Plant Archives Vol. 26, No. 1, 2026 pp. 2271-2284 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.315

DECIPHERING PESTICIDE-INDUCED GUT MICROBIOTA DYSBIOSIS IN *BOMBYX MORI* L. THROUGH 16S *RRNA* AMPLICON SEQUENCING

K. Nisarga[1*], K.S. Vinoda[1], R. Narayana Reddy[1], B. Shivanna[2], K. Ranganatha[1] and K. G. Banuprakash[1]

[1]Department of Sericulture, College of Agriculture, UAS, GKVK, Bengaluru-65, Karnataka, India
[2] Department of Agricultural Entomology, College of Agriculture, UAS, GKVK, Bengaluru-65, Karnataka, India
*Corresponding author E-mail: nisarganisarga207@gmail.com
(Date of Receiving : 21-01-2026; Date of Revision : 07-03-2026; Date of Acceptance : 26-03-2026)

ABSTRACT

The gut microbiota engages in complex interactions with its host, influencing essential physiological processes such as digestion, nutrient assimilation, immunity, and detoxification. Pesticide residues can disrupt this microbial equilibrium by altering the composition and structure of the gut microbial community. The present study investigated the impact of pesticide residues on the gut microbiota of the silkworm, *Bombyx mori* L., with the objective of determining a safe post-application waiting period. Gut bacteria were isolated from fifth-instar larvae and characterized using morphological and molecular approaches. A total of 74 bacterial isolates were obtained, representing three major phyla: Pseudomonadota (Proteobacteria), Bacillota (Firmicutes) and Bacteroidota (Bacteroidetes). The dominant genera identified were *Serratia*, *Acinetobacter*, *Bacillus* and *Chryseobacterium*. Phylogenetic analysis grouped the isolates into two major clades corresponding to Proteobacteria and Firmicutes. Exposure to pesticide residues caused significant alterations in the gut microbial community, characterized by a decline in beneficial taxa and an enrichment of pesticide-resistant genera. Temporal analysis revealed that microbial diversity reached its lowest level at 8 days after spraying (DAS) and gradually recovered by 21 DAS, indicating a transient disruption of gut microbial homeostasis. Based on these findings, a safe waiting period of 15/21 days after pesticide application is recommended to minimize adverse effects on gut microbiota and to ensure optimal silkworm health and growth.
Keywords: *Bombyx mori L*, pesticide residues, microbial diversity, safety period.

Introduction

The insect gut represents a highly complex and dynamic microbial ecosystem composed of bacteria, fungi, archaea, protozoa and viruses among which bacteria constitute the most dominant and functionally significant group (Kaltenpoth and Florez, 2020). These gut-associated microbes form intimate symbiotic relationships with their hosts and play a central role in maintaining host homeostasis by regulating essential physiological processes such as digestion, nutrient assimilation, immunity and detoxification (Shan *et al*., 2021; Arasakumar *et al*., 2018). In particular, gut bacteria contribute to host growth and development through the synthesis of essential amino acids, vitamins and metabolic enzymes involved in carbohydrate and lipid metabolism, while also enhancing immune competence by limiting pathogen colonization, degrading harmful compounds and modulating inflammatory responses (Koboziev *et al*., 2014; Michaudel and Sokol, 2020). In addition, these microbes play a crucial role in activating host immune responses by inducing the synthesis of antimicrobial peptides and modulating key signaling pathways that protect against pathogenic invasion (Zhang *et al*., 2024). In lepidopteran insects such as the silkworm, *Bombyx mori* L., this microbial consortium plays a crucial role in facilitating efficient utilization of mulberry leaf nutrients and supporting physiological stability under both normal and stress conditions.

The composition and stability of gut microbiota in silkworms are highly influenced by multiple factors, including diet quality, rearing environment, developmental stage and exposure to chemical agents (Li *et al*., 2020). In recent years, due to environmental pollution and pesticide drift, the composition of the insect gut microbiota has been disrupted (Bundschuh *et al*., 2012; Yang *et al*., 2020; Zhu *et al*., 2020). Exposure to phoxim has altered the structure of the silkworm gut bacterial community, significantly reducing the relative abundance of dominant bacteria, making silkworm more susceptible to disease (Li *et al*., 2020). The microbiota composition and diversity of bees are also affected by pesticides (Kakumanu *et al*., 2016) and exposure to fipronil results in pesticide absorption in the gut, causing an imbalance in the gut microbiota and affecting the growth and development of bees (Rouze *et al*., 2019) furthermore, widespread pesticide use can lead to lepidopteran pests developing resistance (Arias *et al*., 2019). The ingestion of pesticide residues through mulberry foliage possess a major risk to the gut microbial balance. Pesticides, though essential for pest management in mulberry cultivation, may persist as residues in the leaves, which are later used as feed for silkworms. The consumption of contaminated foliage can lead to significant disturbances in the gut microbial community, causing a reduction in beneficial taxa, alteration in enzymatic activity and impairment of detoxification and immune mechanisms (Bhosale *et al*., 1988; Bing *et al*., 2010).

Chemical pesticides such as organophosphates, carbamates and pyrethroids exert toxic effects not only on pest species but also on non-target organisms like *B. mori*. Sublethal exposure to these residues can disrupt gut microbial homeostasis, resulting in dysbiosis that compromises larval growth, nutrient metabolism and silk productivity. The imbalance in gut microbiota may also weaken disease resistance and interfere with the physiological adaptation of the silkworm to environmental stress. Furthermore, pesticides may selectively favor the growth of resistant microbial strains while suppressing symbiotic bacteria, thereby altering the ecological balance of the intestinal system (Tian *et al*., 2025).

Recent advancements in molecular biology and sequencing technologies have provided valuable tools for characterizing the microbial diversity within insect guts. The 16S rRNA gene sequencing approach, in particular, has enabled accurate identification and phylogenetic classification of bacterial isolates, offering insights into how chemical exposure influences microbial composition. Studies on pesticide–microbiome interactions in insects suggest that certain gut bacteria may participate in pesticide degradation but chronic exposure tends to suppress microbial diversity and metabolic activity (Zhu *et al*., 2024). Understanding these effects is crucial to evaluate the toxicological implications of pesticide residues and their long-term consequences on silkworm health and silk production.

Material and Methods

Experimental Design

The chawki silkworms of bivoltine double hybrid (*FC2* × *FC1*) were sourced from a registered Chawki Rearing Centre (CRC) and reared at the Department of Sericulture, College of Agriculture, GKVK, Bengaluru, following the recommended package of practices Dandin and Giridhar (2014). Each treatment group consisted of three biological replicates, with fifty larvae per replication. The silkworms were fed on mulberry leaves (*Morus alba* L.) of V-1 variety collected from the treatment plots sprayed with different pesticides (Diafenthiuron 50 % WP; Abamectin 1.9 % EC; Dimethoate 30 % EC; Cyanopyrafen 30 % SC; Azadirachtin 1 % EC). The leaves were harvested at varying intervals after spraying of the chemicals (8, 15 and 21 DAS). The leaves from untreated plots served as control. Feeding of treated leaves commenced from the second feed of the third instar onwards.

Collection and Preparation of Gut Samples

The fifth instar larvae were collected on the third day after the fourth moult from each replication of different treatments and brought to the Insect Molecular Biology Laboratory, Department of Agricultural Entomology, College of Agriculture, GKVK, Bengaluru for gut microbial analysis. The larvae were surface sterilized with 70 % ethanol for one minute, followed by 0.1 % sodium hypochlorite and rinsed thrice with sterile distilled water to remove external contaminants. The midgut was dissected aseptically under a laminar airflow chamber using sterile instruments.

Isolation of Gut Microbes

Each dissected gut was transferred into a sterile 1.5 mL microcentrifuge tube containing 1 mL of phosphate-buffered saline (PBS, pH 7.4). The gut tissues were homogenized using a sterile micropestle. Serial dilutions were prepared up to 10^{-7} and 100 µL from each dilution was plated onto nutrient agar (NA) for bacterial isolation. The spread plate technique was employed to evenly distribute the inoculum across the agar surface. The plates were incubated at 28°C for 24

hours in a BOD incubator and observed for bacterial growth and colony morphology.

Purification and Maintenance of Isolates

Distinct bacterial colonies exhibiting variations in shape, size, margin, elevation, and color were selected and purified by subculturing on NA plates using the streak plate method. The purified isolates were incubated at 28°C for 24 hours. The pure cultures were maintained on nutrient agar slants and stored at 4°C for short-term use. For long-term preservation, bacterial cultures were suspended in 50 % glycerol and stored at −20°C (Jorquera *et al*., 2019).

Morphological and Gram Characterization

The colony morphology of the isolates was recorded based on visual parameters such as color, size, texture and margin. Gram staining was performed following the standard procedure of Becerra *et al*. (2016). The stained smears were observed under a light microscope at 100× magnification using immersion oil to differentiate Gram-positive and Gram-negative bacteria.

DNA Extraction by CTAB Method

The total DNA was extracted from bacterial colonies by inoculating a single colony into nutrient broth and incubating at 37 °C for 24 h. A 1.5 ml of culture was centrifuged at 10,000 rpm for 3 min to obtain a pellet that was resuspended in 400 µL sucrose buffer, vortexed, treated with 32 µL lysozyme and incubated at 60 °C for 10 min. Subsequently, 45 µL of 10% SDS and 5 µL proteinase K were added and the mixture was incubated again at 60 °C for 10 min followed by the addition of 240 µL NaCl and 140 µL freshly prepared 10% CTAB and incubation in a water bath for 10 min. To the suspension, a 500 µL chloroform: isoamyl alcohol (24:1) was added, mixed thoroughly and centrifuged at 12,000 rpm for 10 min. The upper aqueous phase was transferred to a new tube and DNA was precipitated by adding 50 µL of 3 M sodium acetate and 300 µL of isopropanol with gentle mixing and overnight incubation at −20 °C, followed by centrifugation at 12,000 rpm for 15 min. The supernatant was discarded and the DNA pellet was thoroughly washed twice with 1 mL of 70% ethanol, air-dried and resuspended in 40 µL TE buffer. The isolated DNA samples were subjected to RNase treatment (2 µL) and incubated at 37 °C for 30 min (Takakura and Nishio, 2012).

The 16S rRNA gene was amplified from bacterial colonies by PCR using universal eubacterial primer pairs 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1495R (5′-ACGGCTACCTTGTTACGACTT-3′). The PCR was carried out in 50 µL reaction volumes containing 1.5 mM of each primer, approximately 15 ng of template DNA, 5 µL Taq buffer and 1.5 µL Taq DNA polymerase. The PCR cycling conditions consisted of an initial denaturation at 95 °C for 1 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Later, the PCR products were subjected to agarose gel electrophoresis where 2 µL aliquots of each PCR product were resolved on 1% agarose gel using 10× TAE buffer, visualized under UV transilluminator and photographed using a gel documentation system (Gel Doc 200, Bio-Rad, USA). The PCR products were stained with 0.5 µg mL^{-1} ethidium bromide. A 1 kb DNA ladder (Promega) was used as a DNA molecular weight marker to determine the size of the amplified fragments (Garibyan and Avashia, 2013).

Sequencing and Phylogenetic Analysis of the 16S rRNA Gene

Nucleotide sequencing was performed at Eurofins Genomics India Pvt. Ltd., Bangalore. The obtained 16S rRNA gene sequences were verified using the Basic Local Alignment Search Tool (BLAST) to determine sequence similarity. High-quality sequences with maximum coverage were deposited in the NCBI GenBank database, and accession numbers were obtained for all bacterial isolates (Matsumoto *et al*., 2013)

Phylogenetic relationships among the bacterial strains were inferred by constructing a Neighbor-Joining tree using MEGA version 11.0.13 software, with 1,000 bootstrap replications to assess the robustness of the tree topology.

Statistical Analysis

The data collected from the experimental mulberry garden and silkworm rearing were subjected to analysis of variance (ANOVA) following the procedures outlined by Sundaraaj *et al*. (1972). The level of significance used in the F-test was P = 0.05 and Critical Difference (CD) values were computed wherever the F-test was found significant to compare treatment means. Statistical analyses were carried out using OPSTAT software and the results were interpreted and graphically represented using Microsoft Excel (Version 2021).

Results

The morphological and molecular characterization of gut microbiota was carried out to assess the impact of pesticidal toxicity on microbial dysbiosis and the results are narrated under this section.

Morphological Characterization of Gut Bacteria of *B. mori*

The gut microbiota of 5th instar *B. mori* larvae showed 74 bacterial isolates that were characterized based on colony morphology, including shape, size, elevation, margin pattern and colour. Majority of the bacterial colonies were red, yellow-orange or white in colour with circular shape and smooth or irregular margins. Most colonies were translucent, while a few showed opaque elevations. Gram staining results indicated that a greater proportion of the bacterial isolates were gram-negative (85.1 %) (Table 1).

Molecular Characterization of Gut Bacteria of *B. mori*

DNA extracted from the gut bacterial isolates when amplified with 16S rRNA gene produced clear amplicons of approximately 1200 bp, as confirmed by agarose gel electrophoresis. The amplified products were sequenced and the obtained sequences were aligned using BioEdit software application and compared with reference sequences in the NCBI GenBank database. BLAST analysis revealed sequence similarity ranging from 95.98 % to 99.93 % with query coverage between 1034 and 1540 bp. The isolates were identified as belonging to the genera *Serratia*, *Acinetobacter*, *Bacillus*, *Chryseobacterium*, *Enterobacter*, *Stenotrophomonas* and *Pseudomonas* and all isolates showing more than 95 % similarity were confirmed at the species level for further phylogenetic analysis (Table 2).

Phylogenetic Tree Analysis of Gut Bacteria of *B. mori*

The phylogenetic relationships of gut bacterial isolates from 5th instar *B. mori* larvae were inferred using the Maximum Likelihood method with the Tamura–Nei model in MEGA11. The analysis (27 nucleotide sequences; 1740 positions in the final dataset) produced a tree with robust support based on 1000 bootstrap replications. Isolates grouped into major clades corresponding to Pseudomonadota (Proteobacteria) and Bacillota (Firmicutes), the Pseudomonadota clade included *Serratia*, *Acinetobacter*, *Stenotrophomonas*, *Enterobacter*, *Pseudomonas* and *Aeromonas*, while the Bacillota clade comprised *Bacillus* and *Clostridium* species. A distinct cluster of Chryseobacterium formed a separate lineage under Bacteroidota (Bacteroidetes). Internal branch bootstrap values were generally moderate to high, confirming the phylogenetic grouping and taxonomic placements of the pesticide-associated gut bacteria (Fig. 1).

Diversity of gut microbes of *B. mori* larva

The gut isolates of *B. mori* larvae reared on mulberry leaves of different treatment plots belonged to eleven genera under three phyla, namely Pseudomonadota (synonym Proteobacteria), Bacillota (synonym Firmicutes) and Bacteroidota (synonym Bacteroidetes). A significantly larger number of bacterial species including *Enterobacter asburiae, Acinetobacter calcoaceticus, Serratia marcescens, Serratia nematodiphila, Stenotrophomonas maltophilia, Pseudomonas fluorescens.*

Table 1 : Morphological characterization of gut bacterial species of silkworm, *B. mori* with selected chemicals

Sl.no	Bacterial species	Colour	Shape	Margin	Elevation	Opacity	Gram staining
1	*Serratia marcescens*	Red	Circular	Smooth	Raised	Translucent	Negative
2	*Serratia nematodiphila*	Red	Circular	Smooth	Raised	Translucent	Negative
3	*Chryseobacterium* sp.	Yellow	Circular	Smooth	Raised	Translucent	Negative
4	*Bacillus cereus*	White	Circular	irregular	Flat	Opaque	Positive
5	*Acinetobacter johnsonii*	Off White	Circular	Smooth	Flat	Translucent	Negative
6	*Acinetobacter* sp.	Off White	Circular	Smooth	Flat	Translucent	Negative
7	*Chryseobacterium bernardetii*	Yellow orange	Circular	Smooth	Raised	Translucent	Negative
8	*Serratia* sp.	Red	Circular	Smooth	Raised	Translucent	Negative
9	*Stenotrophomonas maltophilia*	White	Circular	Smooth	Raised	Opaque	Negative
10	*Stenotrophomonas* sp.	White	Circular	Smooth	Raised	Opaque	Negative
11	*Pseudomonas fluorescens*	Off white	Circular	Smooth	Flat	Opaque	Negative
12	*Serratia surfactantfaciens*	Red	Circular	Smooth	Raised	Translucent	Negative
13	*Aeromonas hydrophila*	Yellow	Circular	Smooth	Raised	Opaque	Negative
14	*Klebsiella pneumoniae*	Creamy white	Circular	Smooth	Raised	Opaque	Negative
15	*Serratia ureilytica*	Red	Circular	Smooth	Raised	Translucent	Negative

16	*Enterobacter cloacae*	White	Circular	Smooth	Flat	Translucent	Negative
17	*Chryseobacterium arachidis*	Yellow orange	Circular	Smooth	Raised	Translucent	Negative
18	*Chryseobacterium cucumeris*	Yellow orange	Circular	Smooth	Raised	Translucent	Negative
20	*Pectobacterium aroidearum*	Pink	Round	Irregular	Yes	opaque	Negative
21	*Staphylococcus aureus*	White	Circular	Smooth	Raised	Opaque	Negative
22	*Clostridium botulinum*	Grey	Circular	Irregular	Flat	Translucent	Negative
23	*Bacillus licheniformis*	White	Circular	irregular	Flat	Opaque	Positive
24	*Bacillus aerius*	White	Circular	irregular	Flat	Opaque	Positive
25	*Bacillus amyloliquefaciens*	White	Circular	irregular	Flat	Opaque	Positive
26	*Bacillus anthracis*	White	Circular	irregular	Flat	Opaque	Positive
27	*Chryseobacterium culicis*	Yellow orange	Circular	Smooth	Raised	Translucent	Negative
28	*Bacillus subtilis*	White	Circular	irregular	Flat	Opaque	Positive
29	*Acinetobacter calcoaceticus*	Off white	Circular	Smooth	Flat	Translucent	Negative
30	*Acinetobacter septicus*	Off White	Circular	Smooth	Flat	Translucent	Negative
31	*Enterobacter asburiae*	White	Circular	Smooth	Flat	Translucent	Negative
32	*Bacillus proteolyticus*	White	Circular	irregular	Flat	Opaque	Positive

Table 2 : Molecular characterization of bacterial colonies isolated from gut of silkworm, *B. mori* as influenced by feeding mulberry leaves sprayed with selected chemicals

Treatment details		**Bacterial species**	**Querry Coverage (%)**	**Identity (%)**	**Accession ID**
Diafenthiuron 50% WP (@ 1g/l)	**8 DAS**	*Serratia nematodiphila*	1353	97.12	PV770128
		Serratia marcescens	1416	97.25	PV770129
		Serratia surfactantfaciens	1357	94.49	PV770131
		Serratia sp.	1416	98.66	PX206231
	15 DAS	*Stenotrophomonas maltophilia*	1449	96.98	PV770043
		Chryseobacterium bernardetii	1348	99.25	PV770044
		Acinetobacter sp.	1367	98.02	PV048655
		Chryseobacterium cucumeris	1343	79.98	PV770045
		Chryseobacterium sp.	1343	95.95	PV770045
		Serratia marcescens	1377	89.61	PV785331
	21 DAS	*Chryseobacterium culicis*	1414	98.79	PV780431
		Bacillus subtilis	1352	98.81	PX062384
		Chryseobacterium sp.	1338	99.16	PV780432
		Chryseobacterium bernardetii	1513	99.25	PV780433
		Stenotrophomonas sp.	1084	99.47	PX048643
		Acinetobacter johnsonii	1383	90.39	PX062477
		Serratia marcescens	1416	99.37	PV780451
		Acinetobacter sp.	1503	99.03	PV780450
		Bacillus cereus	1453	94.50	PX048648
Abamectin 1.9 % EC (@ 0.75ml/l)	**8 DAS**	*Serratia* sp.	1408	99.69	PV804156
		Bacillus licheniformis	1459	99.35	PV804154
		Bacillus cereus	1524	98.43	PV804151
		Bacillus aerius	1397	99.14	PV804153
		Serratia marcescens	1512	98.45	PV804159
		Serratia nematodiphila	1348	99.55	PV804155

	15 DAS	*Serratia marcescens*	1430	98.19	PV781254
		Bacillus proteolyticus	1034	95.66	PV209282
		Chryseobacterium arachidis	1333	92.10	PV781247
		Serratia sp.	1442	96.38	PV781257
		Chryseobacterium sp.	1450	97.57	PV781255
	21 DAS	*Pectobacterium aroidearum*	1445	98.73	PV785329
		Serratia marcescens	1449	99.21	PV785339
		Serratia nematodiphila	1367	99.93	PV785335
		Bacillus cereus	1360	95.43	PX226019
		Serratia ureilytica	1485	94.29	PV785341
		Bacillus amyloliquefaciens	1330	99.86	PX048686
		Staphylococcus aureus	1161	95.45	PX048892
Diamethoate 30% EC (@ 2ml/l)	**20 DAS**	*Acinetobacter septicus*	1362	86.60	PV804224
		Acinetobacter johnsonii	1402	97.19	PX057984
		Chryseobacterium sp.	1425	95.55	PV779099
		Stenotrophomonas sp.	1361	98.08	PV779695
		Serratia marcescens	1280	90.88	PV779697
Cyanopyrafen 30% SC (@ 0.5ml/l)	**20 DAS**	*Acinetobacter johnsonii*	1402	90.61	PV770162
		Serratia marcescens	1414	92.53	PV770163
		Chryseobacterium sp.	1425	97.27	PV804995
		Acinetobacter sp.	1361	97.54	PV770164
		Serratia surfactantfaciens	1464	94.21	PV770165
		Serratia nematodiphila	1400	96.46	PV770166
		Bacillus cereus	1380	99.60	PV804879
Azadirachtin 1% EC (@ 1ml/l)	**20 DAS**	*Serratia marcescens*	1417	96.58	PX063560
		Acinetobacter johnsonii	1365	98.68	PX057724
		Acinetobacter sp.	1343	99.23	PV778050
		Chryseobacterium sp.	1350	97.73	PX057773
		Klebsiella pneumoniae	1372	99.70	PV778060
		Chryseobacterium bernardetii	1361	99.17	PV778057
Control (water spray)		*Enterobacter asburiae*	1540	96.75	PX067004
		Acinetobacter calcoaceticus	1453	99.22	PX067359
		Serratia marcescens	1440	98.25	PX063835
		Bacillus anthracis	1428	98.68	PX063890
		Clostridium botulinum	1393	97.03	PX206233
		Serratia nematodiphila	1044	97.63	PX063852
		Stenotrophomonas maltophilia	1393	94.52	PX213455
		Pseudomonas fluorescens	1353	96.60	PV048676
Absolute control		*Serratia nematodiphila*	1456	97.63	PX063866
		Serratia marcescens	1360	98.45	PX063837
		Aeromonas hydrophila	1455	95.39	PX215263
		Pseudomonas fluorescens	1464	96.47	PX050479
		Stenotrophomonas maltophilia	1370	91.93	PX218890
		Acinetobacter johnsonii	1445	99.35	PV779696
		Bacillus cereus	1425	96.36	PX241577
		Enterobacter cloacae	1375	92.35	PX222340

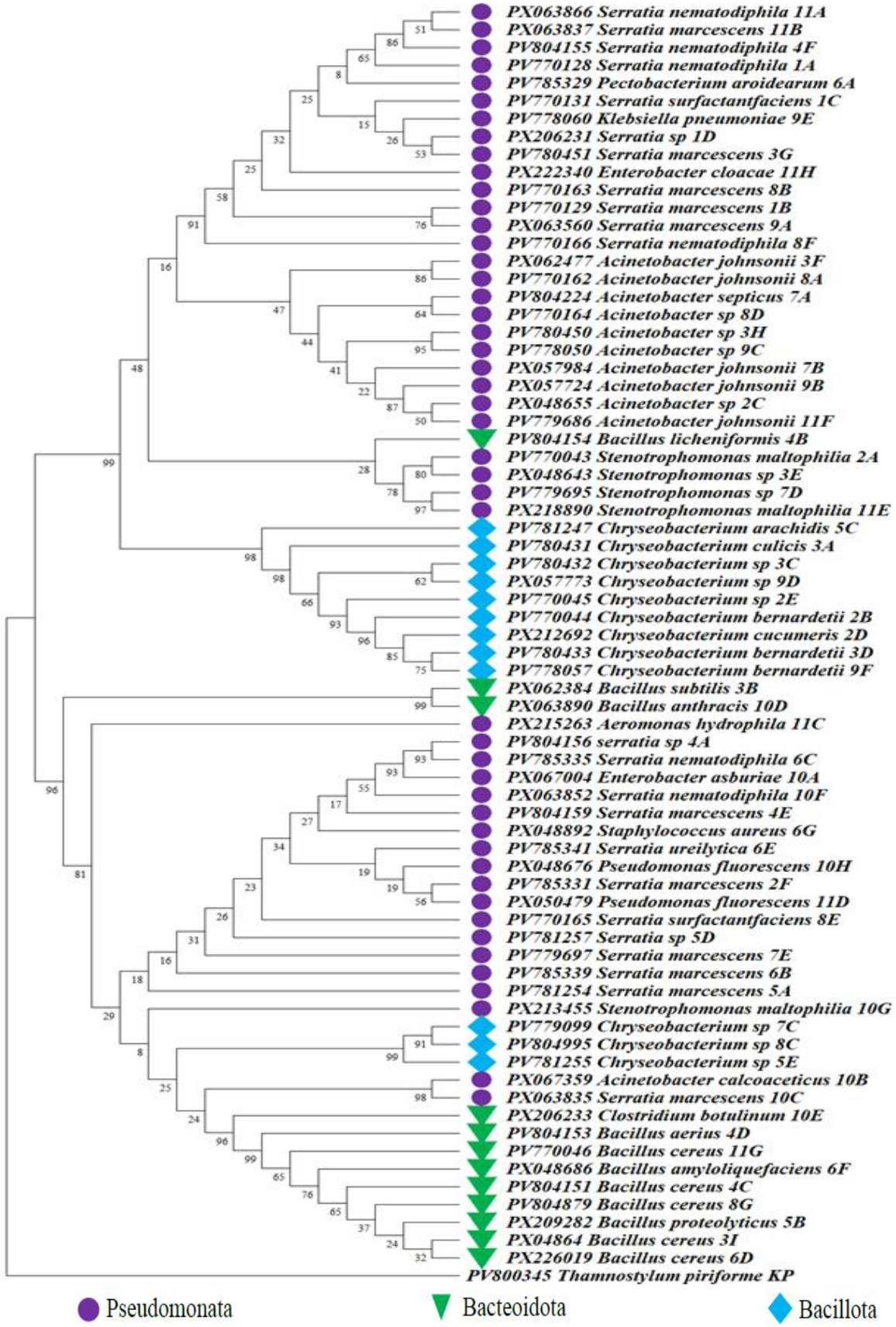

Fig. 1 : Genetic relatedness of gut bacterial isolates among different treatments of silkworm, *B. mori*

Table 3 : Diversity of gut bacterial isolates of *B. mori* silkworm reared on mulberry sprayed with selected chemicals

Sl.no	Bacterial isolates	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
1	*Serratia marcescens*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	*Serratia nematodiphila*	✓	×	×	✓	×	✓	×	✓	×	✓	✓
3	*Chryseobacterium* sp.	×	✓	✓	×	✓	×	✓	✓	✓	×	×
4	*Bacillus cereus*	×	×	✓	✓	×	✓	×	✓	×	×	✓
5	*Acinetobacter johnsonii*	×	×	✓	×	×	×	✓	✓	✓	×	✓
6	*Acinetobacter* sp.	×	✓	✓	×	×	×	×	✓	✓	×	×
7	*Chryseobacterium bernardetii*	×	✓	✓	×	×	×	×	×	✓	×	×
8	*Serratia* sp.	✓	×	×	✓	✓	×	×	×	×	×	×
9	*Stenotrophomonas maltophilia*	×	✓	×	×	×	×	×	×	×	✓	✓
10	*Stenotrophomonas* sp.	×	×	✓	×	×	×	✓	×	×	×	×

11	*Pseudomonas fluorescens*	×	×	×	×	×	×	×	×	×	□	□
12	*Serratia surfactantfaciens*	□	×	×	×	×	×	×	□	×	×	×
13	*Aeromonas hydrophila*	×	×	×	×	×	×	×	×	×	×	□
14	*Klebsiella pneumoniae*	×	×	×	×	×	×	×	×	□	×	×
15	*Serratia ureilytica*	×	×	×	×	×	□	×	×	×	×	×
16	*Enterobacter cloacae*	×	×	×	×	×	×	×	×	×	×	□
17	*Chryseobacterium arachidis*	×	×	×	×	□	×	×	×	×	×	×
18	*Chryseobacterium cucumeris*	×	□	×	×	×	×	×	×	×	×	×
20	*Pectobacterium aroidearum*	×	×	×	×	×	□	×	×	×	×	×
21	*Staphylococcus aureus*	×	×	×	×	×	□	×	×	×	×	×
22	*Clostridium botulinum*	×	×	×	×	×	×	×	×	×	□	×

23	*Bacillus licheniformis*	×	×	×	□	×	×	×	×	×	×	×
24	*Bacillus aerius*	×	×	×	□	×	×	×	×	×	×	×
25	*Bacillus amyloliquefaciens*	×	×	×	×	×	□	×	×	×	×	×
26	*Bacillus anthracis*	×	×	×	×	×	×	×	×	×	□	×
27	*Chryseobacterium culicis*	×	×	□	×	×	×	×	×	×	×	×
28	*Bacillus subtilis*	×	×	□	×	×	×	×	×	×	×	×
29	*Acinetobacter calcoaceticus*	×	×	×	×	×	×	×	×	×	□	×
30	*Acinetobacter septicus*	×	×	×	×	×	×	□	×	×	×	×
31	*Enterobacter asburiae*	×	×	×	×	×	×	×	×	×	□	×
32	*Bacillus proteolyticus*	×	×	×	×	□	×	×	×	×	×	×

Aeromonas hydrophila, Acinetobacter johnsonii and *Enterobacter cloacae* under the phylum Pseudomonadota; *Bacillus anthracis* and *Clostridium botulinum* under the phylum Bacillota and *Chryseobacterium bernardetii* under the phylum Bacteroidota were identified based on 16S rRNA analysis. The pesticides sprayed treatments, T_1 and T_4 at 8 DAS recorded least diversity of bacteria, while both diafenthiuron 50 WP and abamectin 1.9 EC exhibited maximum diversity at 21 DAS similar to control treatments (T_{10} and T_{11}) (Table 3).

Relative abundance of gut bacterial isolates

At phylum level

The gut bacterial community of *B. mori* comprised three major phyla Pseudomonadota, Bacillota and Bacteroidota. Pseudomonadota was the most dominant, represented by the genera *Serratia*, *Acinetobacter*, *Stenotrophomonas*, *Enterobacter*, *Klebsiella*, *Aeromonas*, *Pectobacterium* and *Pseudomonas*. This was followed by Bacillota mainly genera *Bacillus* spp. and least abundant *Bacteroidota* represented by *Chryseobacterium* spp (Fig. 2A).

At class level

The isolated gut bacterial community of *B. mori* predominantly composed of *Gammaproteobacteria*, *Flavobacteriia*, *Bacilli* and *Clostridia* at the class level. Among these, *Gammaproteobacteria* was the most dominant class across all treatment, exhibiting the highest relative abundance. *Flavobacteriia* was the second most abundant class, followed by *Bacilli*, while *Clostridia* occurred in very low proportions (Fig. 2B).

At genus level

The gut bacterial community isolated from *B. mori* predominantly composed of the genus *Serratia*, *Chryseobacterium*, *Acinetobacter* and *Bacillus* at the genus level. Among these, *Serratia* was the most dominant genus across all samples, exhibiting the highest relative abundance. *Chryseobacterium* was the second most abundant genus, followed by *Acinetobacter* and *Bacillus*, while *Stenotrophomonas*, *Pectobacterium*, *Klebsiella*, *Enterobacter* and *Clostridium* occurred in minor proportions (Fig. 2C).

At family level

The gut bacterial community isolated from the silkworm, *B. mori* belonged to the families, *Yersiniaceae*, *Weeksellaceae*, *Moraxellaceae*, *Bacillaceae* and *Enterobacteriaceae*. The *Yersiniaceae* was the most dominant family, followed by *Weeksellaceae* and *Moraxellaceae*. Families such as *Bacillaceae*, *Enterobacteriaceae* and *Micrococcaceae* occurred in moderate proportions, while *Staphylococcaceae*, *Pectobacteriaceae*, *Pseudomonadaceae*, *Lysobacteraceae* and *Clostridiaceae* were present in relatively lower abundances. The predominance of *Yersiniaceae* and *Weeksellaceae* indicates their major contribution to the gut microbial composition of the silkworm (Fig. 2D).

Table 4 : Systematic position of gut bacterial isolates of silkworm, *Bombyx mori* L. sprayed with selected chemicals

Phylum	Class	Order	Family	Bacterial species
Pseudomonadota	Gammaproteobacteria	Moraxellales	Moraxellaceae	*Acinetobacter* sp.
				Acinetobacter johnsonii
				Acinetobacter calcoaceticus
				Acinetobacter septicus
		Enterobacterales	Yersiniaceae	*Serratia marcescens*
				Serratia nematodiphila
				Serratia surfactantfaciens
				Serratia ureilytica
				Serratia sp.
			Enterobacteriaceae	*Enterobacter asburiae*
				Aeromonas hydrophila
				Klebsiella pneumoniae
				Enterobacter cloacae
			Pectobacteriaceae	*Pectobacterium aroidearum*
		Lysobacterales	Lysobacteraceae	*Stenotrophomonas maltophilia*
				Stenotrophomonas sp.
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas fluorescens*
Bacillota	Bacilli	Bacillales	Bacillaceae	*Bacillus subtilis*
				Bacillus cereus
				Bacillus licheniformis
				Bacillus aerius
				Bacillus amyloliquefaciens
				Bacillus anthracis
				Staphylococcus aureus
	Clostridia	Eubacteriales	Clostridiaceae	*Clostridium botulinum*
Bacteroidota	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Chryseobacterium culicis*

				Chryseobacterium bernardetii
				Chryseobacterium sp.
				Chryseobacterium arachidis
				Chryseobacterium cucumeris

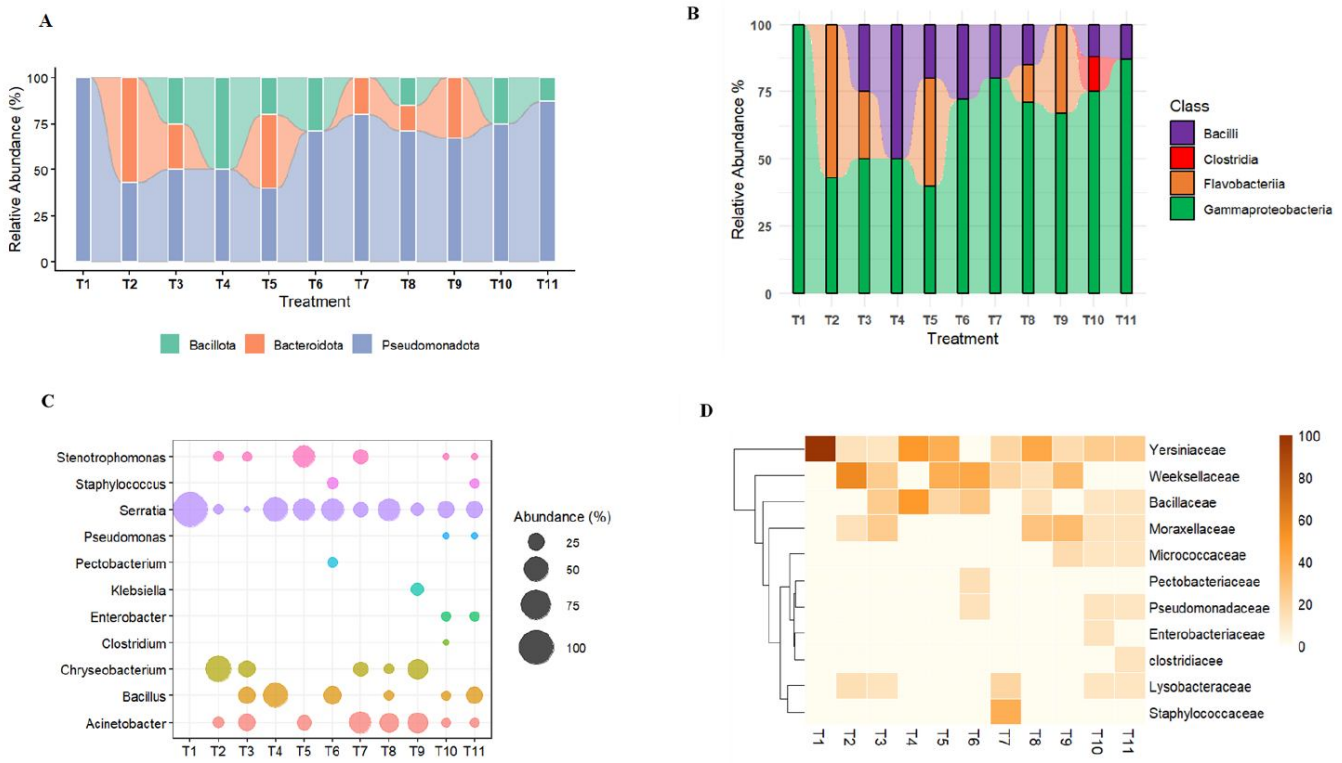

Fig 2. Relative abundance of the gut microbial community at the Phylum (A), Class (B), Genus (C) and Family level (D). Heatmap display major taxa and bubble plots show genera exhibiting notable changes across treatments.

Discussion

The gut microbiota plays an indispensable role in digestion, nutrient assimilation, detoxification and immune regulation in living organisms and so in the silkworm, *B. mori*. The chemical environment in the gut created by pesticide residues in mulberry leaves influenced the structure, diversity and relative abundance of microbial communities in the larvae feeding on these leaves. The morphological characterization of the bacterial isolates indicated the dominance of rod-shaped, gram-negative bacteria, consistent with earlier reports that the gut of *B. mori* is primarily inhabited by facultative anaerobes belonging to *Proteobacteria* and *Firmicutes* (Chen *et al*., 2020; Li *et al*., 2020). The predominance of translucent, smooth and circular colonies with varied pigmentation reflected the adaptation of gut bacteria to diverse microhabitats influenced by pesticide exposure.

The Proteobacteria, Firmicutes and Bacteroidetes were relatively high, with the majority of sequences belonging to Proteobacteria in the silkworm gut (Chen *et al*., 2018; Tian *et al*., 2024). and the abundance of these Proteobacteria increased significantly following exposure to pesticide residues, which probably is the adaptation of the insect to detoxify the pollutants. Several reported studies mention that the Proteobacteria play an important role in carbohydrate degradation, vitamin synthesis and insecticide detoxification (Delalibera *et al*., 2005; McCutcheon *et al*., 2005) On the other hand, the proportions of Firmicutes and Bacteroidetes decreased disturbing the gut microbiota equilibrium and influence the host immune and metabolic functions. Therefore, changes in the gut microbiota composition of silkworm induced by pesticide exposure may mutually affect the host's physiological functions.

Molecular characterization of gut bacterial isolates through 16S rRNA gene sequencing confirmed

that the dominant bacterial genera associated with the gut of *B. mori* were *Serratia*, *Acinetobacter*, *Bacillus*, *Chryseobacterium*, *Enterobacter*, *Stenotrophomonas* and *Pseudomonas*. Similar genera have been reported as core symbionts in the silkworm gut and are known to contribute to digestion and defense functions (Yuan *et al*., 2023). The sequence similarity (95.98-99.93 %) and high query coverage observed in the present study confirm reliable species-level identification.

The phylogenetic analysis revealed two major clades corresponding to *Pseudomonadota (Proteobacteria)* and *Bacillota (Firmicutes)*, with a minor lineage of *Chryseobacterium* under *Bacteroidota (Bacteroidetes)*. The clustering pattern indicated a strong evolutionary relationship among dominant gut isolates and reflected selective enrichment of pesticide-tolerant taxa. The dominance of *Serratia marcescens*, *Acinetobacter johnsonii*, and *Stenotrophomonas maltophilia* in pesticide-treated larvae supports earlier findings that these bacteria possess genes for xenobiotic degradation and resistance to environmental stress (Tian *et al*., 2025).

Diversity analysis revealed that pesticide treatments influenced microbial richness and evenness. Early exposure (8 DAS) reduced bacterial diversity in increasing order that showed recovery comparable to control treatments as the molecules degraded (15 and 21 DAS) suggesting that higher accumulation of pesticide temporarily suppress sensitive symbionts such as *Bacillus* and *Chryseobacterium*, while tolerant strains proliferate as residue levels decline. Such dynamic shifts in the microbiome are known to affect the physiological performance of *B. mori*, including feed efficiency, digestion and silk quality (Rong *et al*., 2024).

Relative abundance studies across taxonomic levels demonstrated that *Pseudomonadota* remained the dominant phylum across treatments, followed by *Bacillota* and *Bacteroidota*. At the class and genus levels, *Gammaproteobacteria*, particularly *Serratia* and *Acinetobacter*, were highly enriched in pesticide-exposed larvae. In contrast, beneficial taxa such as *Bacillus* and *Chryseobacterium* decreased significantly. The enrichment of *Yersiniaceae*, *Weeksellaceae* and *Moraxellaceae* families under stress conditions highlights their role in maintaining gut homeostasis during chemical exposure. These findings align with previous studies that reported pesticide-induced dysbiosis leading to compositional shifts favoring stress-tolerant and detoxification-associated bacteria (Li *et al*., 2020)

Overall, the results indicate that pesticide residues at recommended doses do not cause acute toxicity in *B. mori*, they significantly modulate the gut microbial community structure. Such shifts may influence nutrient metabolism and immune function, indirectly affecting larval growth and cocoon quality. The observed resilience of certain microbial taxa after 15 to 21 days of pesticide application suggests that maintaining a safe waiting period is crucial before feeding the mulberry leaves harvested from sprayed gardens in order to sustain microbial equilibrium and optimal silkworm performance.

Conclusion

A strong relationship exists between the pesticide residues and silkworm gut that inturn impacts the growth and productivity in *Bombyx mori L*. Though pesticide residues do not eliminate the gut bacterial communities, a strong microbial dysbiosis is observed with increased toxicity levels. The test chemicals viz., abamectin 19.5 EC (@ 0.75 ml/L) and diafenthiuron 50 WP (@1 g/L) were safe to silkworms at 15 and 21 DAS while effectively managing both thrips and mites in mulberry with single application.

Acknowledgments: We gratefully thank Prof. Dr. Shivanna B for their valuable contributions and for providing all the necessary materials for the molecular part of this study.

Authors contributions

Conceptualization, writing-original draft preparation, KSV, KGB; methodology, BS; Investigation, KN, KSV; validation, BS ; data curation, KN; software, KN; supervision, KSV, RN, KGB. The final version of the manuscript has been read and approved by all authors

Conflict of interest disclosure: The authors confirm that there are no conflicts of interest associated with this publication.

Data availability: All sequences of bacterial isolates have been deposited in the GenBank database, National Center for Biotechnology Information, accession nos PV770128 TO PX222340. The data are accessible at https://www.ncbi.nlm.nih.gov/nuccore/al

References

Arias, O., Cordeiro, E., Correa, A. S., Domingues, F. A., Guidolin, A. S. and Omoto, C. (2019). Population genetic structure and demographic history of *Spodoptera frugiperda*. *Pest Manag. Sci.*, **75**(11), 2948–2957.

Arasakumar, E., Vasanth, V. and Swathiga, S. V. G. (2018). Role of microorganisms in the gut of silkworms. *J. Pharm. Innov.*, **12**(4), 2691–2696.

Becerra, S. C., Roy, D. C., Sanchez, C. J., Christy, R. J. and Burmeister, D. M. (2016). Staining technique for Gram-

positive and Gram-negative bacteria. *BMC Res. Notes*, **9**(1), 1-10.

Bhosale, S. H. and Kallapur, V. L. (1988). Changes in metabolic fuel reserves of V instar *Bombyx mori* following endosulfan treatment. *Entomon*, **10**(6), 281–283.

Bing, L., Yanhong, W., Haitao, L., Yaxiang, X., Zhengguo, W. and Yuhua, C. (2010). Resistance comparison of domesticated and wild silkworms to phoxim. *Afr. J. Biotechnol.*, **9**(12), 1771–1775.

Bundschuh, R., Schmitz, J., Bundschuh, M. and Brühl, C. A. (2012). Does insecticide drift adversely affect grasshoppers (*Orthoptera: Saltatoria*) in field margins? *Environ. Toxicol. Chem.*, **31**(8), 1874–1879.

Chen, B., Du, K., Sun, C., Vimalanathan, A., Liang, X. and Li, Y. (2018). Gut bacterial and fungal communities of silkworm. *ISME J.*, **12**(9), 2252–2262.

Chen, B., Zhang, N., Xie, S., Zhang, X., He, J. and Muhammad, A. (2020). Gut bacteria facilitate resistance against organophosphate insecticides in *Bombyx mori*. *Environ. Int.*, **143**: 105886. https://doi.org/10.1016/j.envint.2020.105886.

Dandin, S. B. and Giridhar, K. (2014). *Handbook of Sericulture Technologies*. Bangalore: Central Silk Board.

Delalibera, I. Jr., Handelsman, J. O. and Raffa, K. F. (2005). Cellulolytic activities of gut microorganisms in insects. *Environ. Entomol.*, **34**(3), 541–547.

Garibyan, L. and Avashia, N. (2013). Polymerase chain reaction (PCR). *J. Invest. Dermatol.*, **133**(3), 1–6.

Jorquera, M. A., Graether, S. P. and Maruyama, F. (2019). Bioprospecting and biotechnology of extremophiles. *Front. Bioeng. Biotechnol.*, 7, 204. https://doi.org/10.3389/fbioe.2019.00204.

Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R. and Williams, M. A. (2016). Honey bee gut microbiome is altered by in-hive pesticide exposures. *Front. Microbiol.*, 7, 1255.

Kaltenpoth, M. and Florez, L. V. (2020). Versatile and dynamic symbioses between insects and *Burkholderia* bacteria. *Annu. Rev. Entomol.*, **65**, 145–170.

Koboziev, I., Webb, C. R., Furr, K. L. and Grisham, M. B. (2014). Role of the enteric microbiota in intestinal homeostasis and inflammation. *Free Radic. Biol. Med.*, **68**, 122–133.

Li, F., Li, M., Mao, T., Wang, H., Chen, J. and Lu, Z. (2020). Effects of phoxim exposure on gut microbial composition in the silkworm, *Bombyx mori*. *Ecotoxicol. Environ. Saf.*, **189**, 110011. https://doi.org/10.1016/j.ecoenv.2019.110011.

Matsumoto, T. and Sugano, M. (2013). 16S rRNA gene sequence analysis for bacterial identification. *Jpn. J. Clin. Pathol.*, **61**(12), 1107–1115.

McCutcheon, J. P. and Moran, N. A. (2007). Parallel genomic evolution in symbiosis. *Proc. Natl. Acad. Sci. U.S.A.*, **104**(49), 19392–19397.

Michaudel, C. and Sokol, H. (2020). The gut microbiota at the service of immunometabolism. *Cell Metab.*, **32**(4), 514–523.

Rong, W., Wei, Y., Chen, Y., Huang, L., Huang, S. and Lv, Y. (2024). Cupric chloride effects on silkworm gut microbiome. *Animals*, **14**(24), 3634.

Rouzé, R., Moné, A., Delbac, F., Belzunces, L. and Blot, N. (2019). The honeybee gut microbiota is altered after chronic exposure to insecticides and *Nosema ceranae*. *Microbes Environ.*, **34**(3), 226–233.

Shan, H., Wu, W., Sun, Z., Chen, J. and Li, H. (2021). The gut microbiota of the insect infraorder Pentatomomorpha in light of ecology and evolution. *Microorganisms*, **9**(2), 464.

Sundararaj, N., Nagaraju, S., Venkataramu, M. N. and Jagannath, M. K. (1972). *Design and Analysis of Field Experiments*. Bangalore: UAS.

Takakura, K. and Nishio, T. (2012). Safer DNA extraction from plant tissues using sucrose buffer. *J. Plant Res.*, **121**(6), 805–807.

Tian, C., Tang, J., Zhu, Q., Guo, X., Shu, Q. and Gu, Z. (2025). Detoxification strategy of *Bombyx mori* to dimethoate based on gut microbiota. *J. Econ. Entomol.*, **118**(2), 858–867.

Tian, C., Zou, H., Guo, X., Shu, Q., Zhang, X. and Cheng, J. (2024). Effects of florfenicol on silkworm gut microbiota. *Entomol. Exp. Appl.*, **172**(10), 953–963.

Yang, Y., Ma, S., Liu, F., Wang, Q., Wang, X. and Hou, C. (2020). Acute and chronic toxicity of insecticides to *Apis mellifera* larvae reared in vitro. *Pest Manag. Sci.*, **76**(3), 978–985.

Yuan, S., Sun, Y., Chang, W., Zhang, J., Sang, J. and Zhao, J. (2023). Gut microbiota involved in detoxification of plant toxins in *Bombyx mori*. *Commun. Biol.*, **6**, 790. 790. https://doi.org/10.1038/s42003-023-05150-0.

Zhang, N., Qian, Z., He, J., Shen, X., Lei, X. and Sun, C. (2024). Gut bacteria of lepidopteran herbivores facilitate digestion of plant toxins. *Proc. Natl. Acad. Sci. U.S.A.*, **121**(42), e2412165121.

Zhu, L., Qi, S., Xue, X., Niu, X. and Wu, L. (2020). Nitenpyram disturbs gut microbiota and influences metabolic homeostasis and immunity in honey bee (*Apis mellifera*). *Environ. Pollut.*, 258, 113671.

Zhu, Z., He, Y., Xu, J., Zhou, Z., Kumar, A. and Xia, Z. (2023). Effects of pesticide application on leaf physiological traits and phyllosphere bacterial communities. *J. Plant Ecol.*, **16**(2), 84. https://doi.org/10.1093/jpe/rtac084.