as determined by bulk screening, were subjected to Sanger sequencing. PCR primers served as sequencing primers to obtain both forward and reverse sequence reads. Chromatograms were manually reviewed, and sequence ends with low-quality base call scores were trimmed. Each sequence was carefully examined for base call accuracy along its entire length. Forward and reverse sequence reads were aligned to identify and resolve any discrepancies. Final sequences were validated through nucleotide BLAST searches against reference *B. tabaci* cytochrome oxidase I sequences to identify any residual inaccuracies.

Phylogenetic tree construction

COI sequences of *B. tabaci* (Kanakala and Ghanim 2019) were retrieved from the Gen Bank database (www.ncbi.nlm.nih.gov/) for phylogenetic analysis. Preliminary analysis indicated that the collected samples corresponded to genotypes from the Asia region. Two phylogenetic trees were constructed: one focused exclusively on Asian genotypes, and the other included genotypes from

Table 1. Locations selected in different agro-climatic zones for collection of adult whiteflies from green gram and black gram crops

S. No.	Zone	Location	Latitude	Longitude	Crop	Sample name	Sample Code
1.	Central Zone (CZ)	Jhansi (Uttar Pradesh)	25.516° N	78.557° E	Greengram	Jhansi_A1_greengram_*B. tabaci*	A1
					Blackgram	Jhansi_B1_blackgram_*B. tabaci*	B1
						Jhansi_B2_blackgram_*B. tabaci*	B2
2.		Raipur (Chhattisgarh)	22.500° N	81.645° E	Greengram	Raipur_I1_greengram_*B. tabaci*	I1
					Blackgram	Raipur_J1_blackgram_*B. tabaci*	J1
3.	North West Plain Zone (NWPZ)	New Delhi	28.641° N	77.164° E	Greengram	Delhi_F1_greengram_*B. tabaci*	F1
						Delhi_F2_greengram_*B. tabaci*	F2
4.	North Eastern Plain Zone (NEPZ)	Kanpur (Uttar Pradesh)	26.492° N	80.277° E	Greengram	Kanpur_C1_greengram_*B. tabaci*	C1
						Kanpur_C2_greengram_*B. tabaci*	C2
					Blackgram	Kanpur_D2_blackgram_*B. tabaci*	D1
5.		Varanasi (Uttar Pradesh)	25.182° N	82.876° E	Greengram	Varanasi_H2_greengram_*B. tabaci*	H2
6.		Khurda (Odisha)	20.188° N	85.626° E	Greengram	Khurda_G1_greengram_*B. tabaci*	G1
7.	South Zone (SZ)	Dharwad (Karnataka)	15.799° N	74.898° E	Greengram	Dharwad_K2_greengram_*B. tabaci*	K2
8		Bengaluru (Karnataka)	12.971° N	77.594° E	Green gram	Bengaluru _M1_ greengram_*B. tabaci*	M1
						Bengaluru _M2_ greengram_*B. tabaci*	M2
9		Pantnagar (Uttarakhand)	29.022° N	79.490° E	Green gram	Pantnagar_E1_ greengram_*B. tabaci*	E1
10		Umiam (Meghalaya)	25.664° N	91.902° E	Green gram	Umiam_L1_ greengram_*B. tabaci*	L1

global distributions. Phylogenetic tree construction was performed using the MEGA X software package (Kumar *et al*. 2018, Stecher *et al*. 2020). Sequences were aligned using the "codon align" option in MUSCLE (Edgar, 2004) with default parameters. The neighbor-joining method (Saitou and Nei 1987) was employed for tree construction, incorporating 1,000 bootstrap replications (Felsenstein 1985) to ensure reliability maximum composite likelihood method was employed to determine evolutionary distances (Tamura *et al*. 2004). The positions that were found to be ambiguous were excluded from analyses using the pairwise deletion approach.

RESULTS AND DISCUSSION

Genetic diversity of whitefly (B. tabaci) infesting green gram and black gram

Genomic DNA was successfully extracted from all collected samples, and the mt COI partial sequence (~850 bp) was amplified using PCR (Figure 1). Sequencing was performed in a bidirectional manner. Among the 20 whitefly samples, two samples collected from the Dharwad region (green gram) exhibited identical sequences in the mtCOI region. Therefore, only one sample (K2) was selected for subsequent analyses.

Phylogenetic analysis of B. tabaci samples

The COI sequences of 19 samples analysed in conjunction with *B. tabaci* sequences from global databases revealed monophyletic relationships with the Asia genetic groups (Supplementary Figure 1). To enhance clarity, phylogenetic analysis was subsequently performed with a representative subset of sequences from the Asia genetic sub-groups (Figure 2). The genetic grouping of our samples aligned with the results obtained from the global dataset analysis.

The analysed samples were categorized into three major genetic groups: Asia I, Asia II-1, and Asia II-8. Whitefly samples collected from black gram belonged exclusively to the Asia II-1 genetic group. In contrast, samples collected from green gram spanned Asia I, Asia II-1, and Asia II-8 genetic groups.

Whitefly samples infesting black gram originated from three agro-climatic zones in India (Table 2) and consistently belonged to the Asia II-1 genetic group. Sampling of whitefly populations from green gram encompassed eight agro-climatic zones, corresponding to three distinct genetic groups. Notably, green gram samples from the Middle Gangetic Plains Region (Zone 4) and Southern Plateau and Hills Region (Zone 10) were

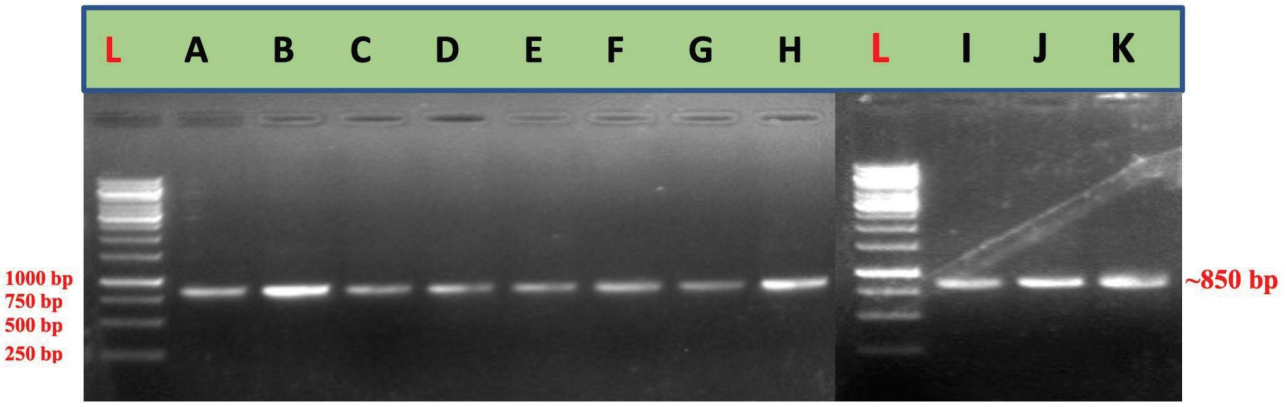

Fig. 1. mtCOI amplicon (~850 bp) from different samples electrophoresed on 1% agarose gel.

L= GeneRuler 1kb DNA Ladder, A-K= Sample name code

distributed across two genetic groups, Asia I and Asia II-8, respectively.

Whitefly samples collected from black gram were exclusively affiliated with the Asia II-1 genetic group, suggesting a narrower genetic diversity and potential host preference for this crop. This aligns with field evaluations that identified distinct whitefly-host associations and varietal susceptibility patterns in black gram under natural conditions (Kumar *et al*. 2020). This might also be due to the highest prevalence of Asia II-1 in India (Ramesh *et al*. 2025). In contrast, green gram samples spanned three genetic groups, including Asia I, Asia II-1, andAsia II-8, indicating broader genetic diversity and adaptation to varying agro-climatic conditions. This disparity highlights the possibility of differential host plant preferences and genetic adaptation among whitefly populations, which may be driven by environmental factors, cropping practices, and local agro-ecological conditions.

The phylogenetic analysis further demonstrated monophyletic relationships between the collected samples and genetic groups from Asia, confirming the genetic coherence of *B. tabaci* within the region. Interestingly, while previous studies have reported significant genetic variability in *B. tabaci* populations worldwide, this study contributes region-specific insights by linking genetic groups to

Table 2. Genetic grouping of *B. tabaci* samples collected from green gram and black gram, along with corresponding NCBI accession numbers and Indian agro-climatic zones.

Genetic Group	Sample ID	NCBI Accession No.	Indian Agro-climatic Zones
Asia II-1	Delhi_F1_G	PQ814769	Trans-Gangetic Plains Region (Zone 6)
	Delhi_F2_G	PQ814770	
	Jhansi_A1_G	PQ814762	Central Plateau and Hills Region (Zone 8)
	Jhansi_B1_B	PQ814763	
	Jhansi_B2_B	PQ814764	
	Kanpur_C1_G	PQ814765	Middle Gangetic Plains Region (Zone 4)
	Kanpur_C2_G	PQ814766	
	Kanpur_D2_B	PQ814767	
	Khordha_G1_G	PQ814771	East Coast Plains and Hills Region (Zone 11)
	Meghalaya_L1_G	PQ814778	Eastern Himalayan Region (Zone 2)
	Pantnagar_E1_G	PQ814768	Western Himalayan Region (Zone 1)
	Raipur_I1_G	PQ814773	Eastern Plateau and Hills Region (Zone 7)
	Raipur_I2_G	PQ814774	
	Raipur_J1_B	PQ814775	
	Raipur_J2_B	PQ814776	
Asia II-8	Dharwad_K2_G	PQ814777	Southern Plateau and Hills Region (Zone 10)
Asia I	Bengaluru_M1_G	PQ814779	Southern Plateau and Hills Region (Zone 10)
	Bengaluru_M2_G	PQ814780	
	Varanasi_H2_G	PQ814772	Middle Gangetic Plains Region (Zone 4)

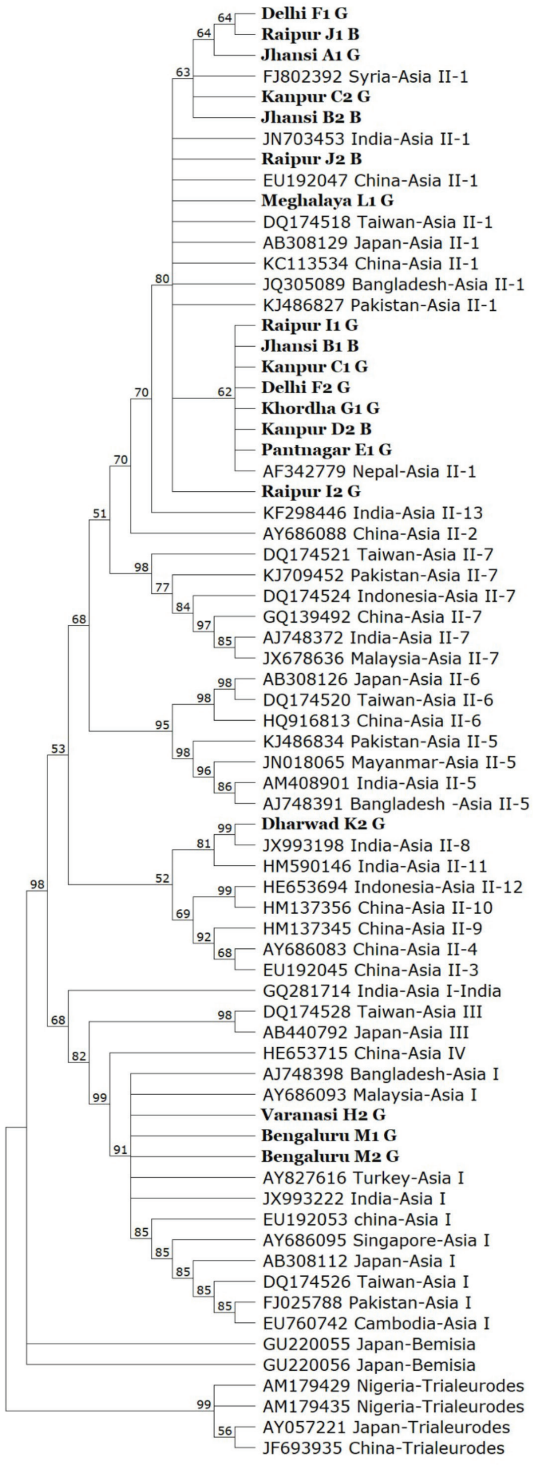

Fig. 2. Neighbor-joining condensed tree of mtCO1 gene sequences of whitefly (*B. tabaci*) with reference samples from Asia group of genotypes showing the grouping of our samples. *Trialeurodes vaporariorum* was used as outgroup in the analysis. Bootstrap values above 50% are represented at the interior nodes. Samples from this study are highlighted in bold letters.

distinct agro-climatic zones in India. For instance, samples from the Southern Plateau and Hills Region (Zone 10) showed affiliations with both Asia I and Asia II-8 groups, suggesting the coexistence of genetically distinct populations within the same region. Our study also identified the Asia I group in samples from Bengaluru, where Ellango et al. (2015) reported the invasive MEAM 1 group in cabbage and cauliflower. The absence of MEAM 1 in green gram raises compelling questions about ecological barriers, host specialization, or other factors influencing its distribution over time.

Comparative studies (Ellango *et al*. 2015, Naga *et al*. 2021, Tamilnayagan *et al*. 2019) have documented the presence of other genetic groups of *B. tabaci* in overlapping regions. However, these groups were absent from the samples in this study, reinforcing the possibility of host-specific adaptations within the *B. tabaci* species complex. These findings underscore the need for further studies to elucidate the ecological and biological mechanisms driving genetic differentiation.

The practical implications of these findings are significant. The genetic variability and host-specific adaptation observed in *B. tabaci* suggest that tailored control measures targeting specific genetic groups may enhance pest management efficacy.

Table 3. Compilation of some *B. tabaci*cryptic species reported from different Agro-climatic zones of India. Agro-climatic zones represented in the present study are emboldened.

Genotype	Indian Agro-climatic Zones
Asia 1	**Trans-Gangetic Plains Region, Middle Gangetic Plains Region,** Lower Gangetic Plains Region, **Central Plateau and Hills Region, East Coast Plains and Hills Region,** and **Southern Plateau and Hills Region**
Asia II-1	Western Himalayan Region, **Trans-Gangetic Plains Region, Middle Gangetic Plains Region,** Gujarat Plains & Hills Region, West Coast Plains and Ghats Region, **Southern Plateau and Hills Region,** and **Central Plateau** and **Hills Region**
Asia II-5	Upper Gangetic Plains Region, Lower Gangetic Plains Region, **Trans-Gangetic Plains Region,** Western Plateau and Hills Region, and West Coast Plains and Ghats Region
Asia II-6	**Southern Plateau and Hills Region**
Asia II-7	**Trans-Gangetic Plains Region,** and West Coast Plains and Ghats Region
Asia II-8	**Southern Plateau and Hills Region,** and West Coast Plains and Ghats Region
Asia II-11	**Southern Plateau and Hills Region**
China 3	Lower Gangetic Plains Region, and **Trans-Gangetic Plains Region**
MEAM1	**Trans-Gangetic Plains Region,** and **Southern Plateau and Hills Region**

The association of black gram populations solely with the Asia II-1 group suggests that interventions specifically addressing this genetic group could mitigate infestations. Meanwhile, the presence of multiple genetic groups in green gram necessitates broader control strategies that account for the genetic diversity of whitefly populations.

The current study also underscores the value of molecular tools, such as *mtCOI* sequencing, in pest monitoring and management. These tools enable precise genotype identification, which could guide region-specific pest control efforts. Future research should delve deeper into the functional implications of genetic variations, such as differences in insecticide resistance, pathogen transmission, and reproductive success among genetic groups. Moreover, environmental factors shaping *B. tabaci* genetic diversity merit further investigation in different *Vigna* species.

Altogether, the present study provides a comprehensive account of *B. tabaci* genetic diversity in green gram and black gram crops in India, offering crucial insights for pest management strategies.Understanding the genetic variationin the populations of *B. tabaci* can inform the development of pest-resistant crop varieties and tailored control measures. Additionally, exploring the transmission dynamics and insecticide resistance profiles of these genetic groups can contribute to more effective disease control. Nonetheless, the study has limitations, notably the relatively small sample size and restricted geographic and host plant scope. Expanding the sampling to encompass more agro-climatic zones in different crop seasons and host crops in future studies will provide a comprehensive understanding of *B. tabaci*'s adaptability and impact.

In conclusion, the observed genetic diversity among *B. tabaci* populations highlights the adaptability of the pest to different host plants and environmental conditions. The linkage of genetic groups to agro-climatic zones opens avenues for region-specific interventions, enhancing the efficiency of pest control measures and supporting the productivity of green gram and black gram crops.Such variability is further influenced by seasonal incidence patterns and cropping practices, as documented in summer mungbean pest dynamics (Singh *et al*. 2022), underscoring the complexity of ecological drivers.

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Authors contributions

AKK, AC and GPD had conceived and designed the research. DBS and SGK aided in the sample collection. VS and VMS had performed the experiments and the data analysis. VS and VMS wrote the main manuscript text and AKK revised the manuscript. All authors reviewed the manuscript.

Compliance with ethical standards

Not Applicable

Conflict of interest

The authors declare that they have no conflicts of interest.

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