Molecular Insights and Seasonal Dynamics of the Pink Mealybug, *Maconellicoccus hirsutus* on custard apple (*Annona squamosa* L.)

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ABSTRACT: The pink mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a highly polyphagous pest that induces leaf curling, fruit malformation, honeydew deposition and significant yield losses in custard apple (*Annona squamosa* L.). Accurate identification of *M. hirsutus* is often difficult because of its pronounced morphological variability, thereby necessitating molecular approaches as a more reliable alternative. In this study, molecular characterization of *M. hirsutus* population infesting custard apple was conducted. High-quality genomic DNA was extracted and the *mitochondrial cytochrome oxidase I* (COI) gene was sequenced. BLAST analysis revealed 100% similarity with sequences available in GenBank and phylogenetic analysis clustered the Vijayapura isolate with other Indian populations, indicating limited genetic divergence. Population monitoring indicated peak densities of 71.28 individuals per fruit during the 47th Standard Meteorological Week (SMW) of 2023 (fourth week of November) and 68.32 individuals per fruit during the 43th SMW of 2024 (fourth week of October). Regression analyses indicated that meteorological factors explained 58–61 per cent of pest severity, underscoring the influence of weather in conjunction with other biotic and management factors. These findings provide baseline information on the molecular identity and seasonal dynamics of *M. hirsutus*, supporting predictive pest management and the development of ecologically based integrated pest management (IPM) strategies in custard apple orchards.

Keywords: *Annona squamosa, Maconellicoccus hirsutus,* molecular characterization, population dynamics, Integrated pest management

INTRODUCTION

Custard apple (*Annona squamosa* L.) is a nutritionally rich tropical fruit valued for its edible pulp and medicinal properties. It is well adapted to arid and semi-arid conditions due to its tolerance to drought, salinity and shallow soils, making it an important fruit crop in several Indian states, including Karnataka, Maharashtra, Telangana and Andhra Pradesh (Pinto *et al*., 2005; Singh *et al.,* 2010). Although custard apple is widely cultivated for its high economic and pharmaceutical value, its productivity remains suboptimal due to severe biotic stresses, particularly insect pest infestations, among which the pink mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), has emerged as a major threat. *Maconellicoccus hirsutus* is a highly polyphagous pest reported from more than 300 plant species worldwide. In custard apple, it infests fruits, leaves and young shoots, causing leaf curling, stunted growth, fruit malformation, and honeydew deposition, which promotes the development of sooty mold. Yield losses of up to 25% have been reported as a result of

its feeding damage (Babu and Azam, 1987; Mani and Krishnamoorthy, 1989). Severe infestations can result in dieback and even plant mortality, posing a major threat to sustainable cultivation. Traditionally, *M. hirsutus* has been identified based on morphological characteristics. However, the high degree of morphological plasticity and the presence of cryptic diversity within mealybug species complicate accurate identification. Molecular approaches- particularly *mitochondrial cytochrome oxidase I* (COI) 'DNA barcoding'- have emerged as reliable tools for confirming species identity and resolving taxonomic ambiguities (Shivakumara et al., 2024a). Despite the widespread application of molecular markers in pest diagnostics, comprehensive studies integrating molecular characterization with seasonal population dynamics of *M. hirsutus* in custard apple ecosystems remain scarce. The pest's population dynamics are closely regulated by climatic variables such as temperature, humidity and rainfall, which influence its seasonal incidence and severity. Thus, understanding temporal fluctuations is critical for forecasting pest outbreaks and implementing timely

management interventions. Although the pink mealybug has been extensively studied on several host plants, information on its molecular identity and seasonal dynamics in custard apple remains limited. In particular, substantial gaps exist in the characterization of *M. hirsutus* populations infesting custard apple, especially when assessed using integrated molecular approaches. Understanding the seasonal population fluctuations of *M. hirsutus* in relation to local agroclimatic conditions and their correlation with weather parameters, is essential for generating predictive insights into pest outbreaks. To address these gaps, the present study was undertaken to molecularly characterize *M. hirsutus* populations infesting custard apple and to assess their seasonal dynamics in relation to weather parameters. This study provides confirmation of *M. hirsutus* on custard apple and insights into its population dynamics, thereby supporting the development of sustainable, ecologically based integrated pest management (IPM) strategies for custard apple orchards.

MATERIALS AND METHODS

Sample Collection and Morphological Identification

Field studies were conducted during the *rabi* seasons of 2023 - 24 and 2024 - 25 in a commercial custard apple (*Annona squamosa* L.) orchard at Ukkali, Vijayapur, Karnataka, India, following a Randomized Block Design (RBD) with three replications. Seasonal incidence of the pink mealybug, *M. hirsutus*, was assessed on ten randomly selected and permanently labelled trees. On each tree, ten fruits were examined, with three fixed areas (2.54 cm² each) on each fruit observed using a 10× hand lens to count live mealybugs. Specimens were preserved in 70% ethanol and forwarded to ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, for morphological and molecular confirmation.

Molecular Characterization

Genomic DNA Extraction and PCR Amplification

Genomic DNA was extracted from individual adult *M. hirsutus* females using the QIAGEN DNeasy® Blood & Tissue Kit (QIAGEN, Germany), following the manufacturer's protocol with minor modifications (Rani *et al.,* 2023; Shivakumara *et al.,* 2024b). DNA concentration and purity was determined using a Jenway 747501 Nano Scanning Micro-Volume Spectrophotometer (Cole-Parmer Ltd., UK). The mitochondrial cytochrome c oxidase subunit I (COI)

gene was amplified at 658bp fragment using primers PcoF1 (5′-CCTTCAACTAATCATAAAAATATYAG-3′) and LepR1 (5′-TAAACTTCTGGATG TCCAAAAAATCA-3′) (Park *et al.,* 2010). The PCR was performed in a Bio-Rad C1000™ thermal cycler (Thermo Fisher Scientific, USA). Each 25 µL reaction contained 12.5 µL of 2× reSource™ Taq Mix (Source Bioscience, UK), 0.5 µL each of 10 µM forward and reverse primers, 3 µL of template DNA, and 8.5 µL of nuclease-free water (Sigma-Aldrich). Thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 min; 5 cycles of 94 °C for 60 s, 45 °C for 30 s, and 72 °C for 60 s; followed by 40 cycles of 94 °C for 40 s, 51 °C for 40 s, and 72 °C for 60 s; and a final extension at 72 °C for 3 min. The PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized using a GelDoc™ Go Imaging System (Bio-Rad) with a 100 bp DNA ladder (Fermentas, Thermo Fisher Scientific, USA) as a reference (Sambrook & Russell, 2001).

Sequencing and Phylogenetic Analysis

Amplified PCR products were sequenced bi-directionally. Consensus sequences were assembled in BioEdit v7.2, checked for insertions, deletions and stop codons and exported in FASTA format. Homology searches were performed using National Center for Biotechnology Information (NCBI) BLAST (https://blast.ncbi.nlm.nih.gov/). The validated sequences were submitted to the NCBI GenBank database for accession numbers. For phylogenetic analysis, homologous sequences of *M. hirsutus*, from diverse geographic origins were retrieved from GenBank. Priority was given to sequences showing high similarity to our isolate, as these constituted the majority of well-curated accessions available in the database. Evolutionary relationships were inferred using MEGA v11 (Tamura *et al.,* 2021), with node support assessed by bootstrap analysis based on 1000 replicates (Felsenstein, 1981). Evolutionary distances were estimated using the Maximum Composite Likelihood model (Tamura *et al.,* 2004), with *Phenacoccus manihoti* (GenBank accession no. OK172179) as an out group.

Seasonal Population Dynamics

Seasonal incidence of *M. hirsutus* was recorded with each meteorological week during 2023-24 and 2024-25. Weekly pest and natural enemy counts were correlated with weather parameters – including maximum and minimum temperature (°C), morning and evening

relative humidity (%), rainfall (mm), wind speed (km h⁻¹), sunshine hours (h) and evaporation (mm) –obtained from Regional Agriculture Research Station, Vijayapur.

Statistical Analysis

Associations between pest incidence and individual weather variables were evaluated using Pearson's correlation coefficients (r). The combined influence of weather parameters was assessed using multiple linear regression analysis, following the model:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_nX_n$$

Where, Y = pest population, a = intercept, $b_1...b_n$ = regression coefficients, and $X_1...X_n$ = weather variables. Significance of correlations and regression coefficients was tested at 5% and 1% probability levels. The coefficient of determination (R^2) was used to estimate the proportion of variation in pest populations explained by weather parameters included in the model. Statistical analyses was performed using SPSS v26 (IBM Corp., USA) and R software.
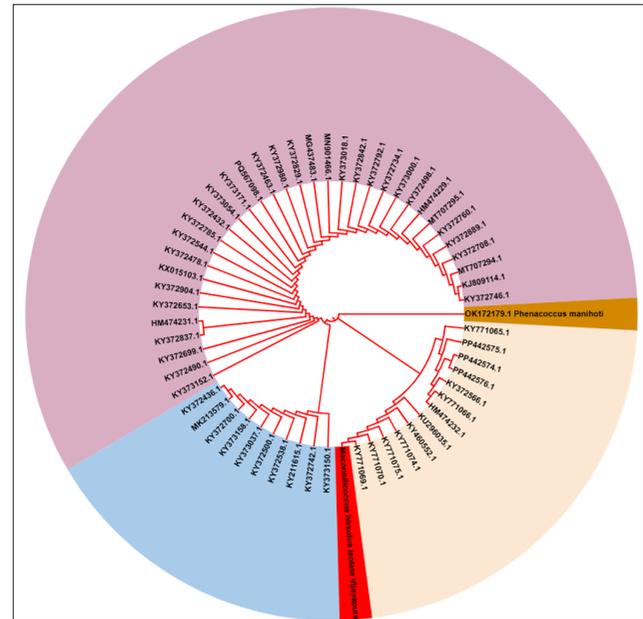
RESULTS

Molecular Characterization

The Nano Drop spectrophotometric analysis at 260 nm indicated A260/280 ratios ranging from 1.50 to 1.80, confirming acceptable DNA purity. The DNA yield reached up to 962 ng/µL. High-molecular-weight genomic DNA was visualized as distinct, intact bands on agarose gel (1.5%) electrophoresis. The mitochondrial cytochrome c oxidase subunit I (COI) region, with an amplicon length of 658 bp, was successfully amplified and sequenced. The resulting sequences were analysed using the NCBI Basic Local Alignment Search Tool (BLASTn) against the GenBank database. The BLASTn analysis revealed 100% sequence similarity with previously reported *M. hirsutus* sequences available in GenBank, with a query length of 614 bp and an E-value of 0.00, thereby confirming the molecular identity of the species. Representative COI sequences generated in the present study were deposited in GenBank under the accession number PX511626.1.

Phylogenetic Analysis

Phylogenetic relationships among *M. hirsutus* populations were inferred using 59 COI gene sequences, including the isolates from Vijayapura. The phylogenetic analysis resolved two distinct clades representing *M.*

hirsutus populations from different geographic regions, while a third clade corresponded to the out group (*P. manihoti*). The Vijayapura isolate clustered closely with sequences from Bengaluru, India (KY771069.1 and KY771070.1), demonstrating high genetic similarity and minimal divergence among Indian populations (Fig. 1). These results suggest that *M. hirsutus* populations across India are genetically conserved, with limited regional differentiation.

Fig. 1. The phylogenetic relationships of the study isolate *Maconellicoccus hirsutus* were inferred using a 658 bp mitochondrial cytochrome c oxidase subunit I (COI) sequence. The Maximum Likelihood tree, constructed under the Tamura–Nei model using MEGA v11, shows the isolate from the present study (PX511626.1), highlighted in a red box.

Field Damage and Nature of Symptoms of *Maconellicoccus hirsutus*

Infestation of *M. hirsutus* results in characteristic damage to different plant parts, with symptoms being most pronounced on leaves, shoots, twigs and fruits. Infested leaves exhibit curling, crinkling, and distortion as a result of sap-sucking during feeding. Under severe infestations, yellowing, drying and premature senescence were commonly observed. Tender shoots and twigs show swelling, malformation and shortened internodes. Prolonged and heavy infestations often result in wilting and dieback of tender shoots. Fruits become malformed and stunted, leading to poor marketability. Honeydew deposition on fruits promotes sooty mold development, which blackens the surface, interferes with photosynthesis, and reduces fruit quality. Colonies

of mealybugs, covered with white, cottony wax masses, were conspicuously observed on leaf axils, fruits and along the twigs. Infested plants were frequently attended by ants for honeydew and protected the mealybugs

from natural enemies. Sustained infestations cause progressive weakening of the plant, reduced vigor and in severe cases, plant death, especially in young or poorly managed orchards (Fig. 2).
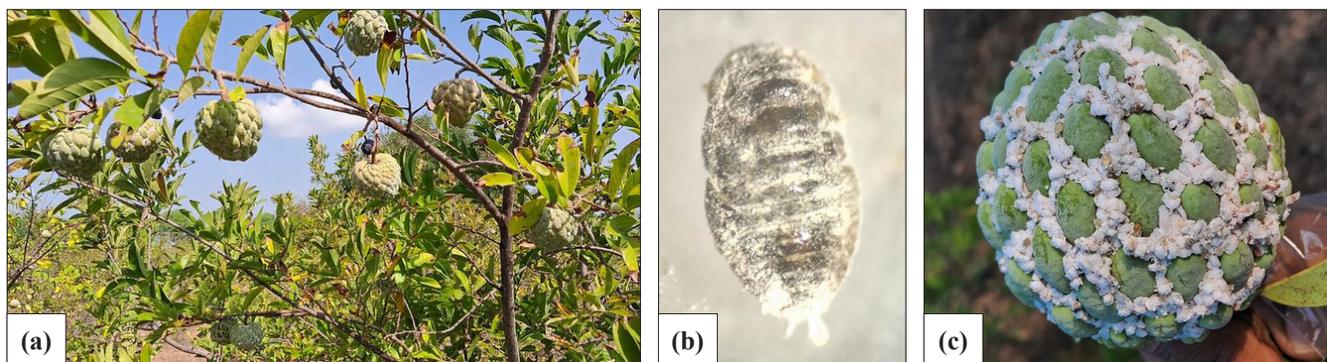

Fig. 2. Field infestation caused by *Maconellicoccus hirsutus* (a) infested plant (b) female *Maconellicoccus hirsutus* (c) colonies of *Maconellicoccus hirsutus* on custard apple fruit

Seasonal Incidence of *Maconellicoccus hirsutus*

The population of *M. hirsutus* exhibited distinct seasonal fluctuations during both cropping years. During 2023 - 24, infestation initiated at the 34th standard meteorological week (SMW), corresponding to the 4th week of August, with a mean density of 0.90 individuals per fruit. The population gradually increased and attained the first minor peak during the 35th SMW (5th week of August) with 2.76 individuals per fruit. A temporary decline in population was recorded during the 36th SMW (first week of September), attributed to heavy rainfall, after which numbers steadily increased, reaching a major peak of 71.28 individuals per fruit in the 47th SMW (fourth week of November). Thereafter, the mealybug density declined gradually from December and remained negligible during February to March 2024, coinciding with fruit maturity and harvest. No infestation was observed during the off-season period from April to July 2023 (Fig. 3). A similar population trend was observed during 2024–25, with densities ranging from 0.00 to 68.32 individuals per fruit. The infestation commenced in late August and progressively increased to reach the seasonal peak of 68.32 individuals per fruit during the 43th SMW (fourth week of October 2024) (Fig. 4). Populations declined steadily from December onwards, with negligible infestation recorded after fruit harvest. Observations across both years indicate that *M. hirsutus* infestation on custard apple is seasonal, initiating in late August, peaking between October and November, and declining sharply after harvest. Rainfall events temporarily suppressed population build-up, whereas dry and warm weather favoured rapid multiplication and persistence until harvest.
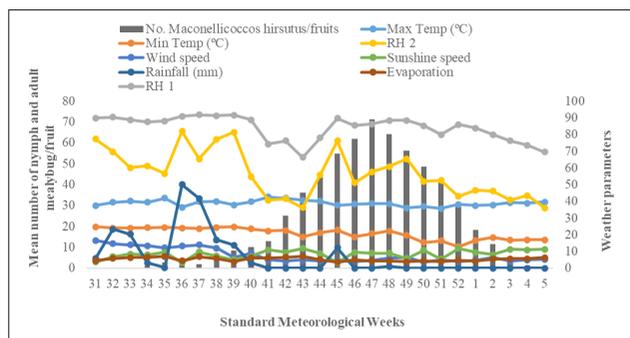

Fig. 3. Severity of Pink Mealybug, *Maconellicoccus hirsutus* population on custard apple fruit during 2023-24
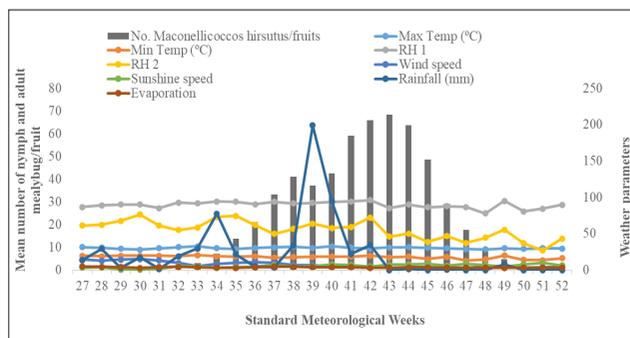

Fig. 4. Severity of Pink Mealybug, *Maconellicoccus hirsutus* population on custard apple fruit during 2024-25

The severity of *M. hirsutus* infestation in custard apple orchards was examined in relation to key weather variables during 2023 - 24 and 2024 - 25. Correlation analysis indicated that among the weather parameters; wind speed ($X5$), rainfall ($X7$) and evaporation ($X8$) exhibited significant negative associations with the severity of *M. hirsutus* ($Y1$), during 2023 – 24, with correlation coefficients (r) of –0.608 (p < 0.01), –0.390

(p < 0.05) and −0.547 (p < 0.01), respectively. Other weather variables such as maximum temperature (X1), minimum temperature (X2), morning relative humidity (X3), evening relative humidity (X4) and sunshine hours (X6) showed weak and non-significant associations with pest severity during year. The multiple regression model for 2023 - 24 revealed that the combined influence of weather parameters accounted for 61.0% of the variation in pest severity. During 2024–25, wind speed (X_5) again showed a significant negative correlation (r = −0.480; p < 0.05), whereas sunshine hours (X_6) exhibited a significant positive correlation (r = 0.429; p < 0.05) with *M. hirsutus* severity (Y_2).

Maximum temperature (X1) and morning relative humidity (X3) showed positive but non-significant correlations, while other variables were weakly or negatively correlated. Overall, the findings highlight that wind speed consistently exerted a suppressive effect on mealybug severity across both years, while sunshine duration enhanced population levels during 2024–25. Other meteorological parameters showed variable or weak influence. The moderate coefficients of determination (R² values of 0.610 and 0.581), indicate that weather factors substantially contribute to pest dynamics, though additional biotic or management factors may also play a role (Table 1).

Table 1. Correlation coefficients and regression model for severity of *Maconellicoccus hirsutus* and abiotic factors.

	X1	X2	X3	X4	X5	X6	X7	X8	Regression model
Y1	-0.304	-0.307	-0.010	-0.078	-0.608**	0.015	-0.390*	-0.547**	$Y= 155.794 - 9.161X_1 + 6.436X_2 + 0.896X_3 - 0.155X_4 - 5.847X_5 + 5.322X_6 - 0.458X_7 - 4.723X_8$ (R²= 0.610)
Y2	0.326	0.007	0.254	-0.038	-0.480*	0.429*	0.165	-0.014	$Y= -77.140 - 2.858X_1 + 3.497X_2 - 0.775X_3 + 2.032X_4 - 3.393X_5 + 10.354X_6 - 0.093X_7 - 13.738X_8$ (R²= 0.581)

**Correlation is significant at 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed).

Where,

Y1= severity of *Maconellicoccus hirsutus* insect population in 2023-24

Y2= severity of *Maconellicoccus hirsutus* insect population in 2024-25

X1=Maximum temperature (ºC), X2=Minimum temperature (ºC), X3= Morning Relative humidity (%)

X4= Evening Relative humidity (%), X5= Wind Speed, X6= Sunshine (hr), X7= Rainfall (mm), X8= Evaporation

Discussion

The present study elucidated the molecular identity and seasonal population dynamics of *M. hirsutus* on custard apple (*A. squamosa* L.) in Vijayapura, India, highlighting the influence of meteorological factors on pest severity. Molecular identification using the mitochondrial COI gene confirmed the identity of *M. hirsutus* with 100% similarity to GenBank sequences. The Vijayapura isolate clustered with other Indian populations, corroborating the findings of Ben-Dov *et al*. (2014), who reported limited genetic divergence among Indian *M. hirsutus* populations. Similar studies in other crops have also

emphasized the utility of COI sequencing for resolving cryptic mealybug species and confirming morphological identifications (Joshi *et al.,* 2012). Seasonal population monitoring revealed a distinct pattern, with infestation initiating in late August and peaking between October and November. Peak densities of 71.28 and 68.32 individuals per fruit were recorded during 2023 - 24 and 2024 - 25, respectively. These results align with the observations of Pinto *et al.* (2005), who documented post-monsoon peaks of *M. hirsutus* in tropical fruit orchards and Singh *et al*. (2010), who noted reduced off-season populations due to unfavourable climatic conditions. The temporary population declines associated with heavy rainfall are

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consistent with previous studies demonstrating that high precipitation can suppress mealybug populations by washing off nymphs and adults and disrupting oviposition (Raman and Chaudhary, 2008).

Meteorological influences on pest severity, correlation and regression analyses revealed that wind speed consistently exerted a suppressive effect on *M. hirsutus* populations, while sunshine hours promoted higher densities during 2024-25. Other parameters, including temperature and relative humidity, showed weak or variable associations. These findings are in agreement with earlier reports indicating that wind and rainfall negatively influence mealybug abundance, whereas warm and dry conditions favour rapid population buildup (McKenzie, 2002; Prasad *et al.,* 2015).

The regression models explained a moderate proportion of the variation in *M. hirsutus* population dynamics, with coefficients of determination (R^2) of 0.610 and 0.581, indicating that abiotic weather variables play a significant role in shaping seasonal abundance patterns. However, approximately 39 to 42% of the observed variability remained unexplained, highlighting the multifactorial nature of mealybug population regulation. This unexplained variation may be attributed to biotic factors such as host plant phenology and nutritional status, natural enemy complexes (parasitoids and predators) and interspecific interactions, as well as agronomic and management practices including pruning, irrigation and insecticide applications. In addition, microclimatic heterogeneity and time-lagged responses of mealybug populations to weather variables may further contribute to deviations from model predictions. These findings underscore that while weather parameters provide valuable predictive insight into *M. hirsutus* dynamics, integrative models incorporating both abiotic and biotic drivers would offer a more comprehensive understanding of pest outbreaks and improve forecasting accuracy under field conditions (Ragsdale *et al.*, 2011; Zappalà *et al.*, 2013).

Understanding the molecular identity and seasonal dynamics of *M. hirsutus* provides a foundation for predictive pest management. The identification of climatic variables influencing population peaks enables growers to implement timely interventions, such as the augmentation of natural enemies or targeted insecticide applications during critical periods. These findings support the development of ecologically based integrated pest management (IPM) strategies tailored to custard apple orchards (Tauber *et al.*, 2000; McEwen *et al.*, 2001).

CONCLUSION

This study elucidates the molecular identity and seasonal dynamics of *M. hirsutus* on custard apple. The *mt* COI gene sequencing confirmed species identity with 100% similarity to GenBank sequences and phylogenetic analysis showed minimal genetic divergence among Indian populations. Seasonal monitoring indicated infestation beginning in late August, peaking in October to November, with maximum densities of 71.28 and 68.32 individuals per fruit in 2023 - 24 and 2024 - 25, respectively. Regression analyses revealed that wind speed and sunshine hours significantly influenced pest severity, explaining 58 to 61 per cent of variation. These findings provide baseline information for predictive pest management and support season-specific, ecologically based IPM strategies in custard apple orchards.

ACKNOWLEDGEMENT

AUTHORS CONTRIBUTION

AS, BAP, SKT: conceptualization, designing and implementation of the experiments and MS preparation implementation of the experiments; USS, PHT, VCK and YGS: planning, supervising and formal analysis and MS finalisation

CONFLICT OF INTEREST

The authors do not have any conflict of interest with respect to the content of the article.

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