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Genetic characterization of MEAM1 *Bemisia tabaci* cryptic species infesting cauliflower in Kolar district of Karnataka

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Abstract
The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has become a significant threat to vegetable cultivation, causing serious issues in Indian agriculture. During April 2023, a sample of the *B. tabaci* adults was collected from cauliflower fields in Seethi village, located in the Kolar district, Karnataka. The genomic DNA of a single adult whitefly was extracted using the Chelex 100 method. To identify the cryptic species of the whitefly, the mitochondrial cytochrome oxidase subunit 1 (*mtCOI)* gene was amplified using species-specific primers. PCR amplification generated an expected *mtCOI* gene fragment of 800 bp. Subsequently, the PCR product was subjected to Sanger sequencing. The *mtCOI* nucleotide sequence of the whitefly sample showed 98-100 percent identity with sequences belonging to MEAMI whitefly cryptic species known to infect different crops worldwide. The sequence demarcation graph and phylogenetic analysis provided substantial evidence for the present findings.

Keywords: *Bemisia tabaci,* genetic characterization, MEAM1, cauliflower, phylogeny

1. Introduction
Cauliflower (*Brassica oleracea* var. *botrytis* L.) belonging to the Brassicaceae family is a widely consumed vegetable cultivated from temperate to tropical regions. It is known for its nutritional richness, and contains significant amounts of dietary fibres, vitamins, minerals, and bioactive compounds (Ahmed and Ali, 2013) [1]. Cauliflower attains its distinct flavour due to Glucosinolates, the sulphur compounds which act as plant defensive metabolites in all brassicaceae plants. Glucosinolates breakdown into isothiocyanates and nitriles which are reportedly noxious and toxic to herbivores. However, epidemiological studies indicate that increased consumption of brassicaceae vegetables may lower the risk of cancer in humans (Higdon *et al*., 2007) [17]. The edible portion of cauliflower is called curd which is packed with phenolic acids, flavonoids, carotenoids, ascorbic acid, minerals, proteins, and amino acids, offering health benefits primarily due to their antioxidant properties (Drabinska *et al*., 2021) [10]. Additionally, cauliflower leaves are rich in flavonoids and phenolic acids, hence used to enhance the shelf life of meat products. Their inclusion in malted wheat biscuits and noodles manufacture can elevate the nutritional value without compromising sensorial quality (Wani *et al*., 2013; Zhang *et al*., 2021) [35, 36].

Globally, cauliflower cultivation spans over 1,458,000 hectares, yielding 25,810,000 metric tons, with a productivity of 18.2 metric tons per hectare. In India, the cultivation area extends over 459,000 hectares, producing 9,282,000 metric tons, with 19.8 metric tons per hectare productivity (Anon., 2023) [2]. Following China, India stands as the second largest cauliflower producer globally, both the nations contributing approximately 70 percent of the total production together. In Karnataka State, notable cauliflower cultivation can be observed in Belgaum, Hassan, Chikkaballapur, Tumkur, Kolar, Mysore, Bengaluru Rural, and Bengaluru Urban areas.

Nevertheless, cauliflower cultivation faces significant challenges due to various insect pests *viz*., diamondback moth (*Plutella xylostella*), common cutworm (*Spodoptera litura*), cabbage butterfly (*Pieris brassicae*), cabbage aphid (*Brevicoryne brassicae*), turnip aphid (*Lipaphis erysimi*), cabbage looper (*Trichoplusia ni*), head borer (*Hellula undalis*) and whiteflies (Mahendran *et al*., 2018) [23].

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) is a worldwide horticultural and agricultural pest that directly damages plants by sucking the sap, excreting honeydew and indirectly by transmission of phytopathogenic viruses (Oliveira *et al.* 2001; Dinsdale *et al*. 2010; Esterhuizen *et al*. 2013) [37, 9, 12]. It is an established polyphagous sucking pest of the tropical and subtropical zones of the world and prevalently reported in Europe, Asia, Africa, North, Central and South America and Oceania. Globally, *B. tabaci* is recognized as a species complex comprising of 46 cryptic species so far, of which the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) are two important invasive species (De barro *et al*., 2011; Rehman *et al*., 2021; Hu *et al*., 2023) [8, 27, 19]. Despite the absence of morphological differences, cryptic species can be accountably distinguished in their genetics, development, and behavioural characteristics. MEAM1 cryptic species is also an efficient vector of several viruses, of which, begomoviruses stand out as economically most significant and the most abundant group (Rajeshwari and Reddy, 2014; Fiallo-Olive *et al*., 2020) [26, 14]. Several economically vital crops fall prey to virus species carried by MEAM1 cryptic species, such as tomato yellow leaf curl virus (TYLCV), tomato chlorosis virus (ToCV), cucurbit yellow stunting disorder virus (CYSDV), cucumber vein yellowing virus (CVYV), squash vein yellowing virus (SqVYV), bean golden mosaic virus (BGMV) and the complex of cotton begomoviruses (Jones, 2003; Hasan *et al*., 2019; Sacilotto *et al*., 2022). Hu *et al.* (2023) [20, 16, 31, 19] reported that MEAM1 cryptic species had negative effects on the settlement and oviposition of subsequent conspecifics and heterospecific MED whiteflies, indicating the degraded quality of MEAM1-damaged peppers as a food resource. Additionally, the high reproduction rates and the short developmental period of *B. tabaci* lead to population outbreaks proving the field management to be highly challenging (Oliveira *et al*., 2001) [37].

Owing to this damage caused, a range of molecular techniques has been employed in the last twenty years to define the *B. tabaci* species complex. Among these methods, the predominant approach in recent times involves utilizing the mitochondrial cytochrome oxidase gene subunit-1 (*mtCOI*). In the present study, the *mtCOI* gene of whitefly species, *B. tabaci* associated with cauliflower crop was analysed and the identification of cryptic species was determined through *mtCOI* gene sequence analysis.

2. Materials and Methods

2.1 Source of whiteflies

Adult whiteflies were collected from a cauliflower field using hand held aspirator and then carefully transferred to a 1.5 mL Eppendorf microcentrifuge tube filled with 70 percent ethanol. The tube was securely sealed using Parafilm, and pertinent information such as the sample number, collection location, date, host plant, and additional details were recorded as mentioned in table 2. The sample was transported to the laboratory and preserved at 4 °C for subsequent procedures. The sample used for current study was labelled as K71 Seethi isolate.

2.2 Extraction of DNA and PCR amplification of *mtCOI* gene

The total DNA from the *B. tabaci* adult sample was extracted following the modified Chelex 100 method previously outlined by Rua *et al*. (2006) [30]. Using a camel hair brush, individual whitefly was picked separately from the collecting tube and positioned on parafilm to facilitate the evaporation of ethanol. Subsequently, flies were taken into a petri dish and subjected to one rinse with sodium hypochlorite (0.1%) and two additional rinses with sterile distilled water. A single whitefly was transferred to a 1.5 mL microcentrifuge tube. Each whitefly was homogenized using a micro pestle by adding extraction buffer comprised of 100 µL TE buffer solution containing 5 percent Chelex 100 resin and 300 µg Proteinase K.

The homogenised whitefly sample was incubated at 60 °C for three hours, followed by 10 minutes phase at 96 °C to denature the proteins. After incubation, the homogenized sample was centrifuged for 10 minutes at 13,000 rpm. The resulting upper aqueous supernatant containing DNA was carefully transferred to a fresh tube and stored at -20 °C. The genomic DNA was checked for intactness and quantity using NanoDrop Microvolume Spectrophotometer based on the optical density value (absorbance ration=260/280 nm). The DNA extracted was subjected to PCR using *B. tabaci*-specific *mtCOI* gene primers, the details on primers used, PCR cycle conditions, and the anticipated amplicon size provided in table 1. The PCR mixture consisted of sterile distilled water (17.7 µL), 10 x PCR buffer (2.5 µL), 25 mM $MgCl_2$ (1.5 µL), 2.5 mM dNTP mixture (0.5 µL), primer-F (10 mM) and primer-R (10 mM) 0.625 µL each, Taq polymerase -5 units/µL (0.3 µL) with 1.25 µL of template DNA (100 ng) making final reaction volme of 25 µL. Four microliters of PCR product was electrophoresed on one percent agarose gel stained with ethidium bromide and visualized under gel documentation system. The amplified PCR product specific to the *mtCOI* gene was extracted from the agarose gel using the Qiaquick gel extraction kit (Qiagen, Hilder, USA), and the purified sample was further sequenced bidirectionally at Eurofins Genomics India in Bangalore, India. Sequence was further used to generate the unique barcode using DNA barcode generator by Bio-Rad.

Table 1: Details of *mtCOI* gene specific primers and PCR conditions used in the current study

Primer sequences (5' to 3')	PCR Cycles	Product size (bp)
F-TTGATTTTTTGGTCATCCAGAAGT R-TCCAATGCACTAATCTGCCATATTA	Initial denaturation: 94 ºC for 1 min. Denaturation: 94 ºC for 1 min. Annealing: 55 ºC for 1 min. Extension: 72 ºC for 1 min. Final extension: 72 ºC for15 min. Number of cycles: 35	800 bp

2.3 Sequence Analysis

The *mtCOI* gene sequence of K71 Seethi isolate obtained after sequencing was subjected to BLASTn search to identify similar sequences in the National Centre for Biotechnology Information (NCBI) database, Maryland, United States. Sequences displaying the highest identity were retrieved from NCBI GenBank, aligned using the BioEdit program (Hall, 1999) [15], and used to calculate pairwise percent identity with the *B. tabaci* K71 Seethi isolate utilizing the Sequence Demarcation Tool version 1.2 (SDTv1.2). Phylogenetic tree was constructed by analysing the sequences using the Neighbor-Joining based on Kimura 2 parameter model (Kimura, 1980) [21] with complete gap deletion and resampled with 100 bootstrap replications in MEGA 7 software to explore the relationships among various cryptic species of *B. tabaci* documented till date (Kumar *et al.* 2016) [25]. Sister species *viz*., *B. subdecipiens, B. afer, B. atriplex* were included along with *Trialeurodes vaporariorum* as an outgroup for phylogenetic analysis.

3. Results

The genomic DNA of *B. tabaci* was subjected to PCR for the amplification of *mtCOI* gene using gene-specific primers cited in previous studies by Dinsdale *et al*. (2010) [9], Himler *et al*. (2011) [18] and Ashwathappa *et al*. (2020). The PCR amplification resulted in 800 bp amplicon specific *mtCOI* gene specific primers (Fig.1), which was further sequenced bi-directionally and the consensus sequences (Accession number OR770106) was deposited in NCBI, GenBank. Moreover, DNA barcoding of the *B. tabaci* sequence denoting Kolar district of Karnataka was carried out to serve as an identification tool in DNA taxonomic studies (Table 2). Comparative analysis was performed by comparing the *mtCOI* gene sequence of the *B. tabaci* K71 Seethi isolate with the corresponding *mtCOI* region of 30 different *B. tabaci* cryptic species sourced from the NCBI database. Results from the sequence comparison revealed a shared identity of 98 to 100 percent between the current *B. tabaci* Seethi isolate *mtCOI* gene sequence with other MEAM1 cryptic species previously reported from various regions such as Pakistan (AJ510075), the USA (MW024941.1, MW024938.1), Israel (AY766373), China (KM821540.1), and different parts of India (AJ748368) (Table 3). Additionally, a comparison was made between *B. tabaci* K71 Seethi isolate and 23 other *B. tabaci mtCOI* gene sequences of different cryptic species obtained from the NCBI database using SDTv1.2 (Fig. 2). The pairwise identity of the query sequence with the retrieved consensus sequences is provided in table 3. Subsequent phylogenetic analysis of the *mtCOI* gene sequences, alongside selected reference cryptic species (Table 3) indicated that the K71 Seethi isolate clustered closely with the MEAM1 cryptic species of *B. tabaci* with 3.5 percent sequence divergence (Dinsdale *et al*., 2010) [9] (Fig. 3).

Table 2: Details of the whitefly, *B. tabaci* sample collected

Sl. No	Sample detail	
1.	Sample name	K71
2.	Place of collection	Seethi, Kolar taluk, Kolar District
3.	Host	Cauliflower
4.	GPS coordinates	13.23N,78.02E
5.	Year of collection	2023
6.	Age of the crop (days)	50 days
7.	Cropping system	Monocropping
8.	Area (Acre)	1.5
9.	Surrounding crops	Pole beans, Gooseberry, Chilli, Bottle gourd
10.	NCBI Accession ID	OR770106
11.	DNA Barcode	

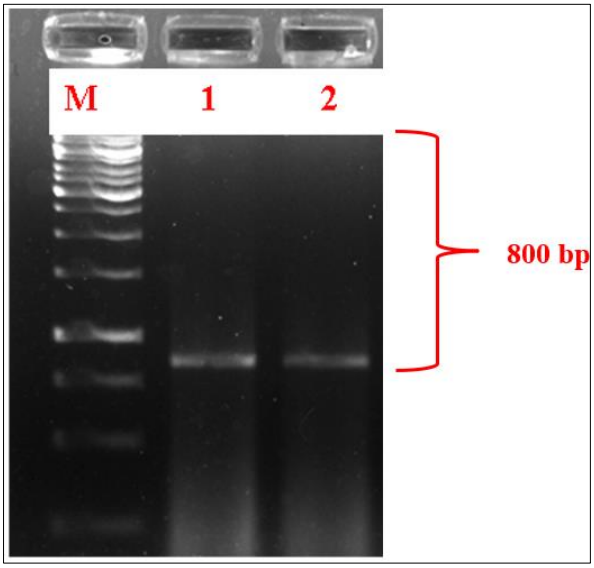

Fig 1: PCR amplification of mtCOI gene of *B. tabaci* K71 Seethi isolate using specific primers

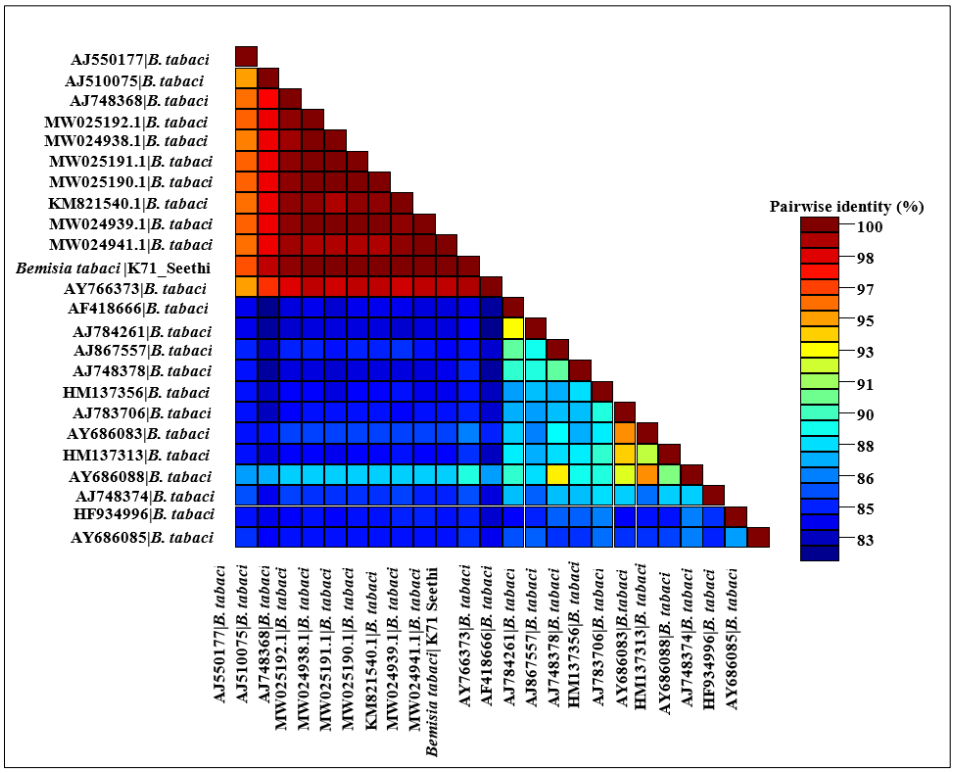

Fig 2: Graphical representation of percentage pairwise genomic scores and nucleotide identity plot of *B. tabaci* K71 Seethi isolate collected from cucumber plot compared with reference sequences using Sequence Demarcation Tool (SDTv1.0)

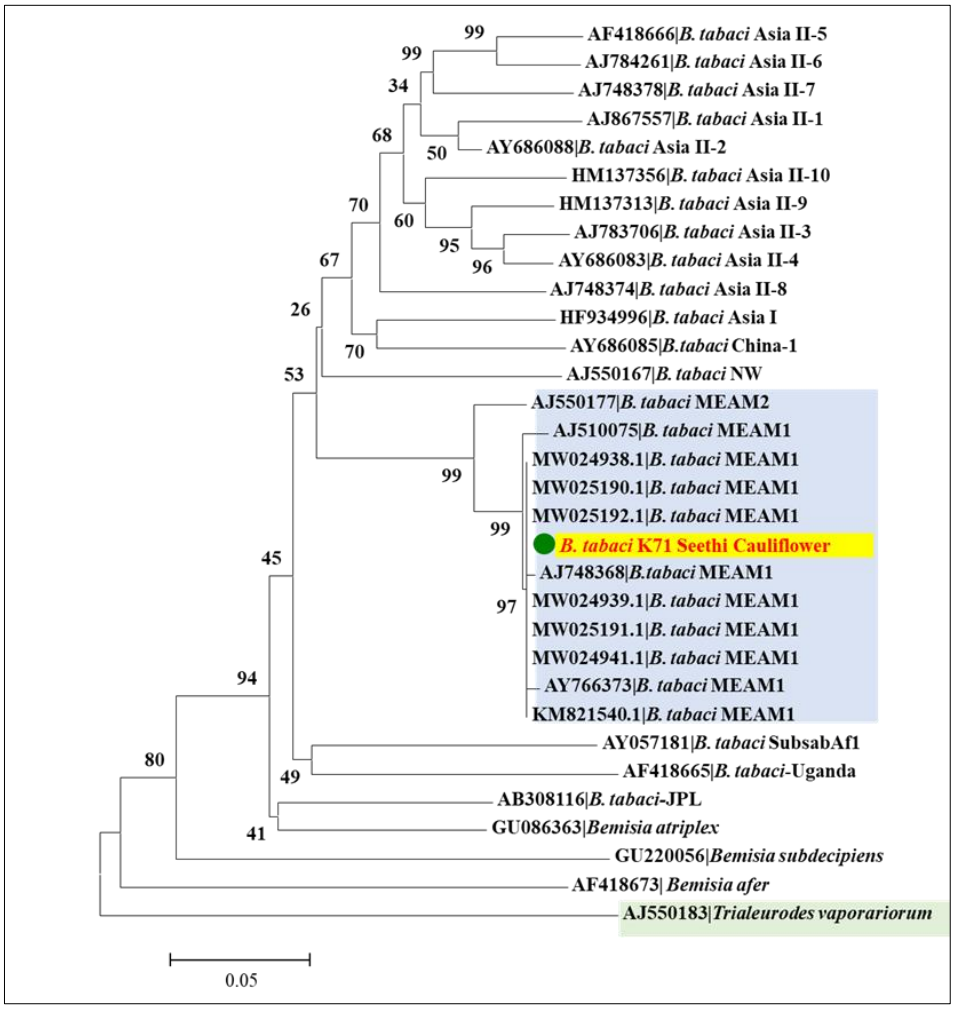

Fig 3: Neighbor-Joining tree constructed with *mt*COI gene nucleotide sequences depicting the phylogenetic relationship of *B. tabaci* K71 Seethi isolate with other cryptic species of *B. tabaci*

Table 3: The *mtCOI* gene sequences of *B. tabaci* cryptic species employed in the phylogenetic analysis and Percent nucleotide identity of *B. tabaci* K71 Seethi isolate with *mtCOI* gene nucleotide sequences of cryptic species retrieved from NCBI

Sl. No.	Organism	Accession Number	Country	Genetic subgroup of reference whitefly	Percent nucleotide identity of *B. tabaci* K71 Seethi isolate (%)
1.	*B. tabaci*	KM821540.1	China	MEAM-1	100
2.	*B. tabaci*	MW024941.1	USA	MEAM-1	100
3.	*B. tabaci*	MW025191.1	USA	MEAM-1	100
4.	*B. tabaci*	MW024939.1	USA	MEAM-1	100
5.	*B. tabaci*	AJ748368	India	MEAM-1	99.71
6.	*B. tabaci*	AY766373	Israel	MEAM-1	99.26
7.	*B. tabaci*	MW025192.1	USA	MEAM-1	99.91
8.	*B. tabaci*	MW025190.1	USA	MEAM-1	100
9.	*B. tabaci*	MW024938.1	USA	MEAM-1	100
10.	*B. tabaci*	AJ510075	Pakistan	MEAM-1	98.82
11.	*B. tabaci*	AJ550177	Reunion	MEAM-2	95.42
12.	*B. tabaci*	AF418666	India	Asia II-5	84.96
13.	*B. tabaci*	AJ867557	China	Asia II-1	85.14
14.	*B. tabaci*	AJ783706	China	Asia II-3	85.33
15.	*B. tabaci*	AY686083	China	Asia II-4	84.52
16.	*B. tabaci*	AY686088	China	Asia II-2	89.78
17.	*B. tabaci*	AJ784261	China	Asia II-6	84.96
18.	*B. tabaci*	AJ748378	India	Asia II-7	85.28
19.	*B. tabaci*	AJ748374	India	Asia II-8	86.28
20.	*B. tabaci*	HM137313	China	Asia II-9	85.64
21.	*B. tabaci*	HM137356	China	Asia II-10	85.19
22.	*B. tabaci*	AY686085	China	China-1	85.69
23.	*B. tabaci*	AY057181	Uganda	SubsabAf1	83.50
24.	*B. tabaci*	AJ550167	Colombia	New world	84.42
25.	*B. tabaci*	AF418665	Uganda	-	83
26.	*B. tabaci*	AB308116	Japan	JPL	85.53
27.	*B. subdecipiens*	GU220056	Spain	-	77.88
28.	*B. afer*	AF418673	Uganda	-	77
29.	*B. atriplex*	GU086363	Spain	-	85.40
30.	*T. vaporariorum*	AJ550183	Reunion	-	73.74

4. Discussion

First incidence of the MEAM1 cryptic species of *B. tabaci* in South India was reported by Banks *et al.* (2001) [5] and subsequently confirmed by Rekha *et al.* (2005) [28] and Shankarappa *et al.* (2007) [32]. This line of investigation was continued by Chowda Reddy *et al.* (2012) [6], who utilized RAPD-PCR to detect the presence of Asia I, Asia II-5, Asia II-7, Asia II-8, and MEAM1 cryptic species in whitefly samples collected across 31 locations in India. Whitefly cryptic species, including MEAM1, Asia I, Asia II-1, Asia II-5, Asia II-7, Asia II-8, and Asia II-11, were also identified based on *mtCOI* gene sequences, as reported by Ellango *et al.* (2015) [11] and Prasanna *et al.* (2015) [25]. A survey conducted by Sujatha *et al.* (2021) [33] in the tomato fields of Tippuru village, Bengaluru Rural district, confirmed the presence of MEAM1. Likewise, our findings align with the findings of Roopa *et al.* (2015) [29], who examined 71 *B. tabaci* samples to assess the prevalence of Asia I, Asia II-7, Asia II-8, and MEAM1 cryptic species across various host crops in India. The similarity observed between the K71 Seethi population with the MEAM1 population from other countries is in consonance with the findings of Ashfaq *et al.* (2014) [3] who reported "Pakistan lineage" MEAM1 cryptic species from cotton fields of Pakistan.

Multiple factors like encompassing fertility, egg to adult stage survival, virus transmission efficiency, and notably, susceptibility to pesticides and sensitivity to parasites, influence the prevalence of distinct cryptic species across various geographical locations. Reports suggest that differential levels of pesticide resistance can result in the redistribution and replacement of specific whitefly populations (Crowder *et al*., 2010) [7].

This study enhances our comprehension of the *B. tabaci* species makeup in Seethi village, situated in Kolar Taluk, Kolar District, Karnataka. A more comprehensive survey in this region could reveal a higher diversity of *B. tabaci* cryptic species than previously documented. Moreover, it is imperative to identify cryptic species in diverse regions within Karnataka State and comprehend the interplay between viruses and associated symbionts. The adoption of innovative methodologies such as genomics, proteomics, metabolomics, and transcriptomics holds promise in unravelling the intricate interactions occurring during virus transmission by vector insects.

5. Conclusion

The findings obtained from PCR amplification *mtCOI* of *B. tabaci* and subsequent nucleotide identity analysis, and phylogenetic investigations confirm the presence of the MEAM1 cryptic species in Seethi village, Kolar district of Karnataka. The data could serve as a valuable resource for monitoring alterations in the abundance of MEAM1 cryptic species and their displacement patterns in the future. Moreover, the DNA barcoding performed in this study assists in understanding the genetic sequences of specimens, serving as a useful tool to quickly identify and confirm insect-pests. This would complement traditional ways of classifying agricultural pest. However, dealing with the challenges arising from variations in species identity and strains makes it hard to effectively manage insect-pest issues within integrated

pest management and monitoring systems. The survey and analysis carried out in this study offer a thorough understanding of identifying the whitefly, *B. tabaci* in Kolar region. This knowledge forms a foundation for better monitoring future patterns related to the diversity, migration, abundance, and displacement of the MEAM1 cryptic species, ultimately contributing to the successful management of *B. tabaci* MEAM1 cryptic species in India.

6. Acknowledgement

6.1 Conflict of interests

The authors declare that they have no competing interests.

6.2 Human and animal rights

This article does not contain any studies with human and animal subjects performed by any of the authors.

6.3 Informed consent

Informed consent was obtained from all individual participants included in the study.

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