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Hemipteran Diversity and Genotoxic Effects of Organophosphate Insecticides (Acephate and Profenofos) in Sialkot, Pakistan

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ABSTRACT

Background. Agriculture is a cornerstone of Pakistan's economy and faces significant pest pressure. Hemipteran insects, including aphids, leafhoppers, and true bugs, cause major crop losses and transmit plant pathogens.

Methods. This study surveyed the foliage-dwelling Hemiptera in Sialkot district and evaluated the toxicity and genotoxicity of two widely used organophosphate (OP) insecticides, acephate and profenofos. The field survey methods (sweep net and hand collection), insecticide bioassays, micronucleus (MN) assay procedures, and statistical analyses were done.

Results. Over a four-month field survey, 1,888 Hemipteran specimens were collected representing 32 species from three suborders. *Drosicha corpulenta* (a mealybug) and *Dysdercus cingulatus* (red cotton stainer) were the most abundant, each comprising ~31.8% of the total. Acephate and profenofos were tested on *D. cingulatus*; acephate showed higher acute toxicity (LC_{50} - 1.2 mL/L) than profenofos (LC_{50} - 2.0 mL/L). A micronucleus (MN) assay on hemocytes revealed a dose-dependent increase in DNA damage. MN frequencies in *D. cingulatus* exposed to acephate at LC_{50} were ~4.45%, compared to ~4.31% with profenofos, versus ~0.3% in controls. These findings indicate that acephate, while being more toxic to target pests, also induced slightly greater genotoxic effects. The results document the diversity of Hemipteran fauna in a key agricultural region and underscore the need for careful insecticide management.

Conclusion. This integrated approach—combining biodiversity assessment with toxicological and genotoxic analyses—highlights potential trade-offs between pest control efficacy and sublethal impacts, informing sustainable pest management and conservation strategies.

Keywords: acephate, *D. cingulatus*, genotoxicity, Hemiptera diversity, integrated pest management, micronucleus (MN) assay, organophosphate (OP) insecticides, profenofos

Highlights

- Two species (*D. corpulenta* and *D. cingulatus*) dominated the community, each representing ~32% of the 1,888 insects collected.
- Acephate was more toxic to the representative pest *D. cingulatus* (LC_{50} = 1.2 mL/L) than profenofos (LC_{50} = 2.0 mL/L), indicating stronger efficacy.

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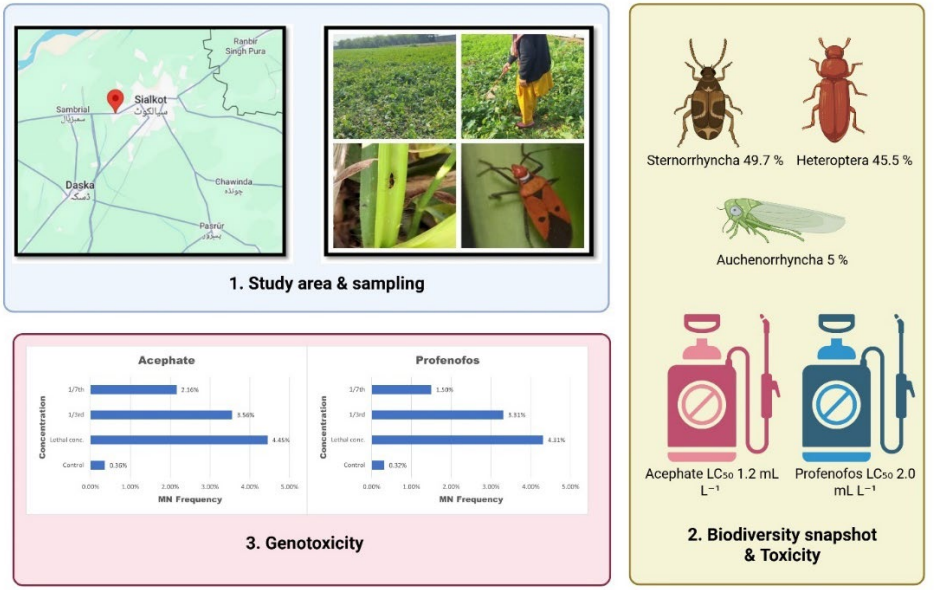
- Micronucleus (MN) assays showed significant DNA damage in insect hemocytes at sublethal exposures, with acephate causing a higher micronuclei frequency (~4.4%) than profenofos (~4.3%) at equivalent doses (control ~0.3%).

GRAPHICAL ABSTRACT



1. INTRODUCTION

Agriculture accounts for roughly one-fifth of Pakistan's Gross Domestic Product (GDP) and employs over 40% of the labor force [1, 2]. The country's major crops (e.g. wheat, rice, cotton) are threatened by a myriad of insect pests, particularly Hemiptera (true bugs), which include sap-feeding insects, such as aphids, whiteflies, and leafhoppers. Hemipteran pests not only cause direct feeding damage but also transmit serious plant pathogens; for instance, phytoplasma diseases spread by leafhoppers may devastate crops [3]. At the same time, beneficial insects (e.g. pollinators) play a crucial role in agriculture – insect pollination contributes significantly to crop yields [4]. This creates a pressing need to manage pest insects while preserving ecological balance.

Chemical insecticides remain the primary means of pest control in many developing regions. Organophosphate (OP) compounds are among the most widely used insecticides due to their broad-spectrum efficacy [5, 6]. Profenofos and acephate are two OP insecticides extensively applied in Pakistan's cotton and vegetable farming [6]. Profenofos is a contact insecticide that is highly persistent in the environment [6] and has been detected as a residue in soils, water, and even air near treated fields [7, 8]. Acephate is a systemic OP that is metabolized into the more toxic compound methamidophos in insects, enhancing its insecticidal activity [6]. Both compounds target the nervous system by inhibiting acetylcholinesterase, causing neurotoxicity in insects. However, profenofos is active in its parent form (a direct acetylcholinesterase inhibitor). On the other hand, many other

OPs require metabolic activation [6]. The heavy reliance on these chemicals has raised concerns about environmental contamination and human exposure [7, 9]. Moreover, repetitive use of a single insecticide or mode of action can select for resistance in pest populations [8, 9]. There is evidence that some field populations of pests have developed tolerance to Ops, such as profenofos and acephate [8], undermining their efficacy over time.

Given these challenges, an integrated understanding of pest ecology and insecticide impacts is crucial. Baseline data on pest diversity and abundance inform targeted management, while toxicological studies reveal the effectiveness and side effects of control measures. To date, limited information exists on the community of foliage-dwelling Hemiptera in Sialkot, a major agricultural district of Punjab, Pakistan. Some recent studies have begun to catalog insect diversity in this region [10] and in neighboring areas [11] but comprehensive surveys of Hemipteran fauna are lacking.

In addition to acute toxicity, the sublethal and chronic effects of pesticides on insects are of growing research interest. Genetic damage in non-target organisms (e.g., pollinators and natural enemies) has been documented when exposed to pesticides or environmental pollutants [12]. MN assays, which detect small extranuclear chromatin bodies in cells, are a well-established method to assess such genotoxic effects [13]. Micronuclei form from acentric chromosomal fragments or whole chromosomes lagging during cell division [13], and their frequency is a reliable biomarker of clastogenic/aneugenic damage in cells [13]. This technique has been widely used in ecotoxicology – for instance, [14] demonstrated via MN testing that herbicide exposure induced genetic damage in a protected butterfly species. However, little is known about whether insecticides induce similar genotoxic stress in pest insects themselves, which could have implications for their population fitness and the potential development of mutations (or, conversely, could indicate impacts on beneficial species that share exposure) [15].

In this context, the current study was designed to (1) survey the diversity and relative abundance of Hemipteran insects on selected crops in Sialkot District, (2) determine the acute toxicity (LC_{50}) of acephate and profenofos on a representative abundant Hemipteran pest species, and (3) assess DNA damage in the insects after sublethal insecticide exposure using the MN assay. By linking ecological data with toxicological and genotoxic endpoints, the study provided a holistic assessment that may inform sustainable pest management. While this study focused on the MN assay as a primary genotoxicity endpoint, future research could benefit from incorporating additional biomarkers, such as the comet assay or chromosomal aberration tests for a more comprehensive DNA damage assessment. Moreover, comparative toxicity studies across insecticide classes would further inform sustainable pest management strategies.

2. METHODOLOGY

2.1. Study Area and Period

Field sampling was conducted in District Sialkot, Punjab, Pakistan. Insect collections were made from four major tehsils (sub-districts): Sambrial, Daska, Pasrur, and Sialkot, all within ~50 km of Government College Women University Sialkot. The region's coordinates are approximately 32°30′ N, 74°32′ E. This area has a humid subtropical climate; average annual rainfall is ~950 mm, with hot summers (peaking >40 °C in June–July) and cool winters (down to ~4 °C). These localities were

chosen because together they account for > 80% of the district's cultivated acreage, differ markedly in crop composition and pesticide-use intensity, and are readily accessible within a 50 km radius of Government College Women University Sialkot, thus providing a representative cross-section of the region's agro-ecosystems. Specimens were collected using a standard sweep net (38 cm diameter, 100 sweeps per site) and hand-picking from foliage at 24 fixed sites across four tehsils. Sites included wheat, cotton, vegetable, and sugarcane fields. Sampling was carried out from early February 2023 to May 2023, spanning late winter to late spring.

2.2. Insect Collection and Preservation

Hemipteran insects (order Hemiptera) were collected from foliage in the above-mentioned crops using standard entomological methods. The study employed a sweep net technique and hand-picking to capture insects from crop canopies and foliage [15]. At each field site (~1 acre in area), we walked an eight-point star pattern to ensure coverage of field edges and center [15]. Sweeps were performed systematically along each transect; insects dislodged or captured in the net were transferred to ventilated plastic jars. Collected specimens were provided fresh host plant leaves in transit to keep them alive. For preservation of vouchers and non-target groups, some specimens were killed and stored in 70% ethanol.

2.3. Identification of Species

In the laboratory, insects were examined under a stereo zoom microscope. High-resolution images were taken for documentation. Specimens were identified to species level using available taxonomic keys and literature [16]. We consulted *"An Introduction to the Study of Insects"* and other entomological references for diagnostic characteristics [16]. For specialized groups, such as lesser-known leafhoppers and bugs, regional checklists and keys (e.g., the *Fauna of British India* series) were used and cross-referenced with recent taxonomic studies. Unidentified or doubtful specimens were sent to entomologists at the University of Agriculture Faisalabad for expert identification and confirmation.

2.4. Laboratory Insecticide Bioassays

The study evaluated the acute toxicity of two OP insecticides – acephate (commercial formulation, 75% SP) and profenofos (50% EC) (Concentrations in mL/L were converted to ppm active ingredient (a.i.) based on formulation purity (e.g., 1.2 mL/L acephate ≈ 900 ppm a.i)– on the most abundant Hemipteran pest from the field survey (the cotton stainer bug *D. cingulatus*). Adult *D. cingulatus* (both sexes, n≈200 total) were collected from local fields and acclimated in the lab for ~24 h with fresh foliage. The insects were divided into groups for treatment. For each insecticide, a series of concentrations were prepared to determine the median lethal concentration (LC_{50}). A modified WHO adult mosquito vial test protocol was adapted for topical spray application to simulate field exposure. A stock solution of acephate was made by dissolving the formulated product in distilled water (e.g. 70 g in 1 L, as per label, to create a high concentration) [17], and dilutions were made to cover a range expected to bracket the LC_{50}.

Similarly, profenofos (an emulsifiable concentrate) was diluted with water. In preliminary range-finding tests, approximate mortality levels were observed and then the test concentrations were narrowed. Spray exposure was used to simulate field application: bugs were placed in mesh-covered plastic cages (10 insects per cage, 3 replicate cages per dose) and misted with a fine

spray of the insecticide solution using a handheld sprayer [18]. Each cage received a standardized 5 mL of solution to ensure even coverage of insects and their food leaves. A control group (3 cages of insects) was sprayed with distilled water only.

2.5. Micronucleus (MN) Assay for Genotoxicity

To assess DNA damage, an MN assay was conducted on hemocytes of *D. cingulatus* following exposure to sublethal insecticide doses. The assay protocol was adapted from Santovito *et al.* [13] with minor modifications. After the acute toxicity tests, surviving insects from the control, 1/7 LC_{50}, 1/3 LC_{50}, and ~ LC_{50} groups were used (we sampled 5–6 insects per group, from each insecticide treatment). Hemolymph was extracted via a gentle abdominal incision, smeared on slides, fixed in methanol:acetic acid (3:1), and stained with 5% Giemsa. Micronuclei were scored according to established cytogenetic criteria – small, non-refractory, chromatin bodies separate from the main nucleus. For each treatment, 1,000 cells per insect were scored from 5–6 individuals.

2.6. Hemolymph Extraction

Each insect was briefly immobilized on ice, and a small incision was made in the intersegmental membrane of the abdomen using fine scissors. A droplet of hemolymph was gently squeezed out and immediately collected onto a clean microscope slide. Using a sterile spatula, epithelial cells were scraped from the incision area to include any circulating cells. The tip of the spatula and the hemolymph on the slide were promptly immersed in a fixative solution of methanol:acetic acid (3:1) kept at 4 °C [18].

2.7. Cell Preparation

The cell suspension in fixative was centrifuged at 8000 rpm for 15 min to concentrate the cells [18]. The supernatant was discarded, and the cell pellet was resuspended in fresh cold fixative (1–2 drops). Clean microscope slides were prepared, and a drop of the fixed cell suspension was poured onto each slide to make smear preparations. Multiple slides were made for each insect (we prepared four slides per insect for control and each treatment group). Slides were air-dried and then fixed again by adding a small volume of 3:1 methanol/acetic acid for 5 min, then air-dried.

2.8. Staining

The slides were stained with 5% *Giemsa* solution (diluted in pH 6.8 Sorensen phosphate buffer) for 10 min [14]. After staining, the slides were gently rinsed with distilled water and destained by briefly dipping in methanol to remove excess stain [18]. Once dry, slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX) and cover-slipped.

2.9. Data Analysis

Field survey data were analyzed to determine the relative abundance of each species. Relative abundance (%) was computed as (number of individuals of a given species/total individuals of all species) × 100 [10]. Diversity indices were calculated using the PAST v4.03 software package [18]. In the MN assay, one-way ANOVA was used to compare the mean MN frequencies among the four treatment groups (control, 1/7 LC_{50}, 1/3 LC_{50}, LC_{50}) for each insecticide. A p-value < 0.05 was considered statistically significant. One-way ANOVA with post-hoc Tukey HSD tests was used to compare treatment effects. Percentage data were arcsine-square root transformed to meet assumptions of homogeneity of variance. While this approach accounts for dose-dependent effects, future studies may consider mixed-effects models

to incorporate spatial and temporal covariates.

3. RESULTS

3.1. Hemipteran Diversity

A total of 1,888 Hemipteran insects were collected from the crop fields of Sialkot during the four-month survey. These belonged to three suborders – Sternorrhyncha, Auchenorrhyncha, and Heteroptera – comprising 32 identified species (across 15 families and several subfamilies; see Table 1). Figure 1 shows the overall composition by suborder. Sternorrhyncha (primarily sap-sucking insects like aphids and mealybugs) accounted for 49.7% of the specimens, followed closely by Heteroptera (45.5%), with Auchenorrhyncha (leafhoppers and planthoppers) being least abundant (4.8%). Despite their lower numbers, Auchenorrhyncha were represented by more species (8 species) than Sternorrhyncha (4 species), whereas Heteroptera had both high species richness (20 species) and high total numbers.

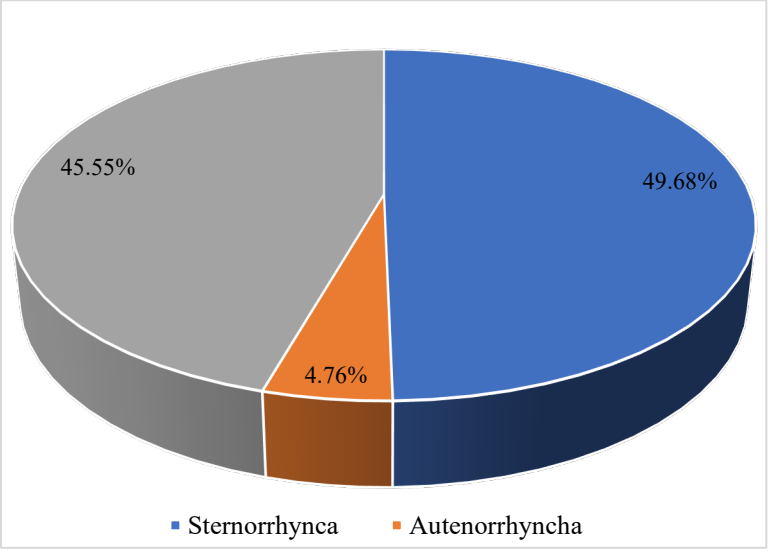


Figure 1. Relative Abundance of Hemiptera by Suborder in Sialkot Samples (in % of Total Individuals). Sternorrhyncha Comprised ~49.7%, Heteroptera 45.5%, and Auchenorrhyncha 4.8%.

In the Auchenorrhyncha (leafhoppers, planthoppers, cicadas), 8 species were identified, however, their individual numbers were low (Table 1). The most frequently encountered Auchenorrhynchans were the sugarcane leafhopper *(Cofana spectra)* (1.37%, 26 specimens) and the maize leafhopper *(Pyrilla perpusilla)* (0.95%, 18 specimens). Other species, such as *(Neotibicen linnei)* (a cicada, ~0.6%, 12 specimens), and *(Psamotettix alienus)* (a leafhopper, 0.37%, 7 specimens), were occasionally found. Each of the remaining Auchenorrhynchan species contributed <0.5% of the total fauna (Table 1). While diverse, this suborder was a minor component in terms of abundance.

Table 1. Diversity and Relative Abundance of Hemipteran Species Collected from Sialkot District

Suborder	Family	Subfamily	Species (Authority)	Relative Abundance (% of total, count)
Sternorrhyncha	Pseudococcidae	Pseudococcinae	*Phenacoccus solani*	15.88% (300)
	Monophlebidae*	—	*Drosicha corpulenta*	31.77% (600)
	Aphididae	Eriosomatinae**	*Prociphilus oriens*	1.90% (36)
	Miridae***	Dicyphinae	*Macrolophus pygmaeus*	0.10% (2)
Auchenorrhyncha	Flatidae	—	*Neotibicen linnei*	0.63% (12)
	Membracidae	—	*Ceresa taurina*	0.52% (10)
	Cicadellidae	Deltocephalinae	*Pyrilla perpusilla*	0.95% (18)
		Deltocephalinae	*Dictylophara nakanonis*	0.26% (5)
	Cicadellidae	Cicadellinae	*Euscelidius variegatus*	0.47% (9)
	Cicadellidae	Cicadellinae	*Cofana spectra*	1.37% (26)
	Cicadellidae	Deltocephalinae	*Psamotettix alienus*	0.37% (7)
	Aphrophoridae	—	*Philaenus spumarius*	0.15% (3)
Heteroptera	Pyrrhocoridae	—	*Dysdercus cingulatus*	31.77% (600)
	Lygaeidae	Orsillinae	*Metochus uniguttatus*	0.21% (4)
	Blissidae	—	*Blissus leucopterus*	0.42% (8)
	Scutelleridae	—	*Chrysocoris stollii*	0.10% (2)
	Tingidae	—	*Corythucha ciliata*	0.42% (8)
	Cydnidae	—	*Sehirus cinctus*	1.05% (20)
	Coreidae	—	*Anasa tristis*	0.63% (12)
	Coreidae	—	*Cletus trigonus*	0.42% (8)
	Coreidae	—	*Leptoglossus occidentalis*	0.21% (4)
	Reduviidae	Peiratinae	*Himacerus apertus*	0.21% (4)
	Lygaeidae	Lygaeinae	*Nysius raphanus*	0.31% (6)
	Lygaeidae	Lygaeinae	*Oncopeltus fasciatus*	0.26% (5)
	Lygaeidae	Lygaeinae	*Spilostethus furcula*	0.37% (7)
	Pentatomidae	Asopinae (predator)	*Andrallus spinidens*	0.21% (4)
	Pentatomidae	Asopinae (predator)	*Podisus maculiventris*	0.84% (16)
	Pentatomidae	Pentatominae	*Halyomorpha halys*	5.19% (98)
	Pentatomidae	Pentatominae	*Piezodorus hybneri*	0.84% (16)
	Pentatomidae	Pentatominae	*Piezodorus lituratus*	0.95% (18)
	Anthocoridae	— (predator)	*Orius insidiosus*	0.63% (12)
	Aradidae	—	*Dimissalna dimissa*	0.42% (8)

* *D. corpulenta* is often classified in family Margarodidae (Giant scale insects). However, here, it was recorded under Monophlebidae (a related group of scale insects). ** *Prociphilus oriens* is an aphid (family Aphididae); subfamily assignment based on its genus (possibly Eriosomatinae). *** *Macrolophus pygmaeus* (predatory mirid) is not a Sternorrhynchan; it was likely an incidental capture included here because it was collected on plants alongside Sternorrhyncha.

Each suborder is listed with its constituent families, subfamilies, and species,

along with the percentage of the total 1,888 specimens (with absolute counts in parentheses).

The family-wise composition of these suborders is illustrated in Figure 2, with dominant representation from Pyrrhocoridae, Pseudococcidae, and Pentatomidae. Further subdivision by subfamily (e.g., Pseudococcinae, Deltocephalinae) is presented in Figure 3, highlighting the internal taxonomic structure of the Hemipteran community. Among the 32 identified species, *D. corpulenta* (a mealybug) and *D. cingulatus* (red cotton stainer) were the most abundant, each comprising 31.77% of the total insect count. Other notable species included *Phenacoccus solani* (15.88%) and *Halyomorpha halys* (5.19%). Rare species (e.g., *Macrolophus pygmaeus*) were found in very low numbers (0.10%).

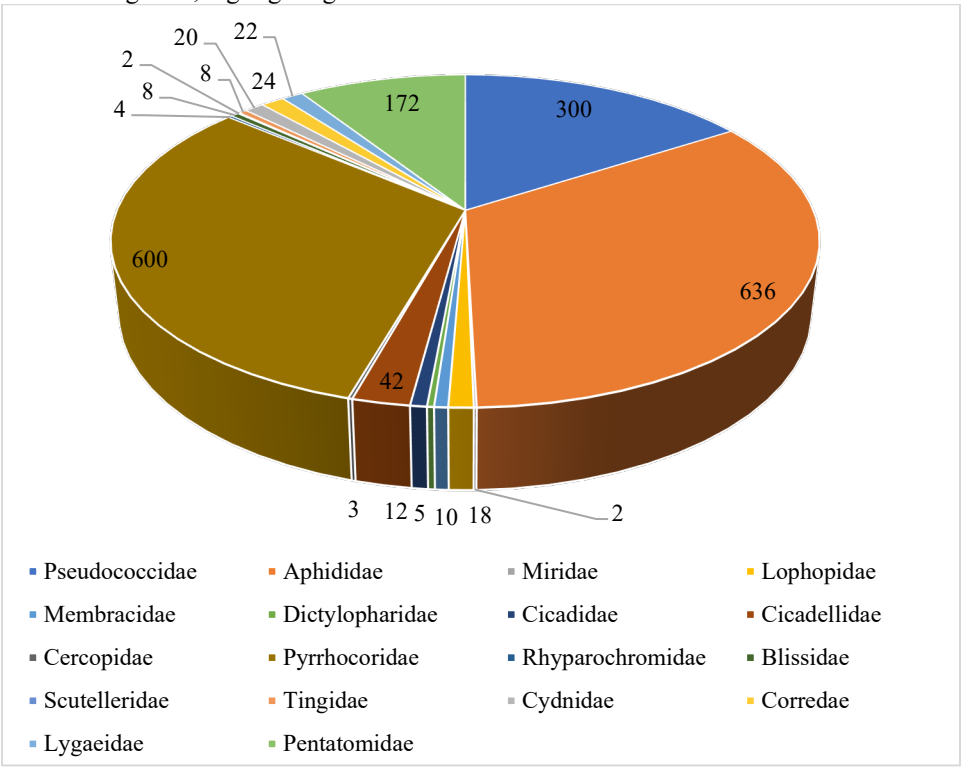


Figure 2. Relative Abundance of Major Hemiptera Families Collected. For instance, Pyrrhocoridae (bugs like *Dysdercus*) and Monophlebidae/Pseudococcidae (Mealybugs Like *Drosicha*, *Phenacoccus*) form the Largest Portions.

The trend in insect abundance is plotted in Figure 4. Bars indicate the proportional contribution of each species to the total number of Hemipteran individuals collected during the study period. This seasonal rise correlates with increasing temperatures and crop maturation. The sharpest rise was seen in *D. corpulenta* and *D. cingulatus* populations during late spring.

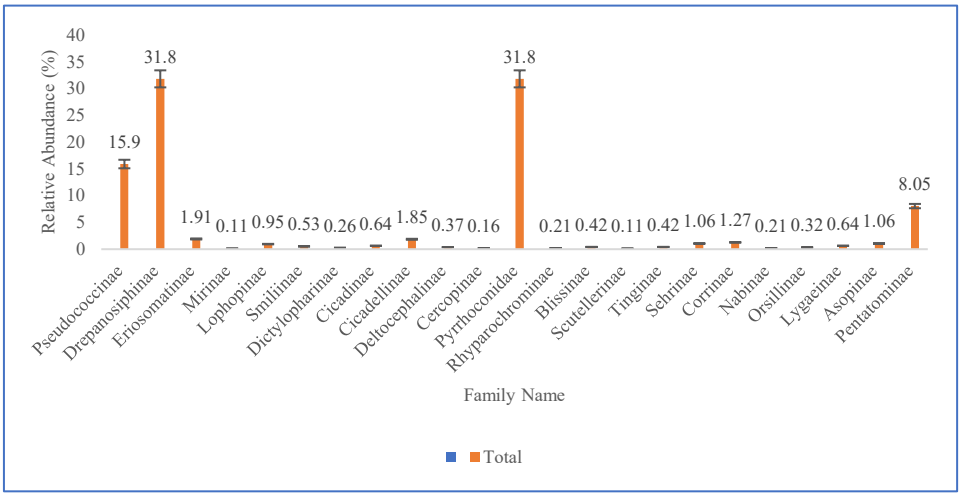


Figure 3. Relative Abundance of Hemiptera Subfamilies (or Significant Groupings) within the Collected Specimens. This Highlights, For Instance, Subfamily Drepanosiphinae (aphids) vs. others.

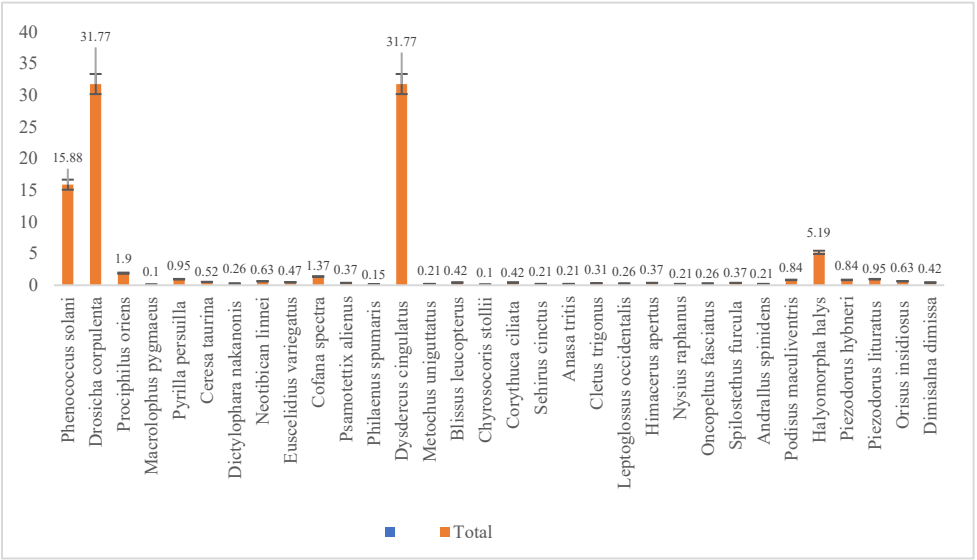


Figure 4. Relative Abundance (%) of Hemipteran Species Recorded from Sampled Crop Fields in Sialkot District.

3.2. Spatial and Temporal Patterns

Hemipteran abundance varied by location, crop, and month. Among the four tehsils sampled, Sambrial yielded the highest number of Hemiptera (811 specimens, ~43% of total), whereas Daska had the lowest (174 specimens, ~9%). Pasrur and Sialkot tehsils were intermediate (approximately 450 and 453 specimens,

respectively). This suggests that local environmental or farming differences (e.g. crop area or pesticide usage intensity) influenced insect population levels. Regarding crop-wise distribution, the majority of insects were collected from vegetable fields (1,437 specimens, ~76% of the total). Vegetables (which included mixed plantings of okra, tomato, etc.) harbored far more Hemiptera than other crops, likely due to a combination of host preference and lower insecticide usage on some vegetable plots. In contrast, sugarcane fields contributed 224 specimens (~12%), wheat fields 159 (~8%), and fodder crops (berseem clover fields) only 68 (~4%). Figure 5 illustrates the percent composition by crop type.

In terms of statistical trends, ANOVA and interval plots (Figure 5) demonstrated significant differences. Figure 5A shows that insect abundance increased significantly over the months. Figure 5B indicates that vegetable crops supported the highest densities of Hemiptera (~76%), followed by sugarcane, wheat, and fodder. Figure 5C highlights that the mean insect abundance was significantly greater in vegetables than other crop types. Figure 5D presents a 95% confidence interval comparison, reinforcing crop-wise abundance differences.

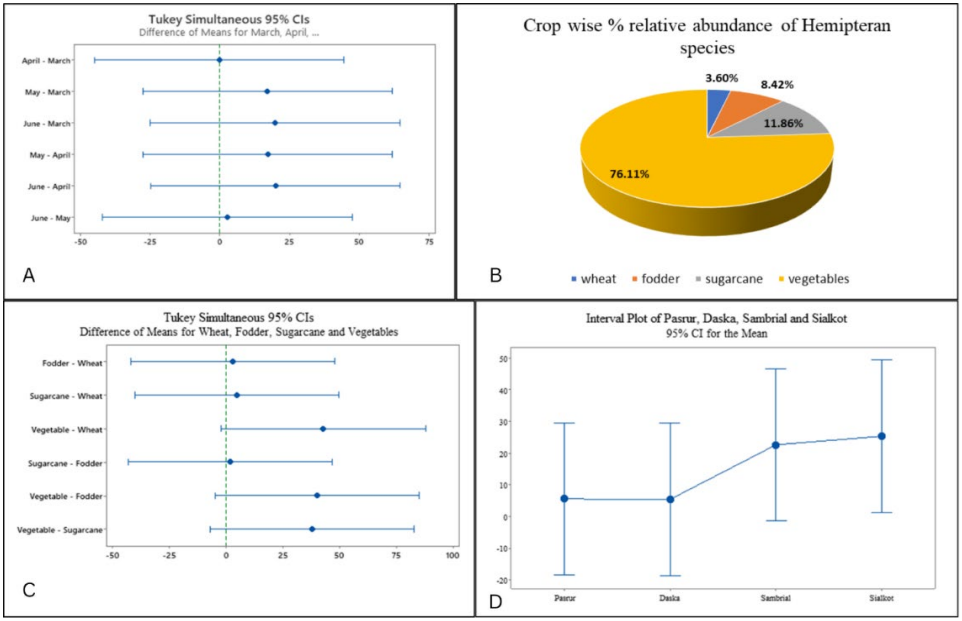


Figure 5. Statistical Comparisons. A) Month-wise Comparison of Mean Hemipteran Abundance, B) Crop-wise Percentage Composition of Hemiptera Collected, C) Differences of Means Plot for Hemipteran Abundance by Crop Type, and D) Interval Plot (95% Confidence) for Hemipteran Abundance across the Four Crop Types.

3.3. Acute Toxicity of Insecticides

The contact spray bioassay results for *D. cingulatus* indicated differential toxicity between acephate and profenofos (Figure 6). Acephate was more potent, yielding an LC_{50} of 1.2 mL/L (95% CI: 1.0–1.4 mL/L) for 8-hour mortality. In contrast, profenofos had an LC_{50} of 2.0 mL/L (95% CI: 1.7–2.3 mL/L) under the same

conditions. The mortality response curves for each insecticide are displayed in Figure 6A and 6B. These values were derived from probit analysis of mortality data and showed clear separation between the two insecticides. For reference, the empirical mortality at test concentrations around these LC_{50} values matched the 50% kill level, validating the probit model. No mortality was observed in the control groups (0% in water-only sprays).

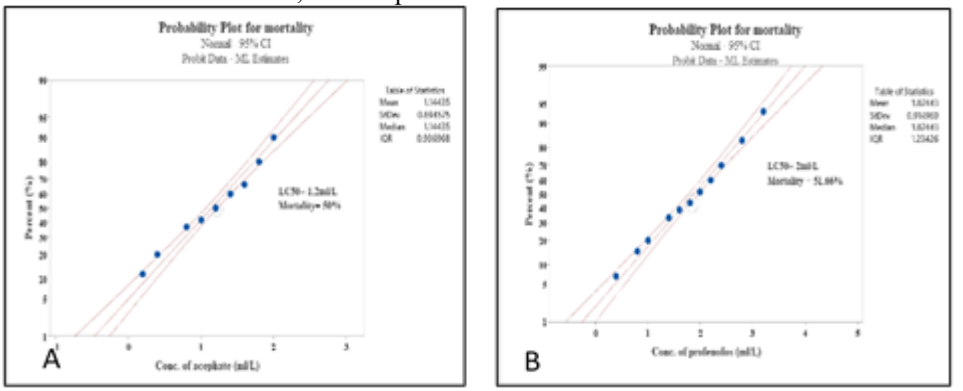


Figure 6. Mortality Percentage A) *D. cingulatus* with Different Doses of Acephate and B) *D. cingulatus* with Different Doses of Profenofos

From the dose-mortality data, the study determined the predefined sublethal concentrations for subsequent genotoxicity tests. For acephate, one-third of the LC_{50} was ~0.4 mL/L and one-seventh was ~0.17 mL/L. For profenofos, one-third LC_{50} was ~0.67 mL/L and one-seventh was ~0.28 mL/L. These values (rounded to two significant decimals) were used to treat insects for the MN assay. By design, exposure to these sublethal doses caused little or no immediate mortality: in the 8 h observation, the 1/3 LC_{50} doses caused <5% mortality, and the 1/7 LC_{50} doses caused virtually 0% mortality. Surviving insects from these treatments were thus available for the assessment of DNA damage.

3.4. Micronucleus (MN) Assay

The MN test revealed a clear increase in genetic damage in insect hemocytes with higher insecticide exposure (Figure 53 and Figure 54). In the control group (unexposed *D. cingulatus*), the MN frequency was very low, at 0.32% (approximately 3 cells with micronuclei out of 1000 scored) for the profenofos control, and 0.36% for the acephate control (essentially baseline levels of DNA aberrations). By contrast, insects exposed to the LC_{50} concentration showed the highest frequencies of micronuclei: 4.31% in the profenofos LC_{50} group, and 4.45% in the acephate LC_{50} group (Table 2). Thus, roughly 1 in 22 cells from these bugs had MN formation after acute high-dose exposure, a striking increase from the ~1 in 300 baseline rate. The 1/3 LC_{50} (medium sublethal) groups also showed elevated MN frequencies: 3.31% (profenofos) and 3.56% (acephate). Even the LC_{50} (low sublethal) exposures induced measurable genotoxic effects: MN frequencies were 1.50% and 2.16% in the profenofos and acephate groups, respectively, which are about 4–6 times higher than control values. These data demonstrate a dose-dependent increase in micronuclei in *D. cingulatus* hemocyte cells with both insecticides (Table 2). Notably, at each comparable fraction of LC_{50} (low, medium,

high), the acephate-treated insects exhibited slightly higher MN percentages than the profenofos-treated insects. For instance, at the LC_{50} level, acephate (4.45%) was marginally higher than profenofos (4.31%); at 1/7 LC_{50}, acephate (2.16%) was higher than profenofos (1.50%). This suggests acephate may induce slightly more genotoxic stress in the insects than profenofos at equivalent toxicity-adjusted doses, though both clearly have significant effects. *Acephate's* systemic action and metabolic activation to *methamidophos* likely contribute to its higher acute toxicity compared to the contact-based activity of *profenofos*.

Table 2. MN Frequency in *D. cingulatus* Hemocytes Following Exposure to Sublethal Concentrations of acephate and profenofos

Treatment Group	Acephate – MN Frequency (%)	Profenofos – MN Frequency (%)
Control (0 mL/L)	0.36%	0.32%
1/7 LC_{50} (low)	2.16%	1.50%
1/3 LC_{50} (medium)	3.56%	3.31%
LC_{50} (high)	4.45%	4.31%

Note. Values represent the percentage of cells with micronuclei out of 1000 cells scored for each treatment group. Control groups were sprayed with water only. LC_{50} = median lethal concentration (1.2 mL/L for acephate, 2.0 mL/L for profenofos). 1/3 LC_{50} for acephate ≈0.4 mL/L, for profenofos ≈0.67 mL/L; 1/7 LC_{50} for acephate ≈0.17 mL/L, for profenofos ≈0.28 mL/L. Each percentage is based on pooled counts from multiple insects; standard errors for MN % were <0.2 percentage points for all groups. ANOVA confirmed a significant increase in MN frequency with higher exposure ($p < 0.001$ for both insecticides).

Statistical analysis confirmed these observations. One-way ANOVA revealed a significant dose-dependent increase in MN frequency ($p < 0.001$ for both insecticides). Post-hoc Tukey tests confirmed all treatment groups differed significantly from controls ($p < 0.01$). One-way ANOVA comparing the four groups (control vs increasing exposure) showed a highly significant effect of treatment on MN frequency for both insecticides ($p < 0.001$ for acephate, $p < 0.001$ for profenofos). Post-hoc comparisons indicated that all insecticide-treated groups differed significantly from the control in MN frequency ($p < 0.01$ in each case). Among the treated groups, the LC_{50} group had a significantly higher mean MN frequency than the 1/7 LC_{50} group ($p < 0.05$), with the 1/3 LC_{50} group intermediate. There was no significant difference between acephate and profenofos in overall genotoxic effect when comparing equivalent groups via two-way ANOVA (insecticide × dose interaction $p > 0.1$), although numerically acephate yielded higher MN counts. The data (Figure 7 and 8) highlight that both chemicals, even at sublethal doses, can cause genetic damage to insect cells. Microscopy images of the hemocyte smears (Figure 7 for acephate, Figure 8 for profenofos) show typical micronuclei appearing as small, round, darkly-stained bodies in the cytoplasm near the main nucleus. These results provide evidence that surviving insects carry cytogenetic damage after exposure, which could have sublethal fitness consequences.

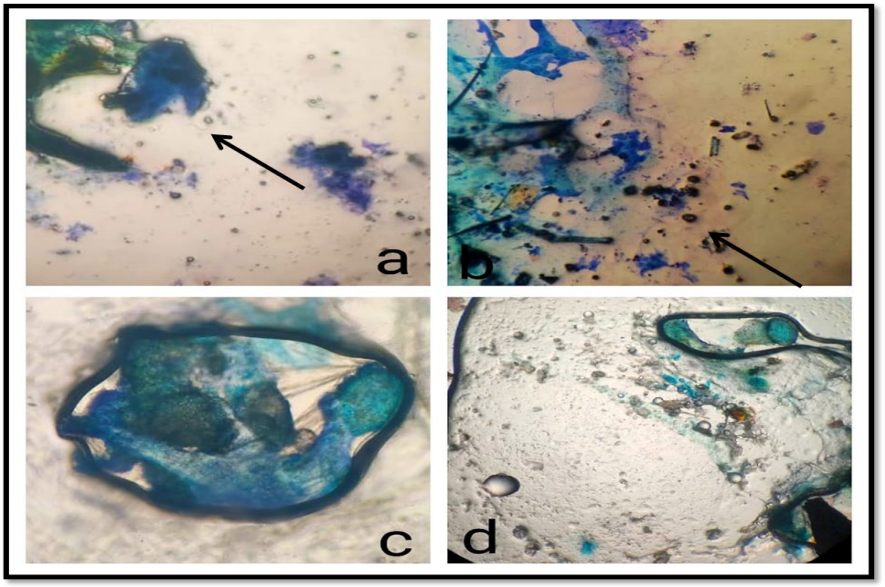


Figure 7. Micronuclei in Hemocytes of *D. cingulatus* after Acephate Exposure. Arrows Indicate Cells with Micronuclei in an Insect from the LC_{50} Acephate Group (Giemsa Stain, 1000×).

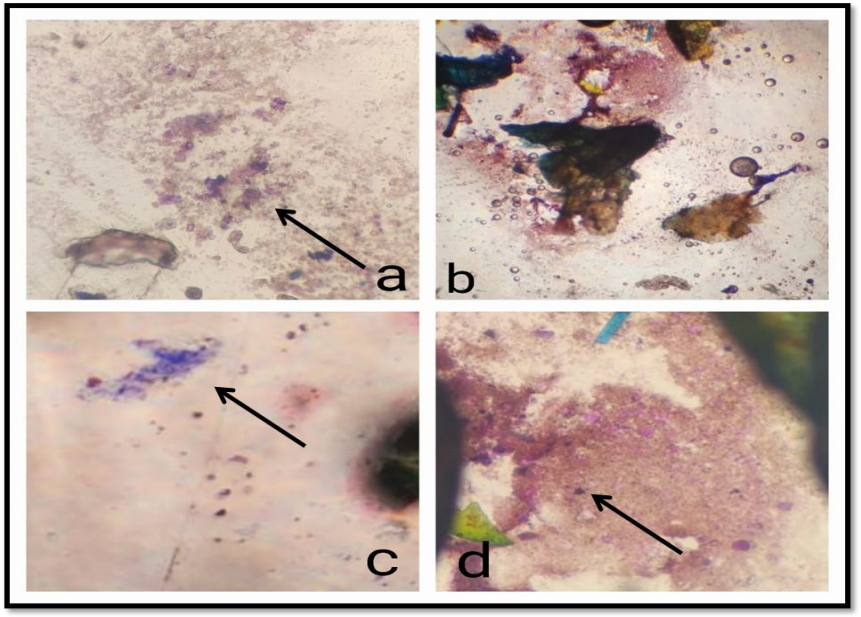


Figure 8. Micronuclei in Hemocytes of *D. cingulatus* after profenofos Exposure. Arrows Point to Micronucleated Cells in an Insect from the LC_{50} Profenofos Group. Micronuclei Appear as Small Dark-Purple Dots Separate from the Main Nucleus.

4. DISCUSSION

4.1. Hemipteran Faunal Composition

The current survey presents one of the first detailed accounts of foliar Hemiptera in Sialkot's croplands. The 32 species documented include notorious pests as well as some predatory and neutral species, reflecting a diverse arthropod community in these agroecosystems. Comparable studies in nearby regions have reported similar orders of diversity: for instance, [19] found 27 hemipteran species in an agricultural park in India, and noted a richness of over 30 insect species (across orders) in Sialkot's industrial-affected farms [19]. The findings are in line with these surveys, though direct comparisons are nuanced by differences in habitat and sampling methods.

4.2. Implications for Pest Management

The dominance of *D. cingulatus* and *D. corpulenta* as pests in Sialkot's agroecosystem underscores an urgent need to manage these species. *Dysdercus* in particular can cause extensive damage to cotton seeds and stain fibers, and its presence on okra suggests it maintains year-round populations on alternate hosts. Traditional control of *Dysdercus* relies heavily on insecticides but our findings of tolerance at low doses and known cases of resistance in related bugs (e.g. *Oxycarenus hyalinipennis*) [19] indicate that sole reliance on chemicals may be unsustainable. Integrated Pest Management (IPM) approaches, including cultural controls (e.g. removal of weed hosts) and biological control (augmenting natural predators like *Podisus*), should be emphasized. Encouragingly, the study found predators co-existing in the fields; conservation of these natural enemies by judicious pesticide use could help naturally suppress pests [20]. The relatively high Shannon diversity (H′ ~1.94) for an agroecosystem suggests that not all natural control mechanisms are lost—there is still a baseline of biodiversity to leverage for IPM.

4.3. Acute Toxicity and Resistance

The bioassay results clearly demonstrated that acephate is more acutely toxic to *D. cingulatus* than profenofos. This aligns with field observations where acephate often provides quick knockdown of sap-feeding insects. Our measured LC_{50} for acephate (1.2 mL/L, roughly equivalent to ~0.09% active ingredient solution assuming 75% SP formulation) is in the same order of magnitude as values reported for other Hemiptera. For instance, Akhtar *et al.* [21] found that acephate had the lowest LC_{50} among tested insecticides against the dusky cotton bug *Oxycarenus hyalinipennis*, indicating high efficacy. They reported acephate LC_{50} around 84 ppm for *Oxycarenus*, which is a different expression but similarly points to acephate's potency. Our profenofos LC_{50} (2.0 mL/L, ~0.1% a.i.) also aligns with expectations; previous research on Hemiptera (e.g. whiteflies and leafhoppers) shows profenofos is effective but often slightly less so than systemic OPs or neonicotinoids [22, 23]. The need for almost double the concentration of profenofos to achieve mortality comparable to acephate might be due to physiological or behavioral factors—acephate, being systemic, might be ingested as well as contacting the insect, whereas profenofos mostly acts through contact and has somewhat lower penetration or slower action [24].

Interestingly, the study observed that *D. cingulatus* exhibited survival at low doses of both insecticides, hinting at partial resistance or tolerance. This mirrors findings in other pest populations; for instance, [25] documented developing resistance to OPs in cotton mealybugs, requiring higher doses for control. The tolerance in our *Dysdercus* population could stem from

metabolic resistance mechanisms (e.g., enzyme detoxification) or target-site insensitivity. [26] have reported biochemical resistance mechanisms (like elevated detoxification enzymes) in insects exposed to OPs and other classes. While resistance ratios were not specifically measured, the low mortality in 1/7 LC_{50} groups reinforces the notion that insecticide resistance management should be a priority. Rotating chemical classes and integrating non-chemical methods can help mitigate this risk [26]. The Insecticide Resistance Action Committee (IRAC) recommends using mixtures or rotations that involve different modes of action to delay resistance development. The data supports this strategy: relying solely on acephate (even though currently effective) could select for resistant individuals, given that some survived significant exposure. A comprehensive IPM plan including crop sanitation, resistant crop varieties (if available), and periodic alternation of acephate with other insecticides (e.g. pyrethroids or neonicotinoids, if appropriate) is advised for managing *Dysdercus* and similar pests [26].

4.4. Genotoxic Effects

A key outcome of this study is the demonstration that sublethal exposure to acephate and profenofos can cause DNA damage in insects, evidenced by increased MN formation in *D. cingulatus* hemocytes. Although MN assays are more commonly applied to non-target organisms (to evaluate environmental safety of pesticides) [26], our application to a pest species reveals that these chemicals have genotoxic potential across taxa. The significantly higher MN frequencies in treated groups (1.5–4.5%) compared to controls (~0.3%) indicate that even doses that do not kill the insect can induce chromosomal breaks or spindle disturbances. This finding aligns with results from studies on other insects and organisms: [27] showed that particulate pollutants caused a dose-dependent rise in micronuclei in butterfly larvae, and similarly found elevated micronuclei in butterflies exposed to an herbicide. Our work extends this to insecticides and pest insects, suggesting a broad genotoxic effect of these OP compounds [28]. The observed DNA damage may result from oxidative stress induced by OP exposure, a known secondary effect of AChE inhibition that generates reactive oxygen species and causes chromosomal aberrations. Although the MN assay provided clear evidence of chromosomal damage, incorporating complementary genotoxicity endpoints—such as the comet assay for DNA strand breaks or detailed chromosomal aberration analyses—would offer a more mechanistic understanding of DNA damage induced by acephate and profenofos. Furthermore, while the current study compared two OPs, future research should expand comparisons to include other insecticide classes (e.g., neonicotinoids, pyrethroids, insect growth regulators) and formulations to better inform resistance management and ecological risk assessments in IPM programs [29].

4.5. Sustainable Pest Management Recommendations

The combined evidence from this survey and bioassays suggests a need for balanced pest management in the region. Given the high efficacy of acephate, it remains a useful tool for outbreak pests, such as *Dysdercus* but should be used judiciously. The detection of possible resistance and the proven genotoxic effects call for rotating acephate with other control measures. Profenofos, while slightly less potent, could be rotated with acephate to delay resistance. However, its heavy use should also be curtailed due to persistence and environmental concerns [30, 31]. Cultural controls, such as timely removal of

crop residues and weed hosts that harbor *D. corpulenta* and *Dysdercus* during off-season, can reduce initial pest influx. Biological controls deserve more attention: the findings of predatory bugs indicate that if protected, these could naturally suppress some pests (for instance, *Podisus maculiventris* preys on caterpillars and could incidentally consume *Dysdercus* nymphs) [32, 33]. Conservation biocontrol – avoiding broad-spectrum sprays early in the season – might allow predator populations to build up [34]. Additionally, augmentation or introduction of biocontrol agents (such as parasitoids specific to mealybugs or fungal entomopathogens for *Dysdercus*) could be explored in future research, as suggested by Sani *et al.* [35] for whitefly control using entomopathogenic fungi.

4.6. Conclusion

In conclusion, the integrated findings emphasized a need for judicious insecticide use and integrated pest management. The study established a baseline of Hemipteran pest diversity in Sialkot and demonstrated that while chemical control (especially with acephate) is effective against key pests, it must be balanced against issues of resistance and sublethal harm. Based on results, the study recommended the following strategies for sustainable pest management in Sialkot and similar agroecosystems, such as insecticide rotation, cultural controls, and monitoring programs. Future research should expand on these results by exploring alternative control measures (biological or cultural) for the dominant pests and assessing the long-term ecological consequences of sublethal pesticide exposure in agroecosystems.

Author Contribution

Fatima Tahir Khan: conceptualization, data curation, investigation, methodology, writing – review & editing. **Rabia Afzal**: software, visualization, validation, writing – original draft.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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