Original Research Article

Bacterial diversity of silverleaf whitefly, *Bemisiatabaci*(Gennadius) collected on jasmine and chrysanthemum

ABSTRACT

The adults and nymphs of *Bemisiatabaci* 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 bacteria) than chrysanthemum (5 bacteria). Maximum bacteria isolated from *B. tabaci*, collected on jasmine and chrysanthemum were belong to the phylum Bacillota (62.5 and 60 per cent). ~~Similarly, Bacilli, Baclillales and Bacillaceae were the dominant class, order and family~~ in *B. tabaci*, collected on jasmine and chrysanthemum. Nymphs harboured more number of bacteria than adults in both jasmine (62.5 and 33.5 per cent, respectively) and chrysanthemum (60 and 40 per cent, respectively). *Bacillus cereus* and *B. pumilus* were found common among the nymphs and adults of *B. tabaci* collected on jasmine. When, insect shifts to different hosts, change in the nutrition and defence system of host plant influences the bacterial diversity of the insect.

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 vector for various plant viruses (*Begomovirus*, *Crini* virus, *Closterovirus etc*.) and 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 as a result the gas exchange by the plants will be interrupted and leads to poor development of plants. The silverleaf whitefly, *Bemisiatabaci*(Gennadius), Central Asia originated and invaded major host crops of this pests are field crops (Green gram, Soybean, Blackgram, etc.), vegetables (Tomato, Chilli, Bhendi, Brinjal, Beans, Gourds, etc.), flower crops (Chrysanthemum, Jasmine, Marigold etc.) and commercial crops (Cotton, Tobacco, Jute etc.) and infestation on plantation crops is rarely seen. Currently there are 40 cryptic species (morphologically indistinguishable but genetically distinct in biological characteristic species) has been recorded in *B. tabaci*. 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].

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 plays major role in their survival, development, reproduction, fecundity, viral transmission and resistance against the various chemicals. About 99 per cent 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, 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.

2.1. Serial dilution and plating

The surface sterilized adults were 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^{-7} 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 incubated at 28 ^{0}C for 24 to 48 h in bio-oxygen demand (BOD) incubator. Further, plates were observed for microbial growth after every 24 hours.

2.2. Purification and storage of colonies of bacteria

Representative colony from each colonies showing similar morphology were selected and pure culture was obtained by sub-culturing it 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 ℃ 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℃ 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 ℃. 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℃.

The sample was spun at 12000 rpm for 15 min on the next day, to pellet down DNA and 1ml of 70 per cent 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 ^{TM}) followed by 2 µl of RNase treatment and incubation at 60 ℃in water bath stored at -20 ℃ until use (Takakura and Nishio, 2012)[11]. The concentration of isolated DNA was quantified by using nanodrop.

2.4. Quality and quantity check of genomic DNA

Quality of genomic DNA was checked by 0.8 per cent (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 ℃. 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 ^{0}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.

3.2. Host-wisebacterialabundanceof*B.tabaci*collectedon jasmine and chrysanthemum

The *B. tabaci*population was collected from Jasmineharbouringthemostbacteria(8 bacteria),than Chrysanthemum (5 bacteria) (Table 1).

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

The phylum Bacillota accounted for 62.5 per cent and 60 per cent of the bacterialpopulation in *B. tabaci* collected on Jasmine and Chrysanthemum. While,Actinomycetota was found only in *B. tabaci* collected on Jasmine with 25 per cent abundance, whereas, Pseudomonadota12.5 per cent in Jasmine and 40 per cent in Chrysanthemum. (Fig.1a and 2 a).

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

TheclassBacillifounddominantin*B. tabaci* collected on jasmine and chrysanthemum(62.5and 60 percent, respectively) followed by Actinomycetes (25 per cent) in Jasmine, Gamma-proteobacteria (40 per cent) in chrysanthemum.Whereas, Gamma-proteobacteria was accounted only 12.5 per cent in jasmine (Fig. 1b and 2b).

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 per cent), Micrococcales in Jasmine (25 per cent). The order Micrococcales observed only in *B. tabaci* collected on jasmine and Enterobacteriales accounted for only 12.5 per cent 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 per cent), respectively. The families, Yersinaceae, Microbacteriaceae were found only on jasmine with 12.5 and 25 per cent abundance respectively. On chrysanthemum, Enterobacteriaceae was found exclusively and accounted for 40 per cent (Fig. 1d and 2d).

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 per cent of the bacteria and adults had 37.50 per cent (Fig.1e). In chrysanthemum, 60 per cent of the bacteria were recorded from nymphs and 40 per cent from nymphs (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).

Thediversityofmicrobesintheinsectsvarieswithhostsonwhichitfeeds,habitat,thepressureofbioticandabioticfactors(EarlyandSax,2014).Inthepresentstudy,the bacterial diversity of*B. tabaci* varied with hosts. Change in the diversity of bacteriawas observed by Pujar *et al*. (2023)[9] in rugose spiraling whitefly, *Aleurodicusrugioperculatus* 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[4]). 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[2]; Mason and Raffa, 2014) and *Leptinotarsadecemlineata* (Chung *et al*., 2017[3]). 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 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 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.

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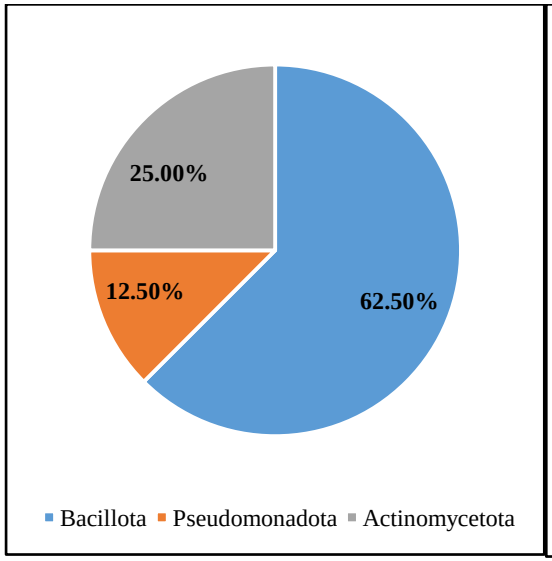
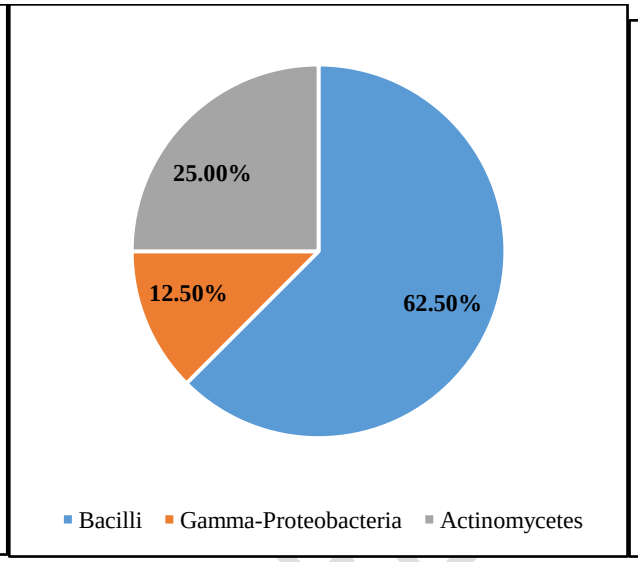
Table 1. Bacterial diversity of *B. tabaci*collected on Jasmine

Jasmine(8)					
Phylum	**Class**	**Order**	**Family**	**Species**	
				Nymphs(5)	**Adults(3)**
Bacillota	Bacilli	Bacillales	*Bacillaceae*(5)	*Bacillus zhangzhouensis*	*Bacilluscereus*
				Bacilluscereus	*Bacillus pumilus*
				Bacillus pumilus	
Pseudomonadota	Gamma-Proteobacteria	Enterobacteriales	Yersinaceae(1)	*Serratiamarcescens*	
Actinomycetota	Actinomycetes	Micrococcales	Microbacteriaceae (2)	*Curtobacterium luteum*	*Microbacterium proteolyticum*
Chrysanthemum(5)					
Phylum	**Class**	**Order**	**Family**	**Species**	
				Nymphs(3)	**Adults(2)**
Bacillota/ Firmicutes	Bacilli	Bacillales	Bacillaceae(3)	*Bacillus pumilus*	
				Terribacillus saccharophilus	
				Bacillus licheniformis	
Pseudomonadota	Gamma-Proteobacteria	Enterobacteriales	Enterobacteriaceae (2)		*Enterobacterhormaechei*
					Klebsiellavariicola

*Values in parenthesis indicates the number of species



a. Phylum level



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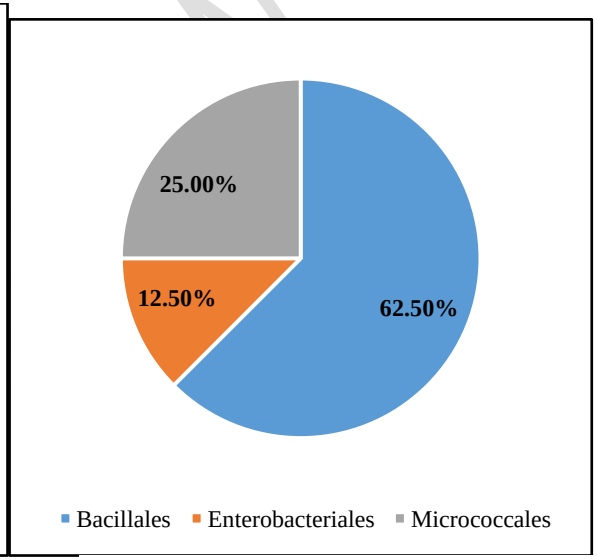
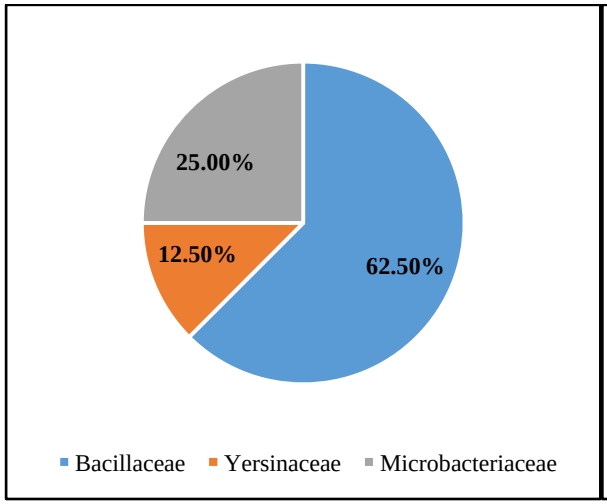
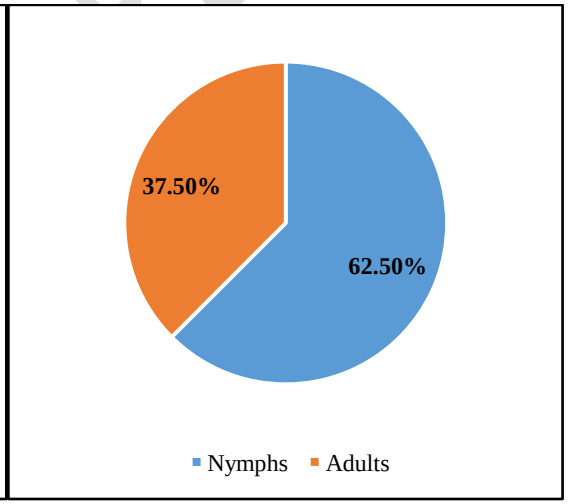


c. Order level

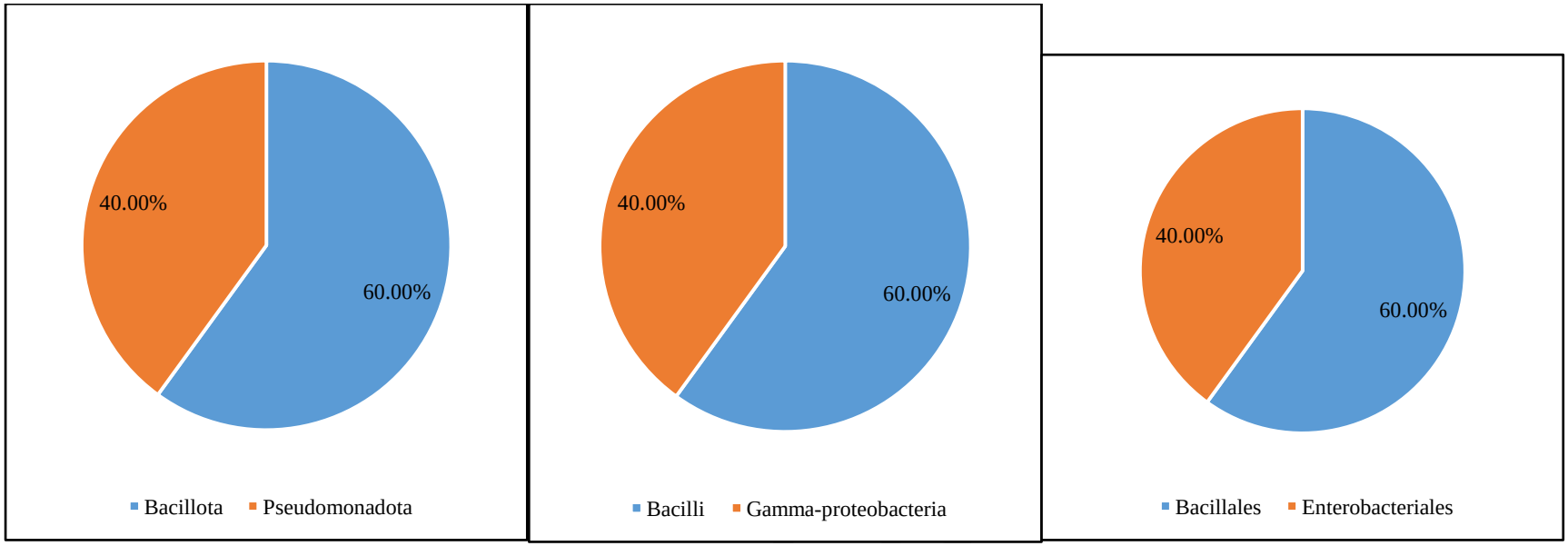


d. Family level

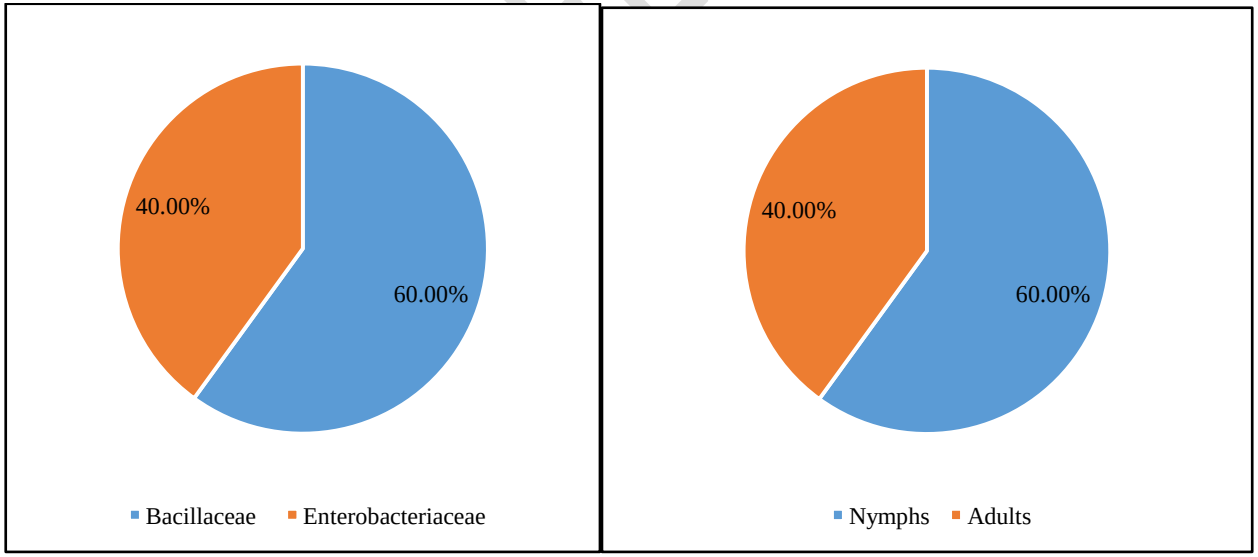


e. Nymphs and adults

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

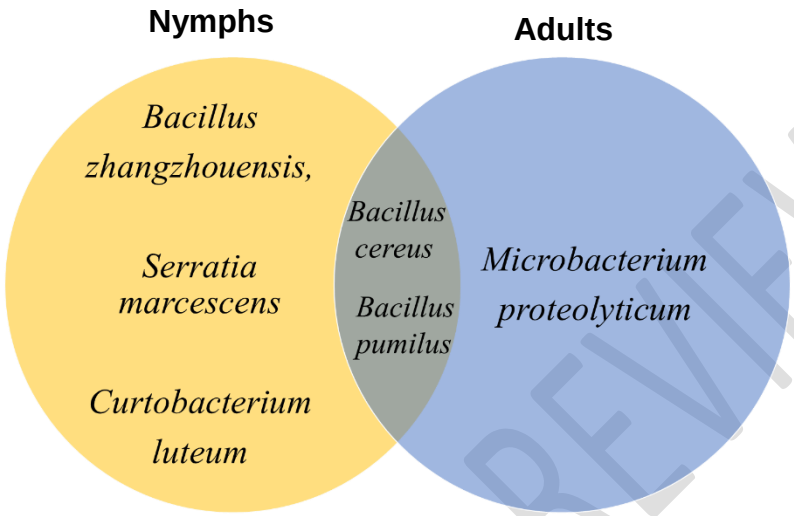


Fig. 3. Species diversity of adults and nymphs of *B. tabaci* collected on jasmine

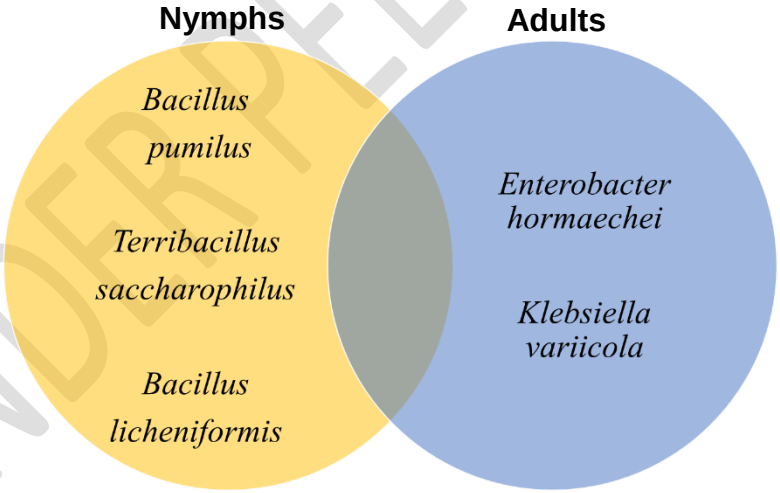


Fig. 4. Species diversity of adults and nymphs of *B. tabaci* collected on chrysanthemum