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K Nisarga
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

KS Vinoda
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

R Narayana Reddy
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

B Shivanna
Department of Agricultural
Entomology, College of
Agriculture, UAS, GKVK,
Bengaluru, Karnataka, India

K Ranganatha
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

KG Banuprakash
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

Corresponding Author:
K Nisarga
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

Effect of selected pesticides on phyllosphere microbial diversity in mulberry (*Morus alba* L.)

K Nisarga, KS Vinoda, R Narayana Reddy, B Shivanna, K Ranganatha and KG Banuprakash

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Abstract

Phyllosphere microbes are associated with diverse microbial communities and these microorganisms influence plant health, nutrient cycling and degradation of agrochemical residues. With this contest, the present study was undertaken to evaluate the effect of recommended pesticides, viz., diafenthiuron, abamectin, dimethoate, cyanopyrafen and azadirachtin on the diversity and composition of phyllosphere bacteria associated with mulberry. The results of the experiment were revealed based on morphological and molecular identification of culturable bacterial isolates. A total of 52 bacterial isolates were identified from the phyllosphere of mulberry. Pesticide application resulted in a marked shift in the bacterial community of phyllosphere, with treated leaves showing dominance of Bacillota (Firmicutes), mainly represented by *Bacillus cereus*, *B. thuringiensis* and *B. subtilis*. In contrast, leaves without pesticide application exhibited higher diversity of Proteobacteria represented by *Acinetobacter*, *Brevundimonas* and *Sphingomonas*. The investigation has thrown light on the influence of pesticide toxicity on phyllosphere microbes, where chemical stress favours spore-forming *Bacillus* species while suppressing non-spore forming Proteobacteria.

Keywords: Phyllosphere microbes, pesticide residue, residual toxicity, bacterial diversity

Introduction

Pesticides are widely employed in agriculture to enhance crop productivity and ensure food security by controlling pests, weeds and pathogens. Despite these benefits, the excessive and unregulated use of pesticides has led to serious environmental concerns posing significant risks to soil health, water resources and non-target organisms, including humans. Among various pesticide groups, organophosphate and organochlorine compounds are of particular concern because of their persistent bioaccumulation and toxicity, which have resulted in their widespread detection in soil, water and crop products (Tudi *et al.*, 2021; Kumar *et al.*, 2025) [24, 11].

The phyllosphere comprising the above-ground plant surface serves as a dynamic ecological niche where microbial communities contribute to broader ecosystem processes like leaf health, degradation of pollutants etc. Further, it harbours diverse microbial communities, predominantly bacteria that play crucial roles in plant health, nutrient cycling and environmental interactions (Bashir *et al.*, 2022; Liu *et al.*, 2020) [2, 12]. When pesticides are sprayed in agricultural fields, the phyllosphere microbes are the first to encounter and interact with the chemicals, often initiating the transformation of xenobiotic compounds. Under such environmental stress, phyllosphere bacteria and fungi secrete extracellular enzymes including hydrolases, transferases, isomerases, ligases etc., which mediate key biochemical reactions such as hydrolysis and redox reactions, thereby contributing to pesticide degradation (Kucharska *et al.*, 2020; Chia *et al.*, 2024) [10, 5].

Mulberry (*Morus* spp.) is an inimitable host plant for the silkworm, *Bombyx mori* L. However, due to it luxuriant and lush green foliage available all through the year attracts several pests and pathogens and hence is often exposed to various chemicals for pest and disease management. Such chemical application can inadvertently modify the natural leaf-surface microbiota, potentially affecting leaf quality, ecological stability and ultimately sericulture productivity (Sunil Kumar and Naika, 2019) [20]. Therefore, understanding pesticide-induced microbial shifts is essential for sustainable mulberry cultivation and sericulture.

Microbial degradation plays an increasingly important role in bioremediation of pesticide-contaminated environments. Several bacterial genera, including *Bacillus*, *Pseudomonas*, *Enterobacter* and *Arthrobacter* have been reported to degrade organophosphate and organochlorine pesticides through enzymatic pathways, converting toxic compounds into less harmful metabolites (Lourthuraj *et al.*, 2021; Ariffin and Rahman., 2020) [14, 1]. Previous studies in several crops have documentary evidence of altered phyllosphere microbial composition and suppression of sensitive taxa while supporting stress-tolerant bacterial groups upon pesticide exposure (Di Benedetto *et al.*, 2017) [7]. However, comprehensive investigations on pesticide-induced changes in the mulberry phyllosphere are limited.

Diafenthiuron and abamectin were chosen as a test chemical due to their extensive use in mulberry pest management and have novel modes of action. Previous studies (Shwetha *et al.*, 2024; Gagana Sindhu *et al.*, 2025) [19, 8] reported their residual behaviour and relative safety to silkworms. However, their influence on mulberry phyllosphere bacterial diversity remains inadequately explored.

In this context, an investigation was carried out on impact of pesticide residue on phyllosphere bacteria communities in mulberry plant. The study adopted both morphological and molecular approaches to assess the influence of selected pesticides on diversity of phyllosphere microbial communities.

Material and Methods

The experiments were conducted in a well-established mulberry garden (Var. V-1) at the Department of Sericulture, College of Agriculture, GKVK, Bengaluru. The field is located in the Eastern dry zone (Zone 5) of Karnataka at 12°58' N latitude and 77°35' E longitude and an altitude of 930 m above mean sea level.

Experimental details

Mulberry leaves positioned at 3rd, 5th and 7th from apex representing different levels of maturity were sampled and processed separately from 11 treatments corresponding to different pesticides and safety durations post spray as mentioned below:

- T₁-T₃: Diafenthiuron 50 WP @ 1 g/L (at 8, 15, 21 DAS, respectively)
- T₄-T₆: Abamectin 1.9 EC @ 0.75 mL/L (at 8, 15, 21 DAS, respectively)
- T₇: Dimethoate 30 EC @ 2 mL/L (20 DAS)
- T₈: Cyanopyrafen 30 SC @ 0.5 mL/L (20 DAS)
- T₉: Azadirachtin 1 EC @ 1 mL/L (20 DAS)
- T₁₀: Control (water spray)
- T₁₁: Absolute control
- DAS-Days After Spray

Collection of Samples and Isolation of Phyllosphere Microbes

Mulberry leaves were collected at 8, 15 and 21 DAS from the experimental plots treated with selected pesticides. Phyllosphere bacteria were isolated using the spread plate technique, followed by quadrant streaking to obtain pure cultures. Leaves were processed by shaking in sterile distilled water and serial dilutions were prepared up to 10⁻⁶ before plating on nutrient agar. Plates were incubated at 28 °C for 24 hours and distinct colonies were purified for further characterization. Culturable microflora were

identified using both conventional morphology-based methods and molecular techniques (Thaochan *et al.*, 2009) [23].

Colonies that differed in appearance were considered to represent different bacterial strains, species, or genera. Based on characteristics such as size, shape, pigmentation, elevation, consistency and margin of the isolated colonies, differentiation and pure culture were obtained by sub-culturing on the same medium.

Molecular identification of phyllosphere bacterial isolates

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 (Swathi *et al.*, 2015) [21].

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 (Swathi *et al.*, 2015) [21].

16S rRNA gene sequence analysis

The nucleotide sequencing of purified PCR fragments was performed following Sanger sequencing method. The DNA sequences corresponding to the 16S rRNA gene obtained from individual bacterial isolates were reverse complemented using BioEdit software and the resulting sequences were analyzed along with reference sequences retrieved from the NCBI (National Center for

Biotechnology Information) GeneBank database using bioinformatics software and bacterial strains were identified accordingly (Matsumoto and Sugano, 2013 and Devaiah *et al.*, 2022) ^[15, 6].

Phylogenetic Analysis

Phylogenetic relationships among the isolates were inferred

using Maximum Likelihood (ML) method with the Tamura-Nei model in MEGA-11 software (Tamura and Nei, 1993) ^[22]. Bootstrap analysis with 1000 replications was performed to evaluate clade confidence. The final tree, constructed from 27 sequences with 1740 positions, grouped isolates into well-supported clades corresponding to the phyla Bacillota and Pseudomonadota.

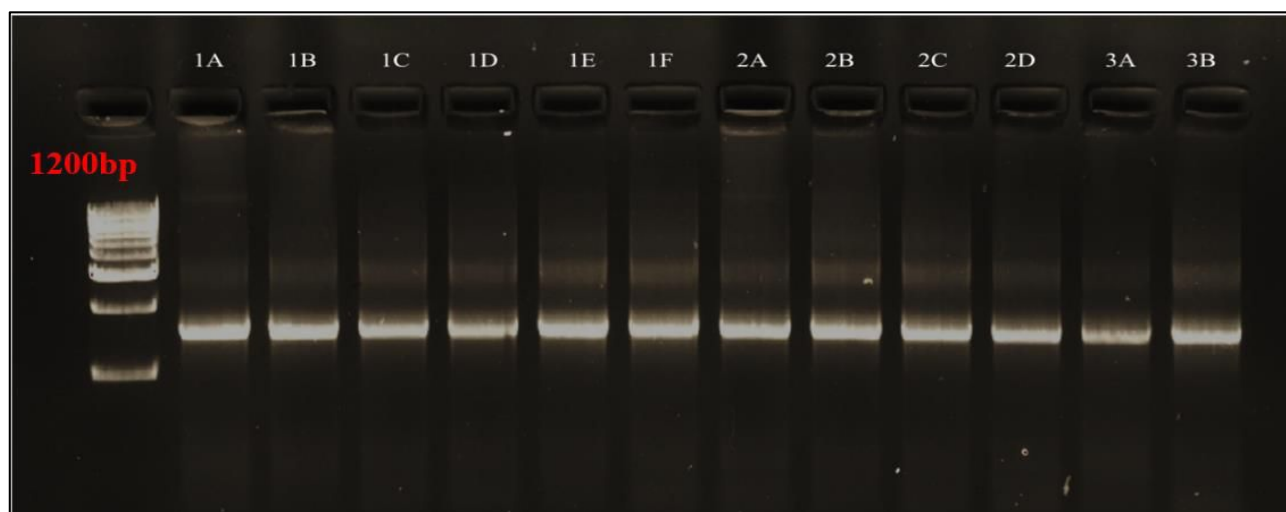


Plate 1: PCR amplified 16S rRNA product of phyllosphere of mulberry sprayed with selected chemicals

Results and Discussion

Isolation and Characterization of bacterial communities

A total of 52 bacterial isolates were obtained from mulberry leaf samples of pesticide-treated and control plots. Morphological characterization based on size, shape, pigmentation, elevation, consistency and margin revealed that most isolates were white to creamish in colour, circular or irregular in shape with flat elevation and opaque

appearance. Gram staining showed a strong predominance of Gram-positive bacteria (94.2 %), mainly belonging to the genus *Bacillus* (Table 1). This dominance suggests that Gram-positive, spore-forming bacteria possess a selective advantage under chemically stressed phyllosphere conditions, as previously reported for pesticide-impacted plant surfaces (Nicholson *et al.*, 2000; Chen *et al.*, 2021) ^[17, 4].

Table 1: Morphological characterization of phyllosphere bacterial species from mulberry leaves sprayed with selected chemicals

Sl. No	Bacterial isolates	Colour	Shape	Margin	Elevation	Opacity	Branching	Gram staining
1	<i>Bacillus cereus</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
2	<i>Bacillus thuringiensis</i>	Whitish	Circular	Smooth	Flat	Opaque	No	Positive
3	<i>Bacillus subtilis</i>	Off white	Irregular	Irregular	Flat	Opaque	No	Positive
4	<i>Bacillus paramycoides</i>	white	circular	Irregular	Flat	Opaque	No	Positive
5	<i>Bacillus licheniformis</i>	Off white	Irregular	Irregular	Flat	Opaque	No	Positive
6	<i>Bacillus velezensis</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
7	<i>Bacillus anthracis</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
8	<i>Bacillus amyloliquefaciens</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
9	<i>Bacillus</i> sp.	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
10	<i>Bacillus megaterium</i>	Creamy white	Circular	Irregular	Raised	Opaque	No	Positive
11	<i>Bacillus tropicus</i>	White	Circular	Irregular	Flat	Opaque	No	Positive
12	<i>Bacillus mycoides</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
13	<i>Brevundimonas vesicularis</i>	Yellow	Circular	Smooth	Flat	Opaque	No	Negative
14	<i>Sphingomonas panni</i>	Off white	Irregular	Irregular	Flat	Opaque	No	Negative
15	<i>Bacillus safensis</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
16	<i>Bacillus stercoris</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
17	<i>Bacillus albus</i>	Whitish	Circular	Irregular	Flat	Opaque	No	Positive
18	<i>Clostridium sporogenes</i>	Grey	Circular	Irregular	Flat	Translucent	No	Positive
19	<i>Acinetobacter</i> sp.	Off white	Circular	Irregular	Flat	Opaque	No	Negative

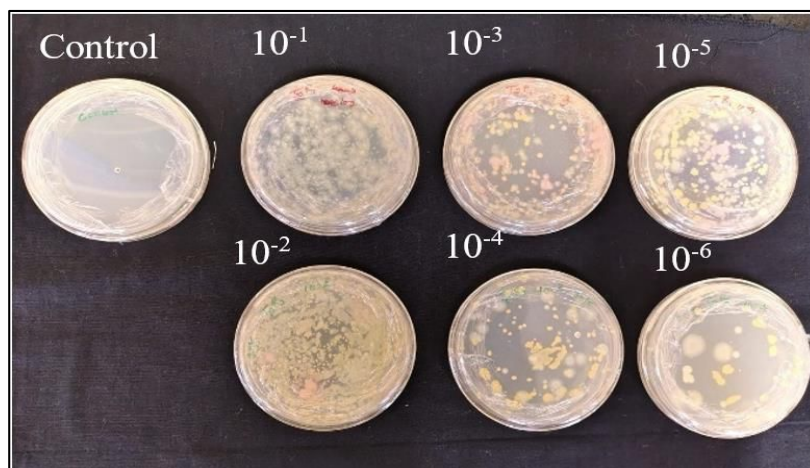


Plate 2: Bacterial colonies at different dilution



Plate 3: Pure culture plates of bacterial strains

Molecular identification using 16S rRNA gene sequencing confirmed the morphological observations. All isolates produced distinct amplicons of approximately 1200 bp. The BLAST analysis revealed a high sequence similarity (95-100 %) with known bacterial species (Table 2). The majority of isolates were identified as *Bacillus cereus*, *B. thuringiensis*, *B. subtilis*, *B. paramycoides* and *B. licheniformis*. In contrast, Proteobacterial members such as *Acinetobacter*, *Brevundimonas vesicularis* and *Sphingomonas panni* were detected exclusively in control samples. These findings are consistent with earlier reports indicating that pesticide exposure suppresses non-spore forming phyllosphere bacteria while selectively favoring stress-tolerant *Bacillus* species (Moulas *et al.*, 2013; Zhu *et al.*, 2023) ^[16, 27].

Diversity of microbial communities exposed to different pesticide concentrations

Microbial diversity varied markedly across different treatments. A high residual concentration of the chemical

lowered species richness as evidenced 8 DAS (T_1 and T_4) where only a few tolerant *Bacillus* species survived (Table 3). High residue levels of both the test chemicals might have exerted strong toxic effects on sensitive bacterial taxa. A similar decline in phyllosphere diversity following pesticide application have been reported earlier in various crops (Moulas *et al.*, 2013; Chen *et al.*, 2021) ^[16, 4]. The microbial diversity gradually increased at 15 and 21 DAS (T_2 , T_3 , T_5 and T_6) when the pesticide residues declined over time. However, despite partial recovery, the diversity of microbial community did not match that observed in chemical free controls of T_{10} and T_{11} (Table 3). Rare *Bacillus* species such as *B. megaterium*, *B. safensis*, *B. stercoris* and *B. albus* were detected exclusively in control treatments (T_{10} and T_{11}), indicating that early chemical exposure may have long-lasting effects on recolonization by sensitive taxa reflecting that the rare microbial members are highly susceptible to disturbance and may not readily recover after pesticide stress (Sangiorgio *et al.*, 2022; Zhu *et al.*, 2023) ^[18, 27].

Table 2: Molecular characterization of phyllosphere bacterial isolates from mulberry leaves sprayed with selected chemicals

Treatment details		Bacterial isolates	Query coverage (%)	Identity (%)	Accession ID
Diafenthiuron 50% WP (@ 1 g/l)	8 DAS	<i>Bacillus paramycoides</i>	1396	99.14	PV810162
		<i>Bacillus subtilis</i>	1398	99.78	PV810163
		<i>Bacillus velezensis</i>	1466	99.50	PV810165
		<i>Bacillus licheniformis</i>	1389	95.01	PV810160
		<i>Bacillus cereus</i>	1399	98.86	PV810159
		<i>Bacillus thuringiensis</i>	1413	87.53	PV810164
	15 DAS	<i>Bacillus subtilis</i>	1425	98.97	PV810371
		<i>Bacillus thuringiensis</i>	1431	95.70	PV810370
		<i>Bacillus anthracis</i>	1330	100.00	PV810367
		<i>Bacillus cereus</i>	1434	99.74	PX148897
	21 DAS	<i>Bacillus amyloliquefaciens</i>	1330	100.00	PX170154
		<i>Bacillus thuringiensis</i>	1396	100.00	PV810372
		<i>Bacillus</i> sp.	1442	92.71	PX123172
		<i>Bacillus cereus</i>	1463	100.00	PV810368
		<i>Priestia megaterium</i>	1414	99.36	PX123157
		<i>Bacillus paramycoides</i>	1452	100.00	PV810369
Abamectin 1.9 % EC (@ 0.75 ml/l)	8 DAS	<i>Bacillus licheniformis</i>	1452	99.70	PX149104
		<i>Bacillus thuringiensis</i>	1384	99.41	PX149106
		<i>Bacillus cereus</i>	1414	99.85	PX149107
	15 DAS	<i>Bacillus thuringiensis</i>	1483	89.99	PX149168
		<i>Bacillus tropicus</i>	1417	94.62	PX149174
		<i>Bacillus</i> sp.	1422	98.66	PX149180
		<i>Bacillus subtilis</i>	1464	98.12	PX149221
		<i>Bacillus cereus</i>	1386	96.55	PV810387
	21 DAS	<i>Bacillus cereus</i>	1011	93.15	PV810435
		<i>Bacillus thuringiensis</i>	1461	92.26	PX287178
		<i>Bacillus mycoides</i>	1395	96.66	PV810161
		<i>Bacillus licheniformis</i>	1420	98.38	PX209284
		<i>Bacillus paramycoides</i>	1050	97.19	PX287179
Diamethoate 30% EC (@ 2 ml/l)	20 DAS	<i>Bacillus subtilis</i>	1425	99.57	PV810389
		<i>Clostridium sporogenes</i>	1434	94.81	PX149317
		<i>Bacillus thuringiensis</i>	1442	94.59	PV810447
		<i>Bacillus anthracis</i>	1050	100.00	PV810438
Cyanopyrafen 30% SC (@ 0.5 ml/l)	20 DAS	<i>Bacillus paramycoides</i>	1050	98.54	PV810444
		<i>Bacillus amyloliquefaciens</i>	1330	100.00	PV810437
		<i>Bacillus cereus</i>	1442	97.72	PV810442
		<i>Bacillus thuringiensis</i>	1431	94.49	PV810448
Azadirachtin 1% EC (@ 1 ml/l)	20 DAS	<i>Bacillus thuringiensis</i>	1104	89.75	PX149859
		<i>Bacillus paramycoides</i>	1050	96.18	PV810445
		<i>Bacillus cereus</i>	1407	99.64	PV810441
Control (water spray)		<i>Acinetobacter</i> sp.	1450	90.00	PX149900
		<i>Brevundimonas vesicularis</i>	1416	99.76	PX148978
		<i>Bacillus subtilis</i>	1370	100.00	PX170172
		<i>Priestia megaterium</i>	1412	98.94	PX123231
Absolute control		<i>Bacillus subtilis</i>	1409	99.86	PX209287
		<i>Bacillus velezensis</i>	1407	98.77	PX123166
		<i>Bacillus safensis</i>	1398	99.50	PX123165
		<i>Bacillus albus</i>	1421	98.75	PX123155
		<i>Bacillus cereus</i>	1387	96.65	PX123156
		<i>Bacillus stercoris</i>	1423	98.60	PX123169
		<i>Sphingomonas panni</i>	1348	93.65	PV810373

Table 3: Diversity of phyllosphere bacterial species from mulberry leaves 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	<i>Bacillus cereus</i>	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓
2	<i>Bacillus thuringiensis</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	×
3	<i>Bacillus subtilis</i>	✓	✓	✓	×	✓	×	✓	×	×	✓	✓
4	<i>Bacillus paramycoides</i>	✓	×	✓	×	×	✓	×	✓	✓	×	×
5	<i>Bacillus licheniformis</i>	✓	×	×	✓	×	✓	×	×	×	×	×
6	<i>Bacillus velezensis</i>	✓	×	×	×	×	×	×	×	×	×	✓
7	<i>Bacillus anthracis</i>	×	✓	×	×	×	×	✓	×	×	×	×
8	<i>Bacillus amyloliquefaciens</i>	×	×	✓	×	×	×	×	✓	×	×	×
9	<i>Bacillus</i> sp.	×	×	✓	×	✓	×	×	×	×	×	×
10	<i>Bacillus megaterium</i>	×	×	✓	×	×	×	×	×	×	✓	×
11	<i>Bacillus tropicus</i>	×	×	×	×	✓	×	×	×	×	×	×
12	<i>Bacillus mycoides</i>	×	×	×	×	×	✓	×	×	×	×	×
13	<i>Brevundimonas vesicularis</i>	×	×	×	×	×	×	×	×	×	✓	×
14	<i>Sphingomonas panni</i>	×	×	×	×	×	×	×	×	×	×	✓
15	<i>Bacillus safensis</i>	×	×	×	×	×	×	×	×	×	×	✓
16	<i>Bacillus stercoris</i>	×	×	×	×	×	×	×	×	×	×	✓
17	<i>Bacillus albus</i>	×	×	×	×	×	×	×	×	×	×	✓
18	<i>Clostridium sporogenes</i>	×	×	×	×	×	×	✓	×	×	×	×
19	<i>Acinetobacter</i> sp.	×	×	×	×	×	×	×	×	×	✓	×

The dominance of *B. thuringiensis* across most pesticide-treated samples suggests that certain *Bacillus* species may proliferate under reduced competition or possess detoxification capabilities that allow them to exploit chemically stressed niches (Jing *et al.*, 2020; Chen *et al.*, 2021) ^[9, 4].

Prevalent Bacterial Taxa in the mulberry Phyllosphere

Five bacterial genera belonging to two phyla, Bacillota (Firmicutes) and Pseudomonadota (Proteobacteria) were recorded from the mulberry phyllosphere with chemical treatments strongly favoring Bacillota, particularly *Bacillus*

species such as *B. cereus*, *B. thuringiensis*, *B. subtilis*, *B. paramycoides*, *B. licheniformis* and *B. velezensis*, while Pseudomonadota members *Acinetobacter*, *Brevundimonas vesicularis* and *Sphingomonas panni* occurred sporadically (Table 4). The predominance of Bacillota is attributed to their high tolerance to environmental and chemical stress, largely due to endospore formation, which enables survival under desiccation, UV radiation and pesticide residues unlike most non-spore-forming bacteria (Nicholson *et al.*, 2000) ^[17]. Functionally, *Bacillus* spp. contribute to mulberry leaf health through the production of phytohormones, siderophores and antimicrobial compounds improving leaf quality, suppressing pathogens and upon ingestion by *B. mori*, they enhance gut microbial balance, digestion, immunity and silk productivity (Li *et al.*, 2022) ^[13]. Although less abundant, the taxa Pseudomonadota contribute to nitrogen metabolism and vitamin synthesis, indirectly supporting leaf quality and silkworm health. Overall, the dominance of Bacillota highlights their strong phyllosphere adaptation and pivotal role in linking mulberry leaf health with improved silkworm performance (Zhang *et al.*, 2024) ^[26].

Phylogenetic Tree Analysis

Phylogenetic analysis clustered the isolates into two major clades corresponding to Bacillota and Pseudomonadota. The Bacillota clade was strongly supported by high bootstrap values and comprised multiple *Bacillus* species, indicating close genetic relatedness and strain-level variation. In contrast, Proteobacterial isolates formed a distinct, smaller lineage reflecting their limited persistence under pesticide stress (Fig 1). The phylogenetic segregation confirms earlier observations that *Bacillus* species dominate stressed environments due to their endospore formation, metabolic versatility and rapid recolonization ability (Nicholson *et al.*, 2000; Sangiorgio *et al.*, 2022) ^[17, 18].

Table 4: Systematic position of phyllosphere bacterial isolates from mulberry leaves sprayed with selected chemicals

Phylum	Class	Order	Family	Bacterial species
Bacillota (Firmicutes)	Bacilli	Bacillales	Bacillaceae	<i>Bacillus cereus</i>
				<i>Bacillus thuringiensis</i>
				<i>Bacillus subtilis</i>
				<i>Bacillus paramycoides</i>
				<i>Bacillus licheniformis</i>
				<i>Bacillus velezensis</i>
				<i>Bacillus anthracis</i>
				<i>Bacillus amyloliquefaciens</i>
				<i>Bacillus</i> sp.
				<i>Bacillus megaterium</i>
				<i>Bacillus tropicus</i>
				<i>Bacillus mycoides</i>
				<i>Bacillus safensis</i>
				<i>Bacillus stercoris</i>
Pseudomonadota (Proteobacteria)	Gammaproteobacteria	Moraxellales	Moraxellaceae	<i>Clostridium sporogenes</i>
				<i>Acinetobacter</i> sp.
	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Brevundimonas vesicularis</i>
		Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas panni</i>

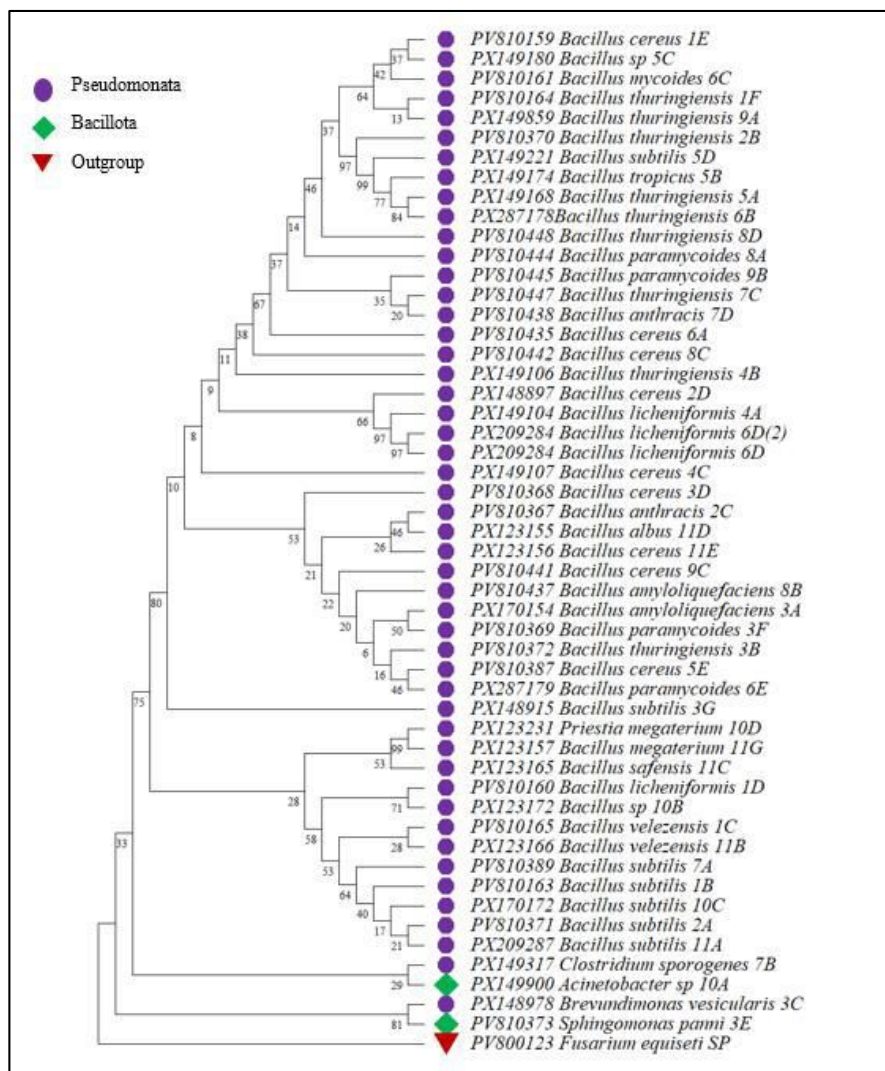


Fig 1: Genetic relatedness of phyllosphere bacterial isolates of mulberry sprayed with selected chemicals

The study enables deep understanding of pesticide-induced changes in the mulberry phyllosphere by showing that pesticide application reduces microbial diversity and causes a lasting shift toward dominance of stress-tolerant *Bacillus* species. The sensitive non-spore forming Proteobacteria were confined to chemical free treatments indicating high vulnerability to chemical stress. Although partial recovery of microbial communities occurred with declined pesticide residue levels, the original community structure restored fractionally reflecting persistent ecological effects. Overall, the findings highlight the long-term impact of pesticides on phyllosphere microbial balance and stress the need for judicious pesticide management in mulberry ecosystem for better leaf health and sustainable sericulture productivity.

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