PLANT SCIENCE TODAY
Vol 12(sp3): 01–08
https://doi.org/10.14719/pst.10324

eISSN 2348-1900

RESEARCH ARTICLE

Prophecising the migratory potential through strainal variation analysis of Fall armyworm

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Received: 27 June 2025; Accepted: 13 November 2025; Available online: Version 1.0: 09 January 2026

Cite this article: Manisha BL, Venkateswarlu NC, Chalam MSV, Saradajayalakshmi DR, Ramana MB, Hemadri T, Rajasri M, Manjula K. Prophecising the migratory potential through strainal variation analysis of Fall armyworm. Plant Science Today. 2026;12(sp3):01–08. https://doi.org/10.14719/pst.10324

Abstract

Fall armyworm is an obliterating pest that is rampantly devastating all the crops in a jiffy, the need to take up this study is to confirm the strain of *Spodoptera frugiperda,* which is very crucial as it can help to better understand the prevalent strain along with assessing the burgeoning trend of attack forehand to the crops that are at formidable invasive threat. In order to avoid ambiguity between C and R strain specimens were collected from Kurnool, Chittoor, Kadapa and Anantapuramu (Rayalaseema region) the major Maize growing belt of Andhra Pradesh and were subjected to molecular analysis. The salient findings of the present study are the genetic similarity among the collected larval populations of Fall armyworm revealed a high A-T rich composition with an average of 41.2 % of thymine (uracil) and Mt COI region studies on populations from Chittoor, Kurnool Kadapa and Ananthapuramu, Ragi in Chittoor showed 89 %, 100 %, 98 %, 98 % and 99 % similarity with C strain Fall armyworm population of France (mw665994.1) respectively which is contrary to several contemporary reports that claim population of Fall armyworm in Andhra Pradesh, India to be predominantly R Strain. As Fall armyworm belonging to C strain are a probable threat to Cotton and sugarcane crops, hence forewarning precautions needs to be taken to curtail the polyphagous pest menace.

Keywords: fall armyworm; forewarning precaution; spread potential; strainal variation

Introduction

Fall armyworms are polyphagous in nature and target Maize from the time seedlings emerge until the ear development stage (1). They like to feed on over 353 plants from 76 different families, primarily Poaceae, Asteraceae and Fabaceae (2). In previous studies, Sorghum was the most popular host among millets (60.1 %), followed by Pearl millet (41.4 %), Barnyard millet (22.9 %) and Finger millet (10.2 %) (3). The insect has quickly expanded to more than 44 African countries, seriously inflicting damage to crops including Sorghum and Maize (4, 5). Maize, rice, sorghum, millet, groundnut, soybean, cotton, sudan grass and other fodder grasses are the main host plants (6). Two populations known as "host strains" are responsible for this wide host range; the C-strain primarily consumes Corn and Sorghum, while the R-strain consumes Pasture, Lucerne and Forage grasses as reported by previous researchers (7, 8). Despite being historically classified as host strains, their evolutionary connections are still unclear, largely since they are physically identical. Genetic markers from sections of the Mitochondrial Cytochrome Oxidase Subunit I (COI) s are the most reliable way to identify the strains (9).

Fall armyworm due to its wider host range caused considerable financial yield loss to farmers along it's invasive pathway which were estimated between 204028.76 crore to 510396.24 crore rupees per annum, translating to a loss ranging from 8.3 to 20.6 million tonnes produce annually in India (10). This can be curtailed to certain extent, when pattern of migratory behaviour is predicted through the strain that is locally prevalent and chances of it attacking the crop however since these two strains are morphologically identical and the taxonomic status remains uncertain, mt coI gene stands as the best definer of strain identity, after surveyed from multiple locations (11).

This inference is critical to assess the crops that are at immediate risk, in order to address these issues, we obtained specimens from roving surveys of Rayalaseema region which is a predominantly Maize growing belt of Andhra Pradesh to confirm the existing strain and litigations associated with crops that are at threat in near future.

Materials and Methods

Sample collection

Larvae of Fall armyworm were collected from Maize and Ragi fields in Rayalaseema region Chittoor where latitude of Chittoor, Andhra Pradesh, India is 13.217096° N and longitude is

79.100677° E and Kurnool is situated at a latitude of 15.83333° N and longitude of 78.0373° E, Kadapa is located at a latitude of 14.477234° N and the longitude of 78.804932° E, in case of Anantapuramu 14.685564° is the latitude and longitude is 77.595406°. Larvae were collected from the above mentioned places during *kharif,* 2021 and 2022 which were identified based on the taxonomic keys. The collected specimens were preserved in 95 % ethanol, labelled and kept in deep freezer (-20 °C). For each species, genomic DNA was extracted from an individual larva separately.

DNA extraction

Each larva's DNA was extracted using the CTAB technique in accordance with extraction protocol (12). Nanodrop® 2000 was used to quantify the DNA samples and a 1 % (w/v) agarose gel was used to verify their quality.

PCR amplification and COI sequencing

A universal COI primer pair (LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) was utilized for PCR analysis of about 100 ng of genomic DNA (13). A 25 µL volume of a mixture including 10X PCR reaction buffer, 2.5 mM MgCl2, 10 mM dNTPs, 10 pM of each primer, 2.5 units of Taq DNA polymerase and100 ng of DNA template was used for the PCR reactions. The Eppendorf thermal cycler (Eppendorf, Germany) was used to carry out the COI PCR cycle program. In accordance with instructions, PCR-amplified DNA was electrophoresed on a 1 % agarose gel (w/v) (14). The movement pattern of the DNA fragments inside the gel was captured using the gel documentation system (Fig. 1) (Alpha Innotech, USA) in auto exposure mode. The DNA sequencing was carried out at (Biokart Pvt. Ltd., Bengaluru).

Analysis of data

To ascertain the sequence similarity for accurate species identification, the sequences of every Fall armyworm that was collected were compared with sequences on GenBank using nBLAST for gene homology. This comparison was based on the total score, expected value, maximum identical query coverage andmaximum score (15). In order to obtain accession numbers, the produced sequences were submitted to NCBI GenBank (Table 1). For a comparative genetic analysis, the sequences from the NCBI database were obtained in FASTA format. Bio Edit version 7.0 software was used for sequence assembly, nucleotide alignment and the percent identity matrix (16). Using the FAW nucleotide sequences from the current investigation and the FAW sequences of the same genus and species that were downloaded from the NCBI database, phylogenetic trees were built. The phylogenetic tree was constructed with a variety of *Spodoptera* spp. included as an outgroup. Molecular Evolutionary Genetic Analysis Version 11.0 (MEGA11) software was used to build the Maximum Likelihood (ML) tree with 1000 bootstrap replications under the distance model Tamura 3-parameter (17). Biorender.com, Clust Vis and the Data tab online statistics calculator was used to create graphs and maps.

Results and Discussion

Larvae obtained from roving surveys were used to determine if the population belonged to the C strain or the R strain to address the Fall armyworm migratory pattern. Since

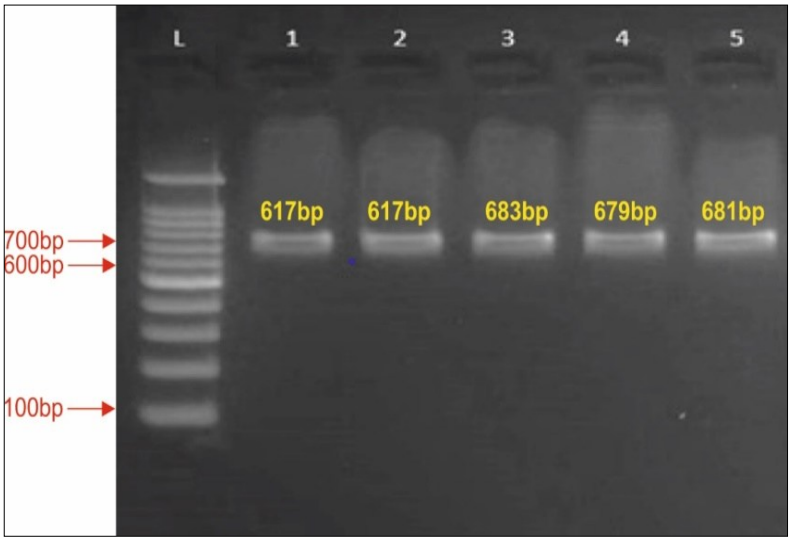


Fig. 1. Agarose gel electrophoresis of PCR amplified DNA of partial mitochondrial CO-I region of Fall armyworm collected from different locations in Andhra Pradesh.

Table 1. Accession numbers acquired from NCBI of Fall armyworm collected from different locations of Andhra Pradesh

S. No	Fall armyworm population collected	NCBI accession number	Population code	Web link
1	Chittoor District, Andhra Pradesh	OQ272112	FAW21CHI	https://www.ncbi.nlm.nih.gov/nuccore/ OQ272112
2	Kurnool District, Andhra Pradesh	OQ272111	FAW21KRNL	https://www.ncbi.nlm.nih.gov/nuccore/ OQ272111
3	Kadapa District, Andhra Pradesh	OQ272110	FAW21KDP	https://www.ncbi.nlm.nih.gov/nuccore/ OQ272110
4	Ananthapuramu District, Andhra Pradesh	OQ272109	FAW21ATP	https://www.ncbi.nlm.nih.gov/nuccore/ OQ272109
5	Chittoor District, Andhra Pradesh	OQ253320	FAW21CHIRG	https://www.ncbi.nlm.nih.gov/nuccore/ OQ253320

mitochondrial cytochrome c oxidase I (mtCOI) shows consistent inter-species differences when compared to other markers, primers associated with the partial mitochondrial cytochrome oxidase I (COI) area were used in this analysis (18). The GenBank database was used to characterize and compare five insect populations: FAW21CHI (Chittoor), FAW21CHIRG (Chittoor), FAW21KDP (Kadapa), FAW21KRNL (Kurnool) andFAW21ATP (Ananthapuramu) (19).

The final assembled sequences were (Fig. 1) of 617 bp, 617 bp, 683 bp, 679 bp and 681 bp in size for FAW21CHI (Chittoor), FAW21CHIRG (Chittoor), FAW21KDP (Kadapa), FAW21KRNL (Kurnool) and FAW21ATP (Ananthapuramu) respectively.

Nucleotide composition of Fall armyworm

Composition of purines (adenine and guanine) and pyridines (cytosine and thymine/uracil) in the sequences of mtCO-I gene among Fall armyworm was computed. The results reveal a high A-T rich composition among the sequences of Fall armyworm. The similarity matrix analysis (Fig. 2) and the multiple nucleotide sequence analysis of mitochondrial gene sequences of five populations under study with other Fall armyworm in NCBI database (Fig. 3, Fig. 4) revealed that Kurnool (FAW 21KRNL) population showed 100 % similarity with population of France (MW665994), Banglore (O4068421.1), West Bengal (LR963467.1), New Delhi (MN541574.1) and Karnataka (OQ068448.1), Kadapa population showed 100 % similarity with population of China (MK860941.1), Indonesia (MW876211.1), Pakistan (MT180097.1), Mexico (ON0384321), West Bengal (OK178018.1), Indonesia (OP692735.1), Banglore (OQ068443.1), China (MK860942.1) Ananthapuramu population (FAW21ATP) showed 100 % similarity with China (MK860941.1), Indonesia (MW876211.1), Pakistan (MT180097.1), Mexico (ON038432.1), West Bengal (OK178018.1), Indonesia (OP692735.1), Banglore (OK068443.1), China (MK860942.1) Fall armyworm population on Ragi in Chittoor (FAW21CHIRG) showed 99 % similarity with France (MW665994.1), Banglore (OQ068421.1), West Bengal (LR963467.1), New Delhi (MN541574.1), West indies (MT881757.1), Karnataka (OQ068448.1), USA (MK318311.1). However Fall armyworm populations in Chittoor (FAW21CHI), Kurnool (FAW21KRNL), Kadapa (FAW21KDP) and Ananthapuramu (FAW21ATP), on Ragi in Chittoor (FAW21CHIRG) showed 89 %, 100 %, 98 %, 98 % and 99 % similarity with C strain Fall armyworm population of France (MW665994.1) respectively. Whereas Fall armyworm populations in Chittoor (FAW21CHI), Kurnool (FAW21KRNL), Kadapa (FAW21KDP) and Ananthapuramu (FAW21ATP), on Ragi in Chittoor (FAW21CHIRG) showed 77 %, 85 %, 86 %, 89 % and 84 % similarity with R strain Fall armyworm population of USA (HM136601.1) (Fig. 5) respectively. Hence it can be concluded from this study that the most predominantly present population of Fall armyworm in Rayalaseema region belongs to C strain. Comprehensive analysis also confirms the dominance of the C strain over the R strain in the samples collected from Rayalaseema region of Andhra Pradesh. However, the genetic similarity of the C-strain populations suggests a more detailed risk associated with the patterns of migration in the region. with the potential damage to crops which are at likely threat of invasion viz. cotton, sugarcane.

Many papers emphasising strainal variation based on

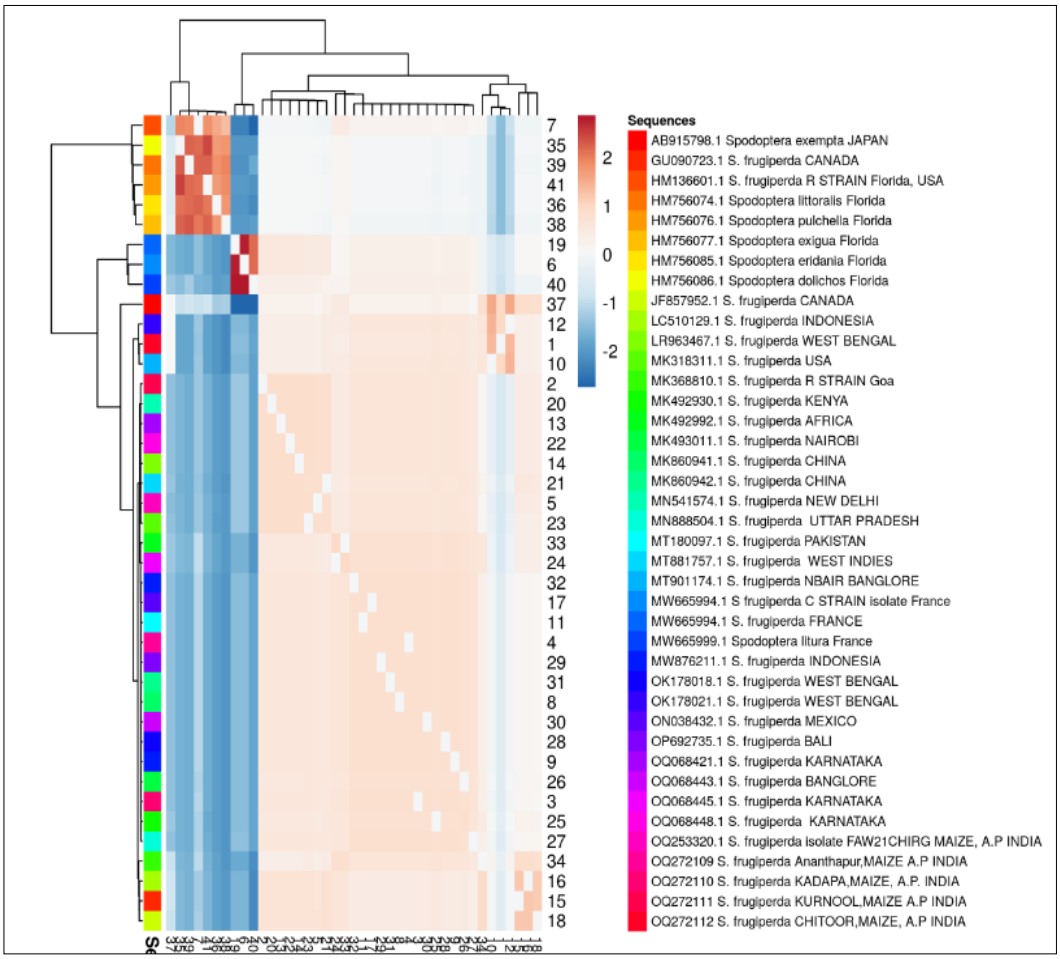


Fig. 2. Sequence similarity index matrix analysis of FAW populations.

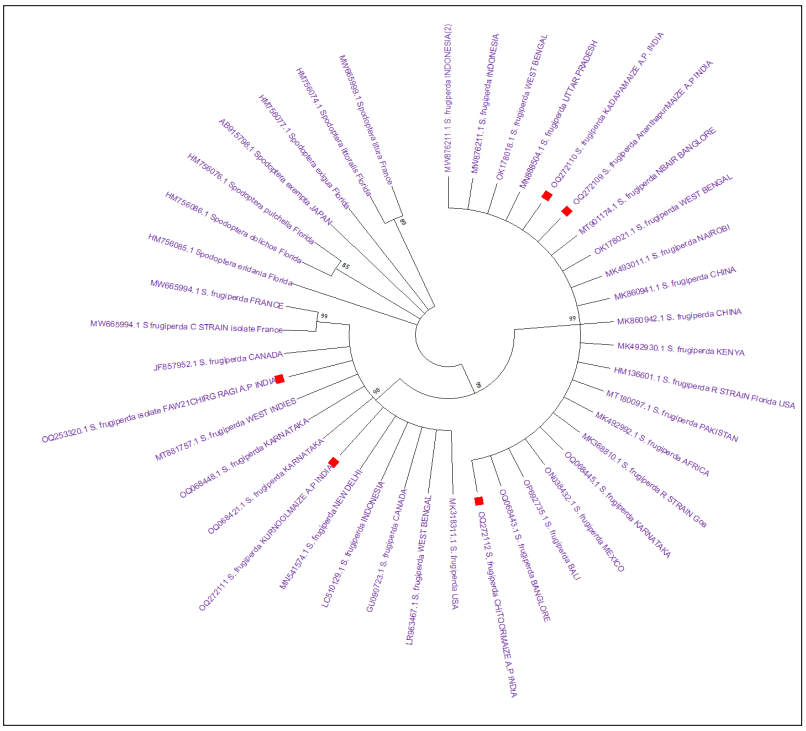
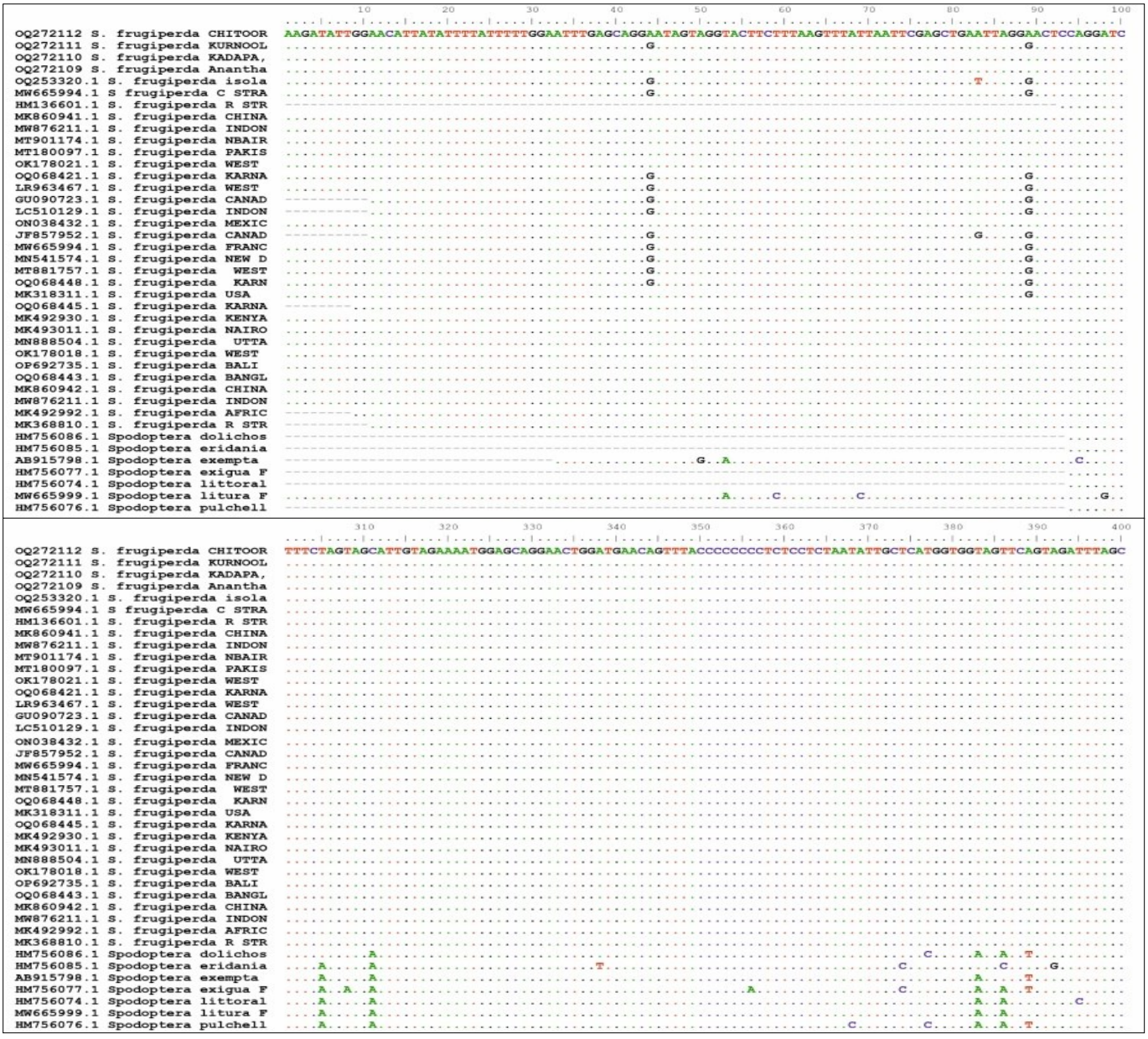
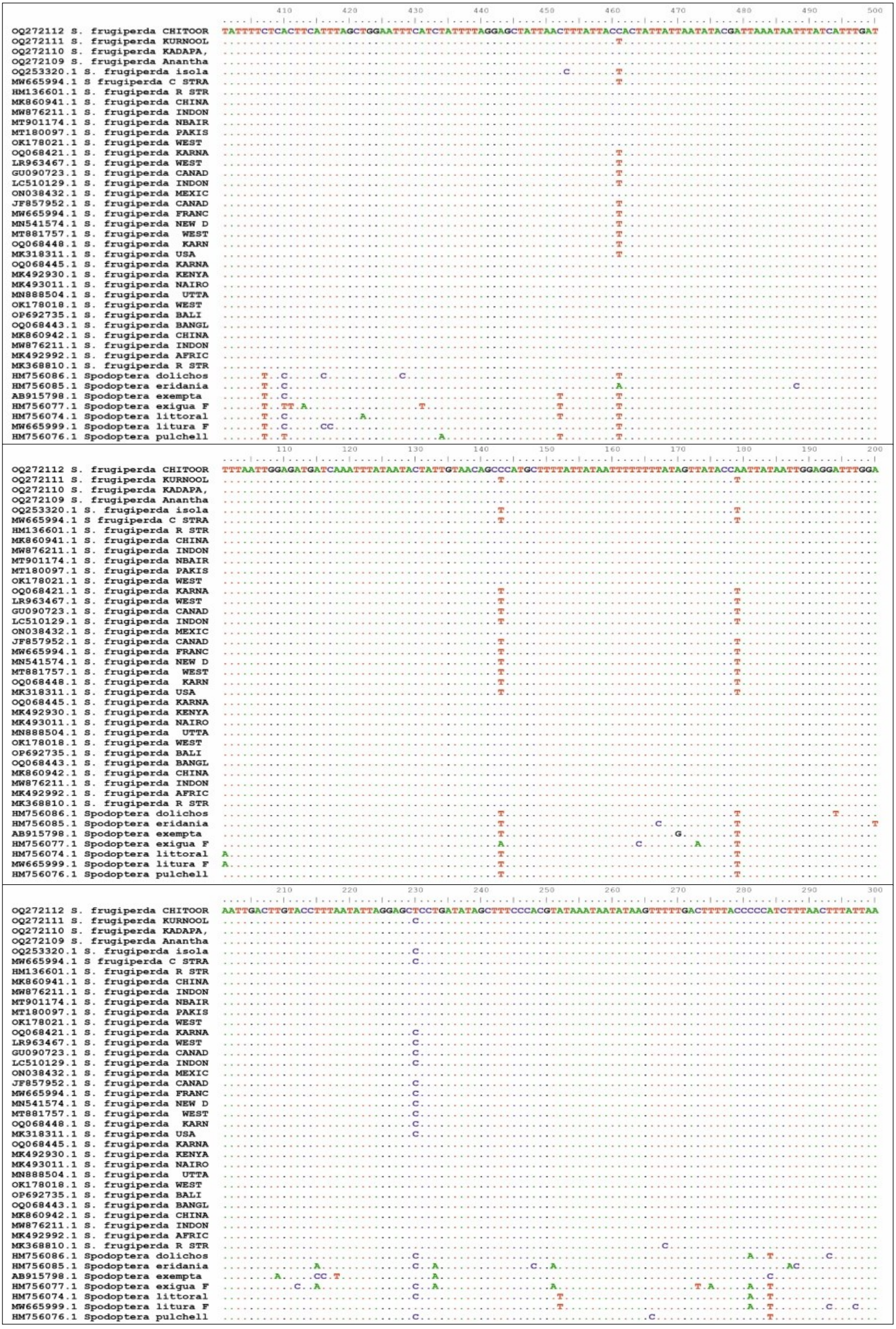


Fig. 3. The circular phylogenetic tree for five *Spodoptera frugiperda* populations collected from India constructed with other *Spodoptera frugiperda* mined from NCBI.




OQ272112 S. frugiperda CHITOOR
OQ272111 S. frugiperda KURNOOL
OQ272110 S. frugiperda KADAPA,
OQ272109 S. frugiperda Anantha
OQ253320.1 S. frugiperda isola
MW665994.1 S frugiperda C STRA
HM136601.1 S. frugiperda R STR
MK860941.1 S. frugiperda CHINA
MW876211.1 S. frugiperda INDON
MT901174.1 S. frugiperda NBAIR
MT180097.1 S. frugiperda PAKIS
OK178021.1 S. frugiperda WEST
OQ068421.1 S. frugiperda KARNA
LR963467.1 S. frugiperda WEST
GU090723.1 S. frugiperda CANAD
LC510129.1 S. frugiperda INDON
ON038432.1 S. frugiperda MEXIC
JF857952.1 S. frugiperda CANAD
MW665994.1 S. frugiperda FRANC
MN541574.1 S. frugiperda NEW D
MT881757.1 S. frugiperda WEST
OQ068448.1 S. frugiperda KARN
MK318311.1 S. frugiperda USA
OQ068445.1 S. frugiperda KARNA
MK492930.1 S. frugiperda KENYA
MK493011.1 S. frugiperda NAIRO
MN888504.1 S. frugiperda UTTA
OK178018.1 S. frugiperda WEST
OP692735.1 S. frugiperda BALI
OQ068443.1 S. frugiperda BANGL
MK860942.1 S. frugiperda CHINA
MW876211.1 S. frugiperda INDON
MK492992.1 S. frugiperda AFRIC
MK368810.1 S. frugiperda R STR
HM756086.1 Spodoptera dolichos
HM756085.1 Spodoptera eridania
AB915798.1 Spodoptera exempta
HM756077.1 Spodoptera exigua F
HM756074.1 Spodoptera littoral
MW665999.1 Spodoptera litura F
HM756076.1 Spodoptera pulchell
410 420 430 440 450 460 470 480 490 500
TATTTTCTCACTTCATTTAGCTGGAATTTCATCTATTTTAGGAGCTATTAACTTTATTACCACTATTATTAATATACGATTAAATAATTTATCATTTGAT
110 120 130 140 150 160 170 180 190 200
TTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCCCATGCTTTTATTATAATTTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGA
210 220 230 240 250 260 270 280 290 300
AATTGACTTGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGTATAAATAATATAAGTTTTTGACTTTTACCCCCATCTTTAACTTTATTAA

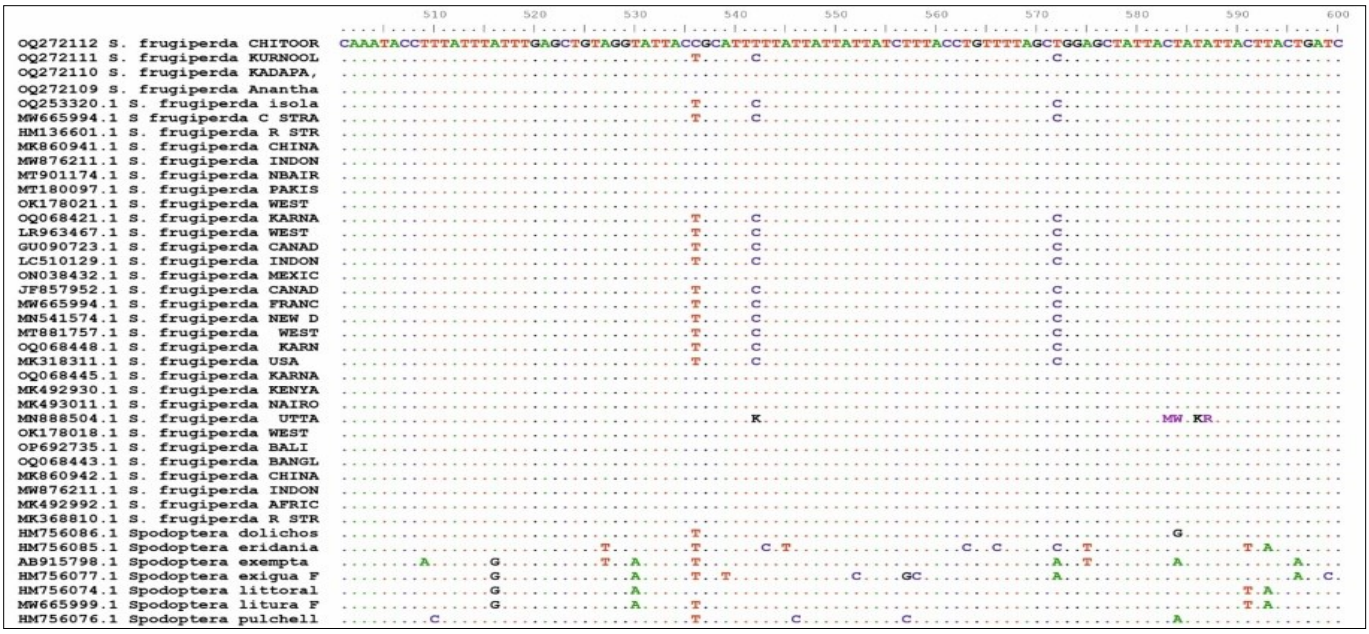


Fig. 4. Multiple sequence alignment of mtCO-I gene of different Fall armyworm species.

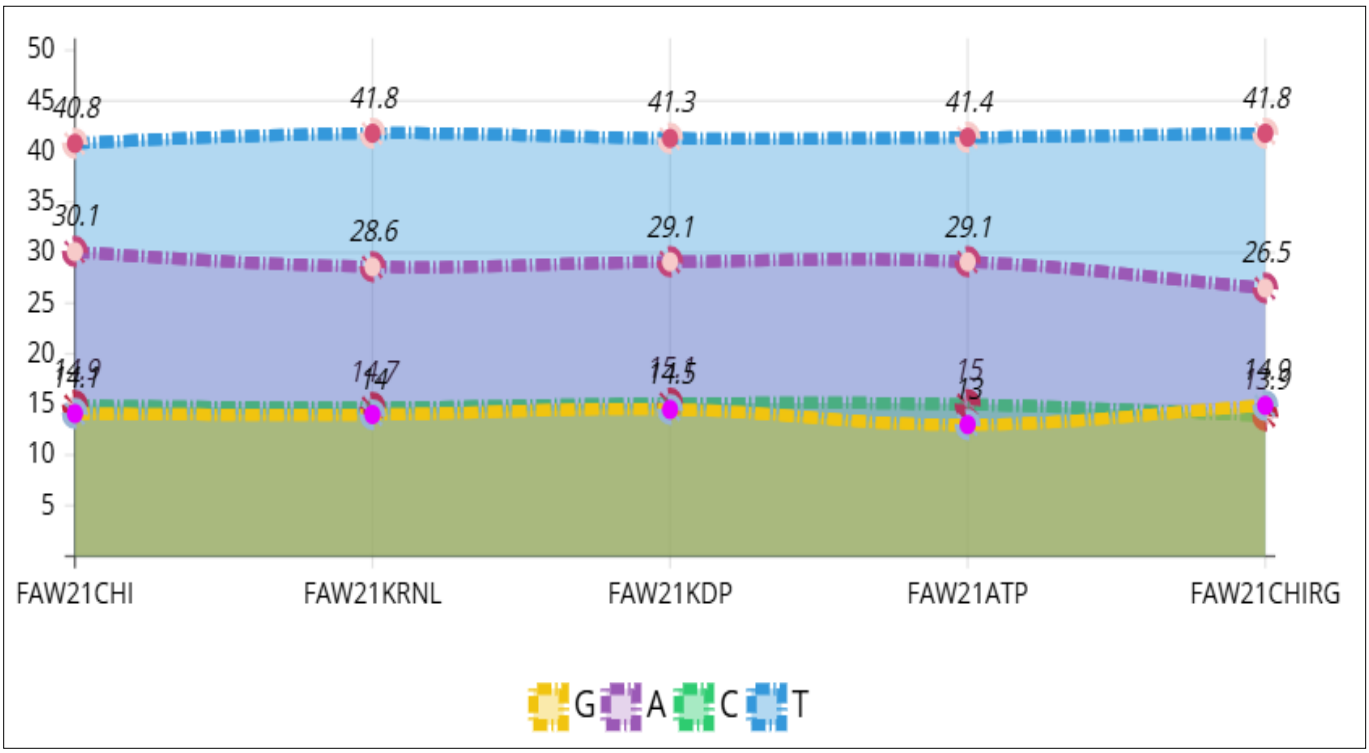


Fig. 5. Nucleotide variation in the strains of FAW in Rayalaseema region.

mtCOI (mitochondrial Cytochrome oxydase I) gene argue that the strain existing dominantly in Andhra Pradesh is R, which was reported in earlier studies, analysed the mtCOI region of FAW, from Andhra Pradesh and confirmed it as R strain but contrarily the salient finding from this study (Fig. 6) prove that Fall armyworm population attacking Rayalaseema region of Andhra Pradesh in India is predominantly C strain (20). Paradoxical trend of findings to the present investigation were also reported from Africa, Nigeria and Tanzania which have shown the predominance of the “R” strain over the “C” strain in Maize (21). Furthermore, experimental studies by previous researchers, also indicated the dominance of the R strain over the C strain in the sample collected from the eastern part of India (22). The reason for C strain can be attributed to the admixture of genes that may have led to invasive FAW hybrids.

There is every threat that if prior care is not taken, then this C strain is likely to ramshackle crops like cotton and sugarcane, these results are also in line with earlier studies and indicated the presence of corn strain on sugarcane in Maharashtra. The present findings are also in conformity with previous studies and proved that “C” strain has started adapting to sugarcane (9).

Conclusion

The reason for polyphagy for a wide range of host plants can be understood by characterizations of strains which in turn helps in assessing potential of FAW in developing resistance to chemical pesticides, this area has to be thrown light on, because if neglected crops viz. cotton and sugarcane which are very

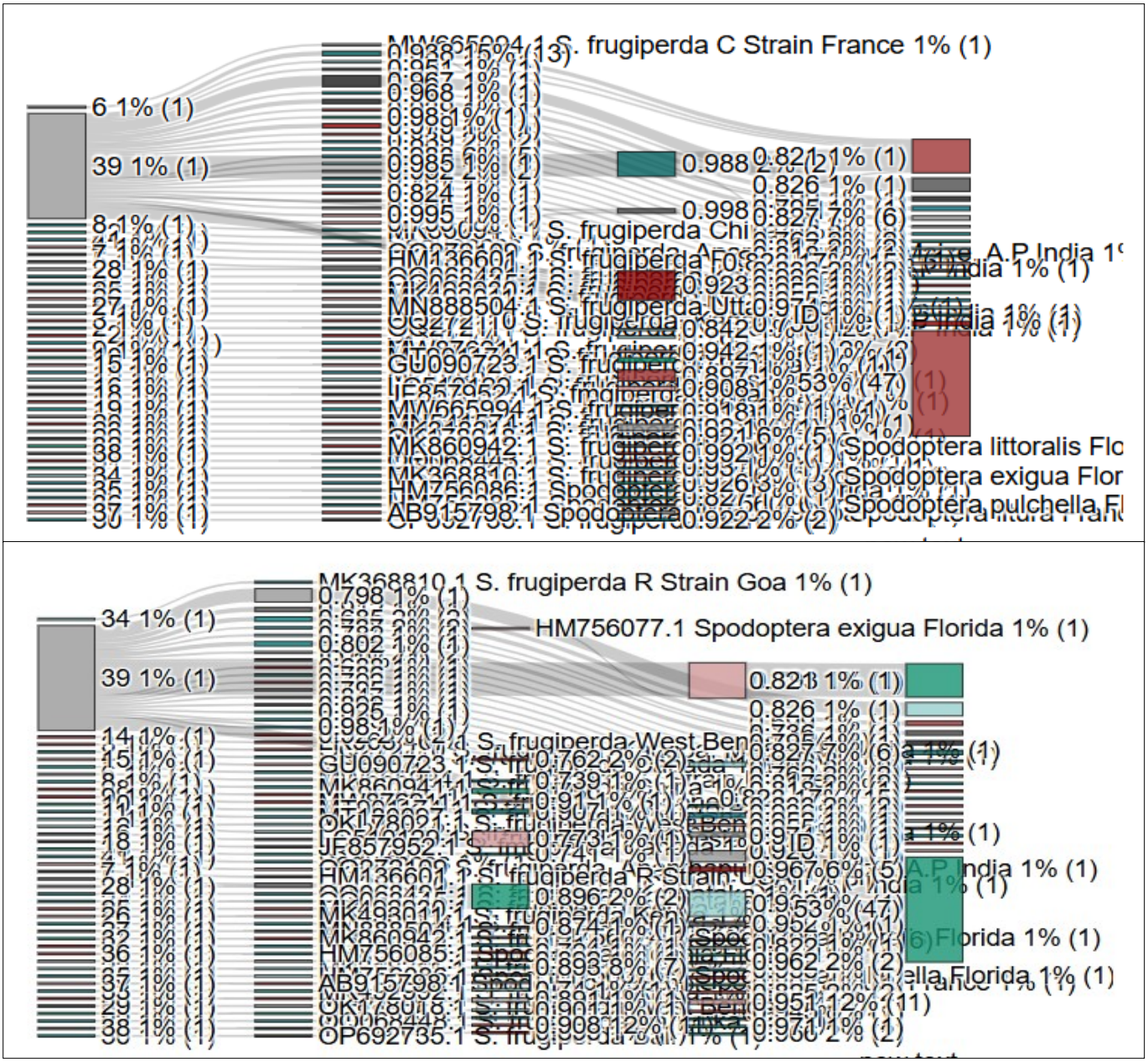


Fig. 6. Per cent C and R strain variation among collected populations of Fall armyworm.

important commercial can be obliterated within no span of time in near future if precautionary measures are procrastinated.

Acknowledgements

The authors are thankful to the Village Agriculture Assistants and Village Horticulture Assistants to help us reach interior villages pertaining to sample collection.

Authors' contributions

BLM carried out the molecular genetic studies, participated in the sequence alignment, molecular analysis, software and drafted the manuscript. NCV formulated the research work, supervised the research and edited the manuscript. MSVC formulated the research, conceptualization of research work drafted the manuscript, RSD, MR and KM conceptualization of research work, data administration, supervision of study, procurement and arrangement of molecular chemicals, reviewed the manuscript. BRM carried out design and methodology and TH formal drafting of manuscript, editing and reviewing of the manuscript. All authors contributed to the interpretation of the results and critically reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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