

## Original Research Article

### Bacterial diversity ~~of-of the~~ silverleaf whitefly, *Bemisia tabaci* (Gennadius), collected on ~~on~~ jasmine and chrysanthemum

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#### ABSTRACT

The adults and nymphs of *Bemisia tabaci* were collected on jasmine and chrysanthemum during ~~2021-2023~~. Bacterial colonies were isolated from adults and nymphs of *B. tabaci* using spread-plate technique and identified through 16srRNA sequencing. The *B. tabaci* collected on jasmine harboured more number of bacteria (8 ~~bacteriaspecies~~) than chrysanthemum (5 ~~bacteriaspecies~~). ~~Most~~ maximum bacterial ~~species~~ isolated from *B. tabaci*, collected on jasmine and chrysanthemum, ~~were~~ belonged to the phylum Bacillota (62.5 and 60 ~~per centpercent~~). Similarly, Bacilli, ~~Bacillales-Bacillales~~ and Bacillaceae were the dominant class, order and family ~~of the bacteria~~ associated with *B. tabaci*, ~~collected on jasmine and chrysanthemum~~. Nymphs harboured ~~more~~ higher number of bacteria than adults in both jasmine (62.5 and 33.5 ~~per centpercent~~, respectively) and chrysanthemum (60 and 40 ~~per centpercent~~, respectively). *Bacillus cereus* and *B. pumilus* were ~~found~~ commonly ~~found species among from~~ the nymphs and adults of *B. tabaci* ~~collected~~ on jasmine. When, insect ~~shifts to different hosts~~ ~~ss~~ shifted, change in the nutrition and defence system of ~~the~~ host plant influences the bacterial diversity of the insect.

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#### 1. INTRODUCTION

Whitefly (Hemiptera: Aleyrodidae) is one of the most economically important groups of pests with global distribution and very wide range of host plants (Kanakala and Ghanim, 2019)[7]. It causes damage ~~in an active way~~ by acting as ~~the~~ vector for various plant viruses (*Begomovirus*, *Crini virus*, *Closterovirus* etc.) and ~~by~~ passively ~~by~~ encouraging sooty mould deposits on plants through honeydew secretion (Head and Savinelli, 2008)[6]. ~~Later, the sooty mould formed by the honeydew~~ ~~secreted by them~~ leads to the closing of stomata ~~and~~ as a result, ~~it as a result the gas exchange by the plants will be~~ interrupted and leads to poor development of ~~the~~ plants. ~~The~~ silverleaf whitefly, *Bemisia tabaci* (Gennadius), ~~Central Asia originated and has~~ invaded major host crops ~~in Central Asia? including of this pests are~~ field crops (Green gram, Soybean, Blackgram, ~~etc.~~), vegetables (Tomato, Chilli, Bhenḍi, Brinjal, Beans, Gourds, ~~etc.~~), flower crops (Chrysanthemum, Jasmine, Marigold ~~etc.~~) and commercial crops (Cotton, Tobacco, Jute ~~etc.~~); ~~and in the area its~~ infestation on plantation crops is rarely seen. Currently, there are 40 cryptic species ~~of B. tabaci~~ (morphologically indistinguishable but genetically distinct ~~in biological characteristic~~ species)

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~~has been~~ recorded in ~~B. tabaci~~...~~where?~~... The Middle East–Asia Minor 1 (MEAM1) and Mediterranean (MED) complexes (previously known as B biotype and Q biotype, respectively) are considered as ~~the most~~ invasive species with broad host range of plants. New world 1 (NW 1, A biotype) is also reported in some parts of the world (~~De-Barro et al., 2011~~)[4].

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**Comment [A8]:** Which bacteria are they?

One of the factors for successful establishment of *B. tabaci* is ~~its nutritional flexibility~~. The two main functions of ~~these endosymbionts~~ of sap sucking insects are; those which are beneficial to the insect under specific ecological conditions and those which play a role in metabolic activities of the insect. (Gosalbes *et al.*, 2010)[5]. Along with this, the microbes inside the insects play a major role in their survival, development, reproduction, fecundity, viral transmission and resistance ~~of the host insects?~~ against the various chemicals. About 99 percent of symbiotic bacteria are non-culturable under laboratory conditions (Amann *et al.*, 1995)[1] but advances in molecular biology have outstandingly improved the culture-independent techniques to study microorganisms, all praises to PCR amplification of bacterial genes straight from environmental samples, pursued by direct sequencing of PCR products. Different gene targets like 16S, 23S, ~~and GroEL etc.~~ have been used to identify bacteria. Several studies have used PCR techniques to identify the different endosymbionts like *Portieraaleyrodidarum* (Primary), *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Fritschea*, *Bacillus*, *Staphylococcus*, *Enterococcus* (Secondary) in *B. tabaci*. The current study is giving special emphasis on the diversity of facultative bacteria in the nymphs and adults of *B. tabaci* on tomato.

## 2. MATERIAL AND METHODS

The whiteflies and nymphs collected on jasmine and chrysanthemum from different ~~locations~~ were starved for 3 h and surface sterilized with 70 per cent ethanol for 1 minute followed by 0.1 per cent sodium hypochlorite for 1 minute and then rinsed with sterile distilled water for 2 to 3 times to remove the external microbes and wax. ~~(...To make up a good paragraph, please add in here two or three more sentences; preferably those specifically pinpointing the locations including the names of the state/province and the country name...)~~

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### 2.1. Serial dilution and plating

~~The surface-sterilized~~ adults ~~B. tabaci?~~ were ~~surface-sterilized and~~ crushed in a sterilized 1.5 ml micro-centrifuge tube using a sterilized micro pestle with 1 ml of phosphate buffer saline (PBS) solution (pH 7.4). Prior to that, micro-centrifuge tubes were labelled with date, host and location. The homogenized samples were centrifuged at 2000 RPM for 10 minutes. Then, 100 µl of the homogenized mixture was added to micro centrifuge tubes containing 900 µl of sterile distilled water and serial dilution of samples was made up to 10<sup>-7</sup> dilutions. 100 µl of aliquot of all the dilutions were plated on both 1M of nutrient agar media and spread using a sterilized glass spreader. ~~Then,~~ Petri plates were ~~then~~ incubated at 28 °C for 24 to 48 h in bio-oxygen demand (BOD) incubator. ~~Further,~~ ~~The~~ plates were observed for microbial growth after ~~wards~~ every 24 hours.

**Comment [A11]:** How many individuals *B. tabaci* that were used?

## 2.2. Purification and storage of colonies of bacteria

Representative colonies from each bacterial colony showing similar morphology were selected and pure cultures were obtained by sub-culturing them in the same media. The pure cultures were added to autoclaved nutrient broth in sterilized test tubes along with respective labels and incubated at 28 °C for 24 h in BOD until the clear nutrient broth turn into turbid by the multiplication of bacterial cells.

## 2.3. Bacterial genomic DNA isolation and quantification

Bacterial culture grown in a nutrient broth was used for genomic DNA isolation by following sucrose buffer method. 1.5 ml bacterial culture was transferred to a sterilized micro centrifuge tube with respective label and centrifuged at 1000 rpm for 3 minutes to get a pellet. Later, supernatant was discarded and pellet was retained. It was repeated with a 1.5 ml culture to collect the sufficient amount of pellet. The pellet was re-suspended into 400 µl sucrose buffer (consists of 1M Tris, 0.5M EDTA and 10 per cent sucrose) and subjected to vortex (SPINIX) to dissolve the pellet. Then, 32µl lysozyme was added and incubated for 10 min at 60°C in hot water bath. 140 µl of freshly prepared 10 per cent sodium dodecyl sulphate (SDS) was added along with 5 µl of protease. Later, 240 µl of NaCl (5M) and freshly prepared 10 per cent CTAB was added and incubated for 10 min at 60 °C. It was followed by addition of 500 µl chloroform: isoamyl alcohol (24:1) and mixed well by inverting the tube until the phase is mixed completely. The mixture was centrifuged at 12000 rpm in a micro centrifuge (SPINWIN MC03) for 10 min. Upper aqueous phase was transferred to a new labelled tube and 50µl of 3M sodium acetate (ice cold) was added and mixed well. Then 300 µl isopropanol (ice cold) was added and gently mixed to precipitate DNA and the sample was incubated overnight at -20°C.

The sample was spun at 12000 rpm for 15 min on the next day, to pellet down DNA and 1ml of 70 percent ethanol was added to the pellet and spinning was done at 12000 rpm for 10 min (twice). Then the supernatant was discarded and the pellet was allowed for air dry. After complete drying, the DNA pellet was re-suspended in 30 µl of protease, DNase, RNase, free water (GeNei™) followed by 2 µl of RNase treatment and incubation at 60 °C in water bath stored at -20 °C until use (Takakura and Nishio, 2012)[11]. The concentration of isolated DNA was quantified by using nanodrop.

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## 2.4. Quality and quantity check of genomic DNA

The quality of genomic DNA was checked by 0.8 percent (0.8g in 100 ml) of agarose which was dissolved in 100 ml of 1X TAE buffer in microwave oven and 5 µl EtBr was added after cooling. This mixture was poured into a pre-set template used with appropriate comb kept on the template, to make wells and the gel was allowed for solidification for 45 minutes. After that, 2µl of DNA was loaded with 2 µl of loading dye (6X Cresol-red DNA loading dye). Electrophoresis was carried at 80 V for 45 min. The

genomic DNA was visualized on UV transilluminator (Bio-Rad, USA) and documented using gel documentation system (GelDoc Go).

The amplification of 16s rRNA was carried out by using the universal primer (Forward-5'AGAGTTTGATCCTGGCTCAG3' and Reverse-5' ACGGCTACCTTGTTACGACTT - 3'). The stocks of primers were prepared as per the instructions given and prepared a working primers by adding 0.1 ml of stock in 0.9 ml double distilled water, further stored at -20 °C. Polymerase chain reactions were performed with 25 µl of PCR mixture in PCR system (ProFlex) with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles each consisting of denaturation for 1 minute at 94°C, annealing for 45 seconds at 59°C with an extension for 1.5 minute at 72°C followed by final extension for 10 minutes at 72 °C and kept hold at 4 °C for infinite time. The amplified PCR products were sent for nucleotide sequencing to Eurofins Genomics India Pvt. Ltd. Bangalore. The obtained DNA sequences corresponding to the 16S rRNA gene was confirmed using BLAST search in NCBI. The obtained forward and reverse sequences were aligned together using the NCBI alignment tool to obtain a contig sequence.

### 3. RESULTS AND DISCUSSION

#### 3.1. Molecular identification of bacterial colonies

Amplification of the 16srRNA gene gave a fragment of about 1500bp. The PCR amplified products were sequenced by using Sanger's Dideoxy sequencing method and obtained results were compared with standard GenBank data base by following NCBI BLAST and species were confirmed.

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#### 3.2. Host-wise bacterial abundance of *B. tabaci* collected on jasmine and chrysanthemum

The *B. tabaci* population was collected from Jasmine harbouring the more rest bacteria (8 bacteriaspecies), than Chrysanthemum (5 bacteriaspecies) (Table 1).

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#### 3.3. Comparison of bacterial diversity of *B. tabaci* collected on jasmine and chrysanthemum at phylum level

The phylum Bacillota accounted for 62.5 percent and 60 percent of the bacterial population in *B. tabaci* collected on Jasmine and Chrysanthemum, respectively. While, Actinomycetota was found only in *B. tabaci* collected on Jasmine with 25 percent abundance. Whereas, whereas, Pseudomonadota was found with 12.5 percent in Jasmine and 40 percent in Chrysanthemum. (Fig. 1a and 2 a).

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#### 3.4. Comparison of bacterial diversity of *B. tabaci* collected on jasmine and chrysanthemum at class level

The class Bacilli was found dominant in *B. tabaci* (Fig. 1b and 2b). Bacilli from *B. tabaci* collected on jasmine and on chrysanthemum contributed to at least 62.5 and 60 percent of bacterial diversity, respectively followed by Gamma-proteobacteria accounted for, respectively, 40 percent (on chrysanthemum) and 12.5 percent (on jasmine) of the diversity. 25 percent of the bacterial diversity was contributed to by the class Actinomycetes or Actinomycetota that were collected from *B. tabaci* on jasmine. (25 percent) in Jasmine, Gamma-proteobacteria (40 percent) in chrysanthemum. Whereas, Gamma-proteobacteria was accounted only 12.5 percent in jasmine (Fig. 1b and 2b).

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### 3.5. Comparison of bacterial diversity of *B. tabaci* collected on jasmine and chrysanthemum at order level

In both, *B. tabaci* collected on jasmine and chrysanthemum, the order Bacillales was found dominant (62.5 and 60 percent, respectively). Followed by Enterobacteriales in Chrysanthemum (40 percent), Micrococcales in Jasmine (25 percent). The order Micrococcales observed only in *B. tabaci* collected on jasmine and Enterobacteriales accounted for only 12.5 percent in jasmine (Fig. 1c and 2c).

### 3.6. Comparison of bacterial diversity of *B. tabaci* collected on jasmine and chrysanthemum at family level

The family, Bacillaceae was recorded abundant in *B. tabaci* collected on jasmine and chrysanthemum (62.5 and 60 percent), respectively. The families, ~~Yersiniaceae, Microbacteriaceae~~ were found only on jasmine with 12.5 and 25 percent abundance respectively. On chrysanthemum, Enterobacteriaceae was found exclusively and accounted for 40 percent (Fig. 1d and 2d).

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### 3.7. Comparison of bacterial diversity of *B. tabaci* collected on jasmine and chrysanthemum at genus and species level

The genus *Bacillus*, was found dominant on both jasmine and chrysanthemum. Overall bacterial species diversity revealed that, nymphs harbored more bacteria than adults. Where, nymphs of *B. tabaci* collected on jasmine had 62.50 percent of the bacteria and adults had 37.50 percent (Fig. 1e). In chrysanthemum, 60 percent of the bacteria were recorded from nymphs and 40 percent from adults (Fig. 2e). The bacterial species, *Bacillus cereus* and *B. pumilus* were found common among adults and nymphs of *B. tabaci* collected on jasmine (Fig. 3). Whereas, in *B. tabaci* collected on chrysanthemum, no such commonality was observed

(Fig. 4).

The diversity of microbes in insects varies with host on which it feeds, habitat, the pressure of biotic and abiotic factors (Early and Sax, 2014). In the present study, the bacterial diversity of *B. tabaci* varied with hosts. Change in the diversity of bacteria was observed by Pujar *et al.* (2023)[9] in rugose spiraling whitefly, *Aleurodicus rugioperculatus* collected on coconut, banana, maize and arecanut. In the present study, *B. tabaci* collected on jasmine and chrysanthemum showed high dominance of Bacillota, Bacilli, Bacillales and Bacillaceae. As the *Bacillus* sp. helps in the production of medium-length sugars from derived sucrose and increase the stickiness of honeydew (Davidson *et al.*, 2000<sup>[4]</sup>). This can be reason for the abundance of *Bacillus* sp. in *B. tabaci*. These results are in accordance with the report of Pujar *et al.* (2024)[10], who recorded bacterial diversity of *B. tabaci* on tomato. Variation in the bacterial diversity in the *B. tabaci* due to change in the host nutrition and defense posed by the host plant against insect. Host plants have a positive impact on the shaping of microbial communities associated with *Spodopteralittoralis* (Tang *et al.* 2012)[12], *Helicoverpa* spp. (Priya *et al.*, 2012[8]; Tang *et al.*, 2012[12]; Xiang *et al.*, 2006[13]), *Lymantria dispar* (Broderick *et al.*, 2004<sup>[2]</sup>; Mason and Raffa, 2014) and *Leptinotarsa decemlineata* (Chung *et al.*, 2017<sup>[3]</sup>). Plant characters such as leaf surface, wax composition and the availability of sugars in plants might influence bacterial community composition in the host insect (Lindow and Brandl, 2003).

#### 4. Conclusion

This study ~~enables with deep~~ presents better understanding of the bacteria associated with ~~the~~ *B. tabaci* on jasmine and chrysanthemum. The change in bacterial diversity in different host crops have made a channel to study about how the bacteria are obtained ~~at from~~ different crops and eliminated from ~~their body?~~. Understanding the specific functions of each bacteria and the transmission patterns will be a suitable area for future research.

#### REFERENCES

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2. Broderick NA, Raffa KF, Goodman RM, Handelsman J., Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Appl Environ. Microbiol.* 2004;70 (1): 293 - 330.

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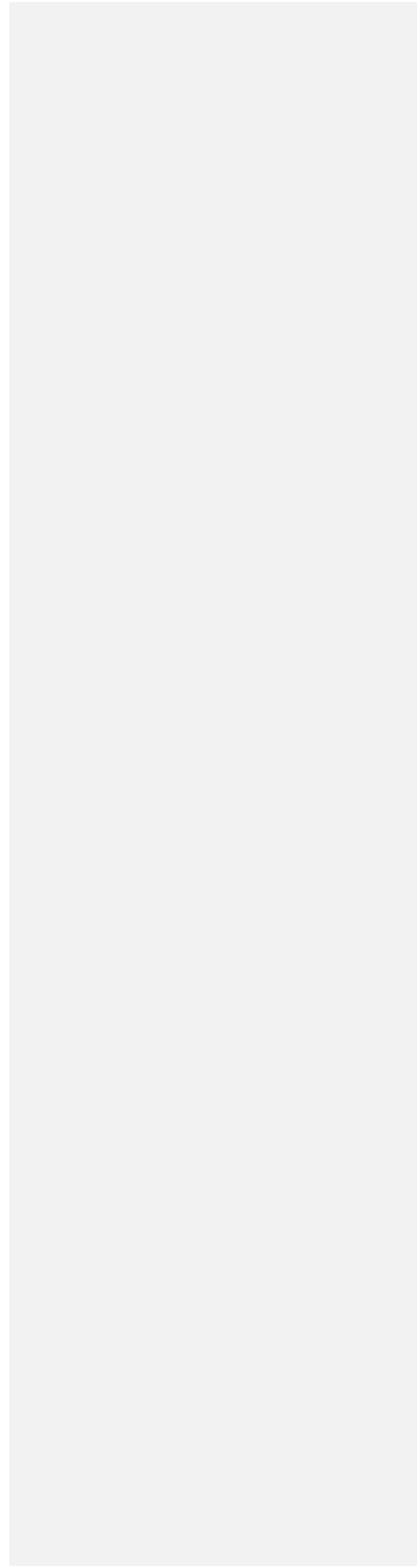
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**Comment [A28]:** Whose body? Insect body as the host of the bacteria or crop or plant body that hosts the insect from which the bacteria are isolated?

**Comment [A29]:** There exist inconsistency between the list in the References and the citations in the text. Please check and re-check .

3. Chung SH, Scully ED, Peiffer M, Geib SM, Rosa C, Hoover K, Felton GW. Host plant species determines symbiotic bacterial community mediating suppression of plant defenses. *Sci. Rep.*, 2017; 7(1): 1 - 13.
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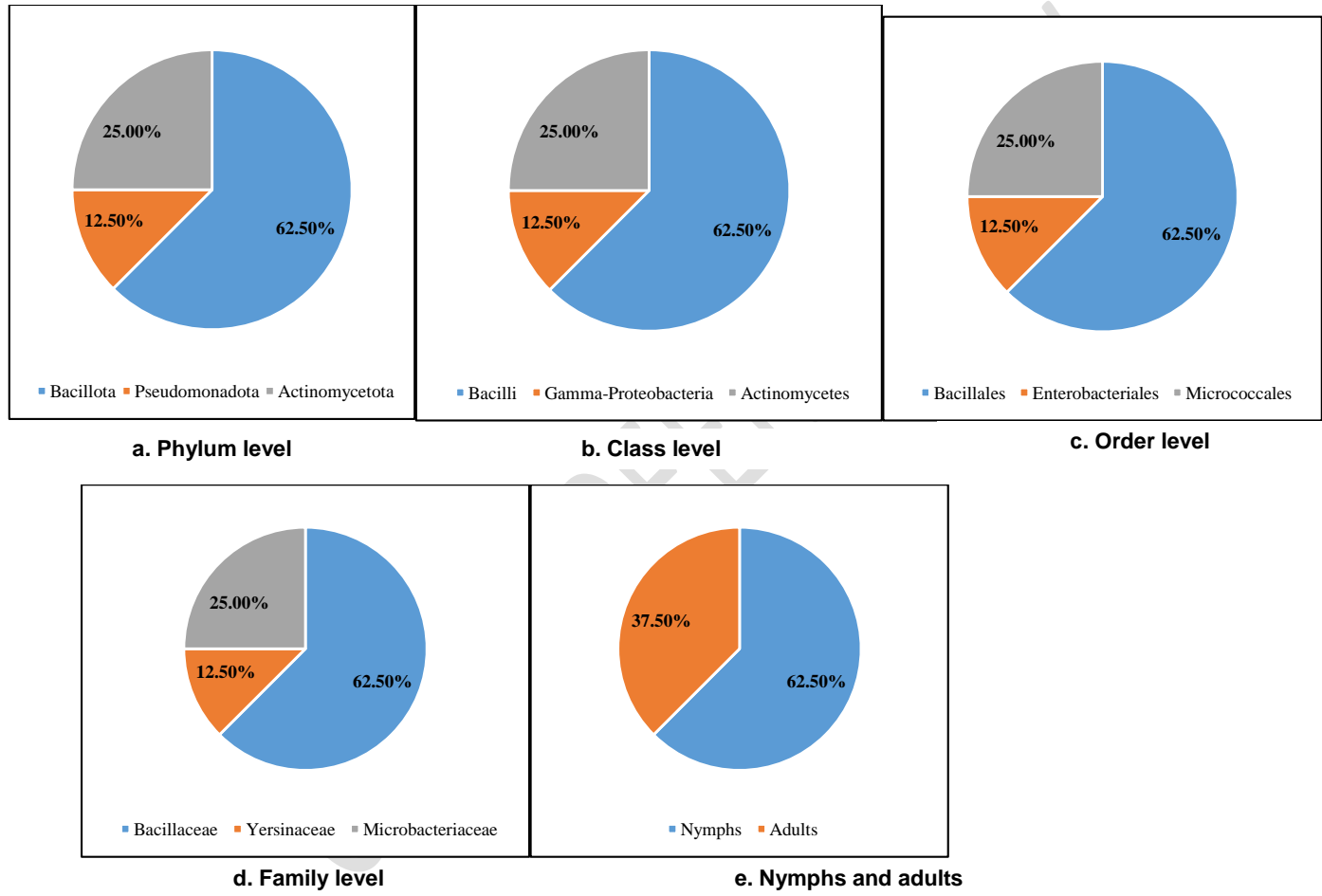
**Table 1. Bacterial diversity of *B. tabaci* collected on Jasmine**

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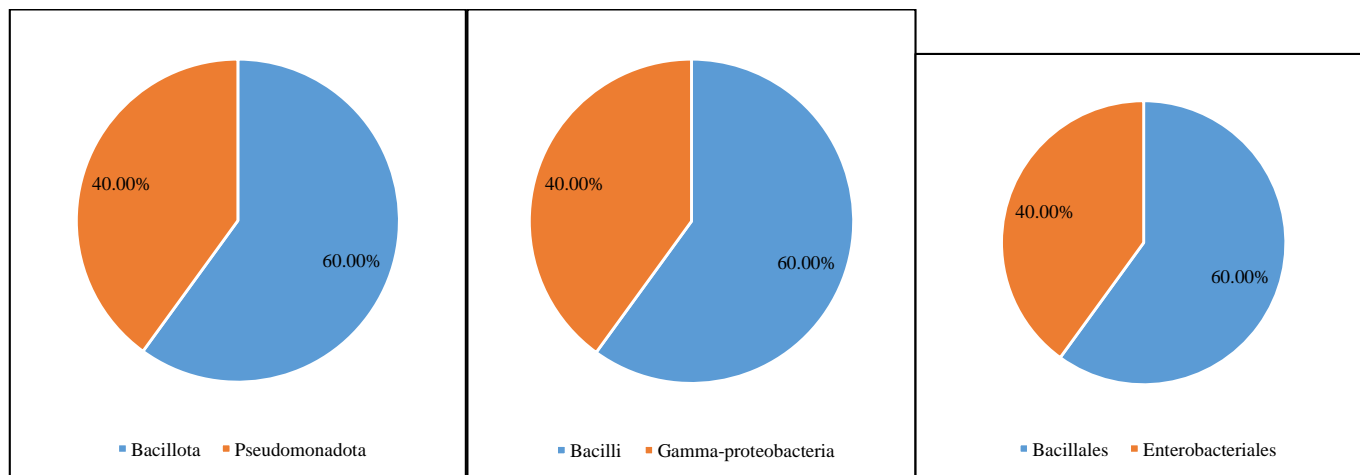
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Jasmine(8)					
Phylum	Class	Order	Family	Species	
				Nymphs(5)	Adults(3)
Bacillota	Bacilli	Bacillales	<i>Bacillaceae</i> (5)	<i>Bacillus zhangzhouensis</i>	<i>Bacillus cereus</i>
				<i>Bacillus cereus</i>	<i>Bacillus pumilus</i>
				<i>Bacillus pumilus</i>	
Pseudomonadota	Gamma-Proteobacteria	Enterobacteriales	Yersiniaceae(1)	<i>Serratiamarcescens</i>	
Actinomycetota	Actinomycetales	Micrococcales	Microbacteriaceae (2)	<i>Curtobacterium luteum</i>	<i>Microbacterium proteolyticum</i>
Chrysanthemum(5)					
Phylum	Class	Order	Family	Species	
				Nymphs(3)	Adults(2)
Bacillota/ Firmicutes	Bacilli	Bacillales	<i>Bacillaceae</i> (3)	<i>Bacillus pumilus</i>	
				<i>Terribacillus saccharophilus</i>	
				<i>Bacillus licheniformis</i>	
Pseudomonadota	Gamma-Proteobacteria	Enterobacteriales	Enterobacteriaceae (2)		<i>Enterobacter hormaechei</i>
					<i>Klebsiella variicola</i>

\*Values in parenthesis indicate the number of species



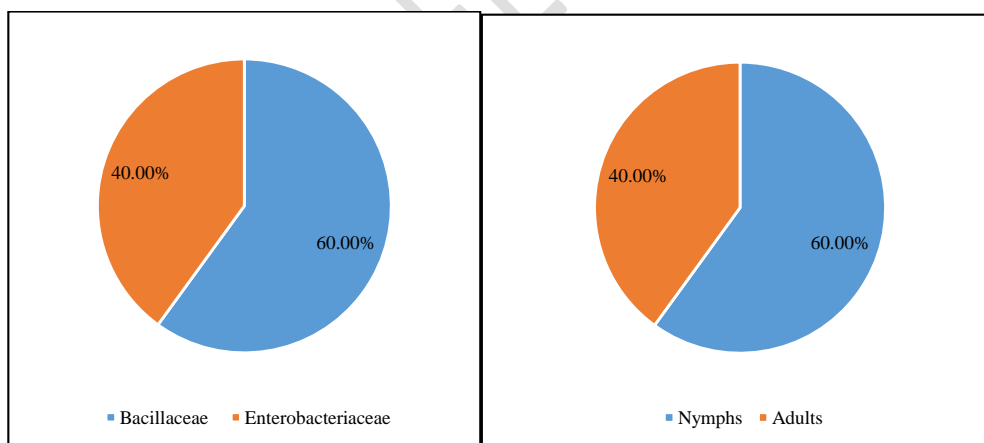
**Fig.1. Diversity of bacteria in *B. tabaci* collected on Jasmine crop**



**a. Phylum level**

**b. Class level**

**c. Order level**



**d. Family level**

**e. Nymphs and adults**

**Fig.2. Diversity of bacteria in *B. tabaci* collected on Jasmine crop**

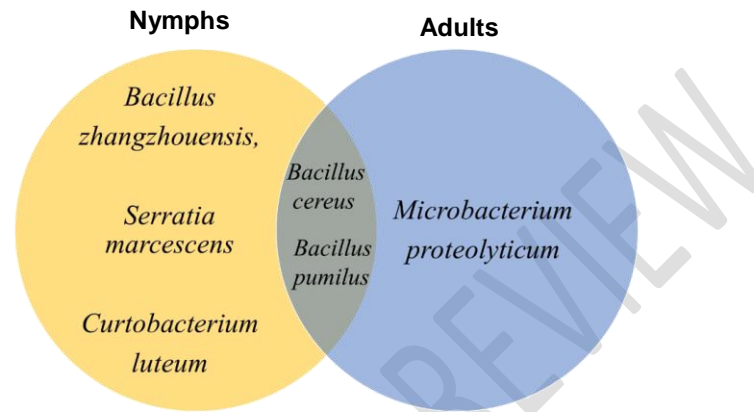


Fig. 3. Species-Bacterial diversity inef adults and nymphs of *B. tabaci* collected on jasmine

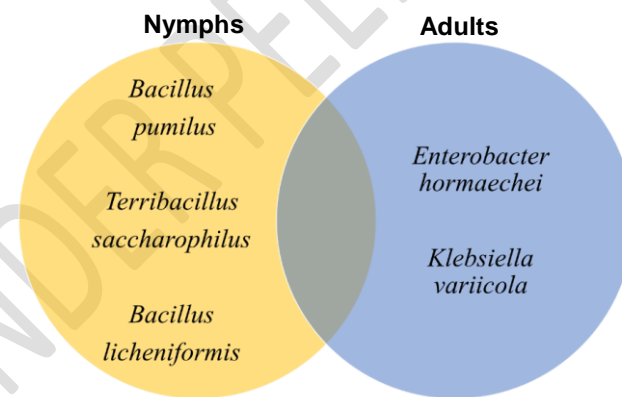


Fig. 4. Species-Bacterial diversity inef adults and nymphs of *B. tabaci* collected on chrysanthemum

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