# Phytochemical Profiling and Insecticidal Potential of *Rosa moschata* Extracts Against Key Agricultural Pests

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**Keywords:** *Rosa moschata*; botanical insecticide; aphicidal activity; *Acyrthosiphon pisum*; GC– MS profiling.

**Submitted 07/02/2026, Published online on 30th April 2026 in the** *Journal of Animal and Plant Sciences (J. Anim. Plant Sci.) ISSN 2071 – 7024*

## 1 ABSTRACT

**This study evaluated the insecticidal potential of ethanolic extracts from *Rosa moschata* (musk rose) leaves and flowers against four agriculturally important insects representing distinct orders: *Acyrthosiphon pisum* (pea aphid, Hemiptera), *Drosophila melanogaster* (fruit fly, Diptera), *Tribolium castaneum* (red flour beetle, Coleoptera), and *Spodoptera exigua* (beet armyworm, Lepidoptera). In 2% (w/v) screening, *A. pisum* was the most susceptible, exhibiting 100% mortality within 24 h, whereas *D. melanogaster* and *S. exigua* showed only moderate, time-dependent effects and *T. castaneum* was unresponsive. GC–MS (Gas Chromatography-Mass Spectrometry) profiling identified 20 constituents in leaf extract and 10 in flower extract, dominated by fatty-acid esters, terpenoids, and aromatic compounds. In dose–response assays against *A. pisum*, the 2% leaf extract was most potent ($LC_{50}$ = 35 ppm; $LC_{90}$ = 101 ppm), and the 2% flower extract also showed activity ($LC_{50}$ = 125 ppm; $LC_{90}$ = 197 ppm). In a focused series on leaf extract concentrations, the 2% treatment achieved even lower lethality thresholds ($LC_{50}$ = 17 ppm; $LC_{90}$ = 47 ppm at 24 h), while 1% and 0.5% were markedly less effective. These findings position *R. moschata* particularly the leaf extract as a promising botanical candidate for integrated pest management targeting aphids, and they motivate fractionation, mechanism elucidation, formulation optimization, and semi-field/field validation.**

## 2 INTRODUCTION

Pesticides are widely used chemical agents applied to control insects, weeds, fungi, and other organisms that threaten agricultural production and human health. They are integral to modern farming, ensuring higher yields and protecting food security in a world where global population growth continues to increase demand( *Hassaan, et al., 2020*). In 2020 alone, global pesticide consumption reached approximately 3.5 million tons, with Asia accounting for nearly half of this use. Despite their benefits, pesticides pose serious challenges, including environmental contamination, bioaccumulation, and adverse effects on non-target organisms such as pollinators and aquatic life (Hassan, 2019). The World Health Organization estimates that pesticide poisoning causes around 385 million cases of acute illness annually, resulting in nearly 150,000 deaths worldwide (Eddleston , 2024) Moreover, the extensive and repeated application of synthetic pesticides has accelerated the evolution of resistant pest species, reducing their long-term efficacy (Mangan *et al.,* 2023). These concerns highlight the urgent need to explore safer, eco-friendly, and sustainable alternatives for pest

management that can reduce dependency on synthetic chemicals while maintaining agricultural productivity Rezende-Teixeira *et al.,* 20222. Botanical pesticides exploit plant secondary metabolites limonoids, alkaloids, terpenoids, phenylpropanoids, coumarins, saponins, fatty acids, and isothiocyanates to deliver insecticidal, antifeedant, oviposition-deterrent, and repellent effects with generally rapid degradation and compatibility with IPM Turchen, *et al.,* 2020; Divekar *et al.,* 2022 *lium* (axonal modulators), rotenone from *Derris/Lonchocarpus* (mitochondrial complex I inhibitor), nicotine from *Nicotiana* (nAChR agonist), ryanodine from *Ryania* (RyR modulator), and veratrum alkaloids from *Schoenocaulon officinale* Khan, S., *et al.,* 2017. Essential-oil monoterpenes such as thymol (*Thymus vulgaris*), carvacrol (*Origanum vulgare*), eugenol (*Syzygium aromaticum*), pulegone (*Mentha pulegium*), linalool (*Lavandula* spp.), 1,8-cineole (*Eucalyptus* spp.), menthol (*Mentha* spp.), limonene (citrus peels), citronellal/citronellol (*Cymbopogon* spp.), and camphor (*Cinnamomum camphora*) act through octopaminergic interference, enzyme inhibition (e.g., AChE), and cuticular or respiratory disruption (Gajger. And Dar, 2021 . Beyond these, limonoids from *Azadirachta indica* (azadirachtin, salannin, nimbin) function as potent insect growth regulators and antifeedants; piperamides (e.g., piperine) from *Piper* spp., allyl isothiocyanate from *Brassica* spp., and triterpenoid saponins from *Quillaja saponaria* provide additional multimodal actions Ramsewak et al. ,2001. Annonaceous acetogenins from *Annona* spp. (annonacin, rolliniastatin analogs) display strong complex I inhibition across diverse pest species, while quinolizidine alkaloids such as matrine and oxymatrine from *Sophora flavescens* show contact and ingestion toxicity with neurophysiological and moulting effects (Kannathasan *et al.,* 2008; Pérez-Gutiérrez *et al.,* 2011. Other promising leads include flavonoids (quercetin, catechin), phenolic acids (gallic, caffeic), fatty acids (lauric, oleic), and glycosides with deterrent or sterilant outcomes. Collectively, the chemical diversity, lower persistence, and potential selectivity of these phytochemicals justify systematic evaluation of additional botanicals setting the stage for a focused examination of *Rosa* spp. *Rosa moschata* Herrm., commonly known as the musk rose, belongs to the family Rosaceae, a diverse family encompassing over 100 genera and more than 3,000 species. Plants of the genus *Rosa* are globally renowned not only for their ornamental and perfumery value but also for their rich reservoir of bioactive compounds. The petals, hips, seeds, and leaves of *Rosa* species contain essential oils, phenolics, flavonoids, tannins, fatty acids, and triterpenoids that have been linked with antimicrobial, antioxidant, insecticidal, and medicinal properties. Essential oils rich in citronellol, geraniol, and phenethyl alcohol exhibit strong repellence and fumigant activity, while seed oils containing linoleic and α-linolenic acids contribute to pest cuticle disruption. In addition, phenolic acids such as gallic and caffeic acid, and flavonoids like quercetin and kaempferol, are known to interfere with insect physiology, feeding behaviour, and moulting processes [16]. Because *Rosa* is widely cultivated and produces substantial amounts of by-products from perfumery and herbal industries, its utilization as a botanical pesticide offers an environmentally friendly and economically viable approach. Within this genus, *Rosa moschata* stands out for its distinctive phytochemical profile, positioning it as a promising candidate for systematic evaluation against key agricultural pests . In light of the increasing demand for sustainable pest management solutions, the present study investigates the insecticidal potential of *Rosa moschata* Herrm. extracts. Both leaf and flower ethanolic extracts were subjected to bioassays against four agriculturally important insect pests representing distinct orders: *Acyrthosiphon pisum* (Hemiptera), *Drosophila melanogaster* (Diptera), *Tribolium castaneum* (Coleoptera), and *Spodoptera exigua* (Lepidoptera). To elucidate the chemical basis of bioactivity, phytochemical profiling of the extracts was performed using gas chromatography–mass spectrometry (GC–MS), enabling the identification of major secondary metabolites with potential insecticidal functions.

Mortality, lethal concentrations ($LC_{50}$ and $LC_{90}$), and dose–response relationships were evaluated to assess comparative efficacy. By linking insecticidal outcomes with phytochemical constituents, this study aims to establish *R. moschata* as a promising botanical resource for integrated pest management strategies, offering both ecological safety and practical utility in agricultural systems.

## 3 MATERIALS AND METHODS

**3.1 Plant material:** Fresh leaves and flowers of *Rosa moschata* Herrm. (Rosaceae) were collected from the lower northern areas of Makkah Al-Mukarramah, Saudi Arabia in April 2024. during the flowering season. Plant parts were shade-dried for three months at ambient conditions, then milled to a fine powder using an electric grinder. Ground material was stored in airtight containers at room temperature, protected from light, until extraction.

**3.2 Preparation of crude plant extracts:** Powdered leaves and flowers were extracted with ethanol using a microwave-assisted extraction (MAE) procedure (method adapted with minor modifications). After extraction, suspensions were filtered, and the combined filtrates were concentrated under reduced pressure on a rotary evaporator at 35 °C. The resulting crude ethanolic extracts (leaf and flower) were transferred to amber vials and stored at 4 °C until bioassay use.

**3.3 Insect cultures:** All target insects were maintained At the Biology Department's Laboratory , Umm Al-Qura University's Faculty of Applied Science in Mecca, Saudi Arabia. Under controlled environmental conditions as specified below. Unless stated otherwise, colonies were kept on standard diets/hosts and monitored daily. Species identities followed current taxonomic usage.

**3.3.1 *Acyrthosiphon pisum* (pea aphid):** A continuous colony was maintained on young *Vicia faba* L. plants at 23 ± 2 °C, 65 ± 5% relative humidity (RH), and a 16:8 h light:dark (L:D) photoperiod For bioassays, adult aphids were placed on fresh leaves in individual boxes; after 24 h, neonates were collected and used as test insects.

**3.3.2 *Drosophila melanogaster* (fruit fly) :** Flies were reared at 25 °C, 65% RH, and a 16:8 h L:D photoperiod on a standard agar–yeast–cornmeal diet (as described by Reynolds, *et al.,* 2014). Adult flies were collected from synchronized cohorts and used for bioassays.

**3.3.3 *Spodoptera exigua* (beet armyworm) :** A laboratory colony was kept at 25 °C, 65% RH, and a 16:8 h L:D photoperiod. Adults emerging in 40 × 25 × 25 cm Plexiglas cages were provided with a 10% (w/v) honey solution. White A4 paper affixed to cage walls served as an oviposition substrate; egg papers were transferred to plastic containers until hatch. Larvae were reared on an agar-based artificial diet [8]. Second-instar larvae were selected for bioassays.

**3.3.4 *Tribolium castaneum* (red flour beetle) :** Beetles were maintained in the dark at 30 °C and 60% RH on wheat flour supplemented with 5% (w/w) brewer's yeast Adults of uniform age were collected and used in bioassays.

**3.4 Insect Bioassays**

**3.4.1 Screening of ethanolic plant extracts (2% concentration):** Bioassays were conducted to evaluate the insecticidal activity of *Rosa moschata* leaf and flower ethanolic extracts against four economically significant pest species: *Acyrthosiphon pisum*, *Drosophila melanogaster*, *Spodoptera exigua*, and *Tribolium castaneum*. Extracts were tested at a 2% (w/v) concentration. Two controls were included in all assays: distilled water (untreated control) and ethanol (solvent control). Mortality was recorded at defined intervals, and each treatment was replicated independently.

- *Acyrthosiphon pisum :* To prepare a 2% extract diet, 8 mg of plant extract (leaf or flower) was dissolved in 8 µL ethanol and mixed with 392 µL liquid artificial diet. Aliquots (100 µL) were pipetted onto a parafilm membrane, which was then overlaid with a second parafilm layer to

form sachets. Ten neonate aphids (<24 h old) were placed on each sachet, confined by a ventilated plastic ring, and arranged upside down in six-well plates. Each treatment was replicated three times. Aphid mortality was assessed after 24 h by gentle probing and observation of post-mortem discoloration

- *Drosophila melanogaster :* For fruit fly assays, 200 µL of extract solution (20 mg in 1 mL ethanol) was applied to the surface of diet placed in 50 mL tubes and allowed to dry under a laminar hood. Ten adult flies were introduced into each tube. Three replicates were maintained for each extract. Mortality was recorded at 24, 48, and 72 h
- *Spodoptera exigua :* Second-instar larvae were exposed individually in diet-treated wells. Each well received 50 µL of extract solution (20 mg in 1 mL ethanol), spread over the diet surface and dried before larval placement. A total of 20 larvae were tested per treatment. Mortality was assessed at 24, 48, and 72 h post-exposure
- *Tribolium castaneum :* Extract-treated flour discs were prepared by dissolving 8.4 mg of extract in 420 µL ethanol, then mixing with 120 mg corn flour [24]. Aliquots (35 µL) of the paste were dispensed into 96-well plates and dried overnight to form discs. Adult beetles (10 per treatment) were confined with five discs inside Falcon tubes. Each treatment was run with two replications (10 discs total). Mortality was assessed at 24, 48, and 72 h post-exposure

**3.5 Insect Bioassays — *Acyrthosiphon pisum***

**3.5.1 Dose–response evaluation of leaf and flower extracts (≤2%) :** Following the 2% screening across four species, the pea aphid (*Acyrthosiphon pisum*) was the most susceptible and was therefore selected for graded-dose assays Artificial diet sachets were prepared as described for screening (parafilm–diet–parafilm). A 1% (w/v) stock was prepared by dissolving 1 mg of leaf or flower crude extract in 100 µL ethanol; this stock served as the diluent source for serial diet dilutions. Five test concentrations 1000, 500, 200, 100, and 50 ppm were prepared by mixing the ethanolic stock with liquid aphid diet to a final volume of 300 µL per treatment (three replicates × 100 µL each). For each replicate, 100 µL of the treated diet was dispensed into a parafilm sachet and sealed. Ten neonate nymphs (<24 h old) were introduced per sachet and confined with ventilated rings in six-well plates. Two controls were included in every run: (i) untreated artificial diet and (ii) solvent control (diet containing the same final ethanol percentage as treated diets). Each concentration and control was tested in triplicate. Mortality (nonresponse to probing and characteristic post-mortem discoloration) was recorded at 24 h to capture acute toxicity. The two most active concentrations identified in this assay were retained for follow-up comparisons.

**3.6 Tissue-specific comparison (leaf vs flower) at operational concentrations:** To compare tissue-specific activity, leaf and flower extracts were assayed at three operational percentages (2.0%, 1.0%, 0.5%) and, in a parallel series, at five graded ppm levels (500, 200, 100, 50, 25 ppm) using the same parafilm-sachet diet system. For both series, a 1% (w/v) ethanolic stock (1 mg/100 µL) was prepared and diluted with *A. pisum* diet to the target concentrations immediately before use [26]. For each treatment level, 100 µL of treated diet was sealed between two parafilm layers and placed in bioassay cages. Ten <24 h nymphs were introduced per cage. All treatments were run with three independent replicates. Controls matched the dose–response assays: untreated diet and solvent control (diet + ethanol at the highest assay-matched final %, without extract). Mortality was assessed at 24 h.

**3.7 Data analysis:** Mortality data were analysed by probit regression (POLO-Plus v2.0, LeOra Software, Berkeley, CA) to estimate $LC_{50}$ and $LC_{90}$ values with 95% confidence intervals (95% CI). Where control mortality was non-zero, data were corrected using Abbott's formula prior to analysis. For each model, the slope (± SE), $\chi^2$ goodness-of-fit, degrees of freedom, and heterogeneity factor were examined to validate probit assumptions. Pairwise comparisons of potency among treatments were based on CI overlap: LC estimates with non-overlapping

95% CIs were considered significantly different. When appropriate (same test species and assay), relative potencies were derived from parallel-slope tests implemented in POLO-Plus.

**3.8 GC–MS analysis of *Rosa moschata* leaf extract:** Phytochemical profiling was performed on a Shimadzu GCMS-QP2010 Plus equipped with a TD20 thermal desorption unit. Electron-impact ionization (EI) was set to 70 eV. Separations used a Restek XTI-5 capillary column (60 m × 0.25 mm i.d., 0.25 μm film; 5% phenyl-95% dimethylpolysiloxane). The GC oven program was: 80 °C (hold 1.0 min), ramp 7.0 °C $min^{-1}$ to 220 °C (hold 3.0 min), then 10 °C $min^{-1}$ to 290 °C (hold 10.0 min). Injector temperature: 290 °C; GC–MS interface (transfer line): 290 °C; source temperature per instrument default. The sample was introduced via glass injector under helium carrier gas (constant flow; split settings as per method), and spectra were acquired in EI full-scan mode. Tentative compound identities were assigned by matching retention times and EI fragmentation patterns to reference spectra and libraries, followed by compositional categorization of major constituents .

## 4 RESULTS

**4.1 Bioassay-guided screening of 2% (w/v) ethanolic plant extracts for insecticidal potential:** In initial screening at 2% (w/v), both leaf and flower extracts caused complete (100%) mortality of *Acyrthosiphon pisum* within 24 h, whereas *Spodoptera exigua* and *Drosophila melanogaster* showed only moderate, time-dependent mortality. No mortality was detected in *Tribolium castaneum* at any recorded interval (Table 1). Based on this clear differential susceptibility, *A. pisum* was selected as the focal species for subsequent dose–response bioassays.

**Table 1:** Screening bioefficacy of 2% (w/v) ethanolic *Rosa moschata* leaf and flower extracts against four pest insects.

| Treatment | *A. pisum* | *D. melanogaster* | | | *S. exigua* | | | *T. castaneum* |
|---|---|---|---|---|---|---|---|---|
| | 24 h | n | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 72 h |
| Leaf extract 2% | 100 | 30 | 0 | 0 | 0 | 5 | 5 | 5 | 0 |
| Leaf extract 1% | 100 | 30 | 26 | 33 | 44 | 0 | 0 | 5 | 0 |
| Leaf extract 0.5% | 100 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flower extract 2% | 100 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flower extract 1% | 100 | 30 | 0 | 6 | 6 | 10 | 17 | 21 | 0 |
| Flower extract 0.5% | 100 | 30 | 0 | 0 | 0 | 0 | 5 | 5 | 0 |
| Solvent control (EtOH) | ≤10 | 30 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 |
| Untreated control (diet/flour) | ≤10 | 30 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 | ≤10 |

Mortality in treated groups was corrected for natural mortality using Abbott's formula; assays were considered valid when concurrent control mortality was <20% at the corresponding time point. [1]*Acyrthosiphon pisum*: All extract treated diets caused 100% mortality by 24 h; thus, observations beyond 24 h were not applicable and the assay was terminated at 24 h (control mortality 0%). [2]*Tribolium castaneum*: No treatment produced measurable mortality at 24, 48, or 72 h (all values 0%).

Mean mortality (%) for *Acyrthosiphon pisum*, *Drosophila melanogaster*, *Spodoptera exigua*, and *Tribolium castaneum* recorded at 24, 48, and 72 h post-treatment. Replication: *A. pisum* and *D. melanogaster*, 3 replicates × 10 individuals (n = 30) per treatment/timepoint; *S. exigua* and *T. castaneum*, 2 replicates × 10 individuals (n = 20). Mortality values are (corrected/uncorrected—specify) using Abbott's formula when control mortality was <20%.

**4.2 Assessment of *A. pisum* toxicity across concentrations:** All active treatments

produced strong 24 h toxicity to *Acyrthosiphon pisum*, except the 0.5% extracts, which were negligible and yielded non-estimable LC values. The 2% leaf extract was most potent ($LC_{50}$ = 35 ppm; $LC_{90}$ = 101 ppm). The 2% flower extract was the next most active ($LC_{50}$ = 125 ppm; $LC_{90}$ = 197 ppm), and its $LC_{50}$ did not differ significantly from the 1% leaf ($LC_{50}$ = 135 ppm) or 1% flower ($LC_{50}$ = 159 ppm) extracts, as indicated by overlapping 95% CIs. Relative to the best performer (2% leaf), $LC_{50}$ potency ratios were 3.57 (2% flower), 3.86 (1% leaf), and 4.54 (1% flower); corresponding $LC_{90}$ ratios were 1.95, 3.69, and 3.75, respectively. Based on these outcomes, the 2% leaf and 2% flower extracts were advanced for bioefficacy evaluation against *A. pisum*.

**Table 2:**Toxicity of ethanolic *Rosa moschata* leaf and flower extracts to 0–24 h nymphs of *Acyrthosiphon pisum* after 24 h diet exposure: probit estimates ($LC_{50}$/$LC_{90}$) and model diagnostics.

| Plant extracts | $LC_{50}$ (95% CI) ppm | Ratio | $LC_{90}$ (95% CI) ppm | Ratio | Slope ± SE | Chi-Square | HF |
|---|---|---|---|---|---|---|---|
| Leaf extract 2% | 35 (17–48) [a] | 0.5 | 101 (79–155) [a] | 1.0 | 1.8±0.7 | 7.1 | 0.5 |
| Leaf extract 1% | 135 (112–162) [b] | 2.8 | 373 (293–527) [d] | 2.7 | 1.9±0.3 | 3.5 | 0.2 |
| Leaf extract 0.5% | - | - | - | - | 0.6±0.5 | 9.1 | 0.7 |
| Flower extract 2% | 125 (112–141) [b] | 2.5 | 197 (170–247) [b] | 1.8 | 5.6±1.1 | 2.9 | 0.2 |
| Flower extract 1% | 159 (135–189) [b] | 3.4 | 379 (301–530) [d] | 2.7 | 2.4±0.5 | 7.2 | 0.5 |
| Flower extract 0.5% | - | - | - | - | 1.4±1.0 | 1.2 | 0.1 |

Values are LC estimates from probit analysis (POLO-Plus v2.0) based on 24 h mortality; CIs are 95%. Chi-square ($\chi^2$) and heterogeneity factor (HF) describe model fit. Mortality was corrected using Abbott's formula when control mortality was <20%.

RP($LC_{50}$) and RP($LC_{90}$) are relative potency ratios vs the most potent treatment (Leaf, 2%): RP = LC_test / LC_Leaf2%; lower values indicate higher potency (Leaf, 2% = 1.00).

± Group letters within each LC column denote statistical groupings based on non-overlapping 95% CIs (different letters ≠ significant difference). NE, not estimable (mortality too low to fit a probit model at 24 h).

**4.3 Bioassay with leaf extract:** Dose–response assays confirmed strong aphicidal activity against *Acyrthosiphon pisum* at 24 h. The 2% leaf extract was most potent ($LC_{50}$ = 17 ppm; $LC_{90}$ = 47 ppm), the 1% leaf extract showed intermediate potency ($LC_{50}$ = 54 ppm; $LC_{90}$ = 145 ppm), and the 0.5% leaf extract was least active ($LC_{50}$ = 164 ppm; $LC_{90}$ = 531 ppm). Consistent with these estimates, RP($LC_{50}$) vs 2% leaf was 3.18 (1% leaf) and 9.65 (0.5% leaf); RP($LC_{90}$) was 3.09 and 11.30, respectively (Table 3). $LC_{90}$ values for 2% and 1% leaf were comparable (overlapping 95% CIs), whereas $LC_{50}$ values were clearly separated. Model diagnostics (slope, $\chi^2$, HF) supported acceptable probit fit.

**Table 3:** Toxicity of ethanolic *Rosa moschata* leaf extracts to 0–24 h *Acyrthosiphon pisum* nymphs after 24 h diet exposure (probit estimates and diagnostics).

| Extracts (Concentrations) | LC50 (95% CI) ppm | Ratio | LC90 (95% CI) ppm | Ratio | Slope ± SE | Chi-Square | HF |
|---|---|---|---|---|---|---|---|
| Leaf extract 2% | 17 (6–25) [b] | 1.0 | 47 (68–163) b | 1.0 | 2.8±0.8 | 4.6 | 0.3 |
| Leaf extract 1% | 54 (44–63) [c] | 3.1 | 145 (114–208) [c] | 2.0 | 2.0±0.4 | 5.3 | 0.3 |
| Leaf extract 0.5% | 164 (125–230) [a] | 9 | 531 (347–1150) [a] | 10 | 2.4±0.3 | 21.5 | 1.6 |

Values are LC estimates from probit analysis (POLO-Plus v2.0) based on 24 h mortality; CIs are 95%. Chi-square ($\chi^2$) and heterogeneity factor (HF) describe model fit. Mortality was corrected using Abbott's formula when control mortality was <20%.

RP($LC_{50}$) and RP($LC_{90}$) are relative potency ratios vs the most potent treatment (Leaf, 2%): RP = LC_test / LC_Leaf2%; lower values indicate higher potency (Leaf, 2% = 1.00).

± Group letters within each LC column denote statistical groupings based on non-overlapping 95% CIs (different letters ≠ significant difference). NE, not estimable (mortality too low to fit a probit model at 24 h).

**4.4 Bioassay with flower extract:** Dose–response assays with the flower extract confirmed clear concentration-dependent aphicidal activity at 24 h. The 2% (w/v) flower extract was the most potent ($LC_{50}$ = 67 ppm; $LC_{90}$ = 156 ppm). In contrast, the 1% ($LC_{50}$ = 684 ppm; $LC_{90}$ = 3556 ppm) and 0.5% ($LC_{50}$ = 695 ppm; $LC_{90}$ = 3567 ppm) treatments were an order of magnitude less active. Relative potency vs 2% was RP($LC_{50}$) ≈ 10.21 (1%) and 10.37 (0.5%), and RP($LC_{90}$) ≈ 22.79 (1%) and 22.87 (0.5%). These outcomes indicate that only the 2% flower extract achieved operationally meaningful toxicity within 24 h, supporting its prioritization for subsequent evaluations.

**Table 4:** Toxicological evaluation of ethanolic *Rosa moschata* flower extracts against 0–24 h nymphs of *Acyrthosiphon pisum* (pea aphid) after 24 h exposure via artificial diet supplementation.

| Extracts (Concentrations) | $LC_{50}$ (95% CI) ppm | Rati o | $LC_{90}$ (95% CI) ppm | Rati o | Slope ± SE | Chi-Square | HF |
|---|---|---|---|---|---|---|---|
| Flower extract 2% | 67 (57–78) [b] | 1.0 | 156 (126–214) [b] | 1.0 | 3.4±0.4 | 7.1 | 0.5 |
| Flower extract 1% | 684 (450– 1540) [a] | 10 | 3556 (1570–21020) [a] | 22 | 1.7±0.3 | 6.5 | 0.4 |
| Flower extract 0.5% | 695 (570– 1260) [b] | 15 | 3567 (1580–21030) [a] | 27 | 1.8±0.4 | 7.5 | 0.6 |

Values are LC estimates from probit analysis (POLO-Plus v2.0) based on 24 h mortality; CIs are 95%. Chi-square ($\chi^2$) and heterogeneity factor (HF) describe model fit. Mortality was corrected using Abbott's formula when control mortality was <20%.

RP($LC_{50}$) and RP($LC_{90}$) are relative potency ratios vs the most potent treatment (Leaf, 2%): RP = LC_test / LC_Leaf2%; lower values indicate higher potency (Leaf, 2% = 1.00).

± Group letters within each LC column denote statistical groupings based on non-overlapping 95% CIs (different letters ≠ significant difference). NE, not estimable (mortality too low to fit a probit model at 24 h).

**4.5 GC-MS profiling of R. moschata ethanolic extracts:** Gas chromatography–mass spectrometry (GC–MS) was used to characterize the bioactive constituents of ethanolic leaf and flower extracts of *Rosa moschata*. Compound lists are provided in Tables 5 and 6 (leaf and flower,

respectively), with chromatograms in Figures 1 and 2. For each detected constituent, we report the CAS Registry Number, library match score (%), molecular formula, chemical class, and literature-reported biological activities. In the leaf extract, 20 constituents were identified: six with match score 99%, two at 97%, two at 96%, one at 95%, three at 91%, four at 90%, one at 89%, and one at 86%. Several of these compounds are known from prior studies to exhibit insecticidal, repellent, or behaviour-modifying effects, consistent with the strong aphicidal activity observed in our bioassays. In the flower extract, 10 constituents were identified: one with match score 99%, one at 94%, six at 91%, one at 90%, and one at 86%. While fewer in number, these metabolites include bioactive classes commonly implicated in pest management. Overall, the GC–MS profiles support the bioefficacy results and motivate targeted follow-up on the highest-confidence constituents (high match score and/or abundance) for structure–activity analysis and formulation development.

**Table 5:** GCMS profiling of bioactive phytoconstituents in 2 % ethanolic leaf extract of *Rosa moschata*

| Sr. no. | CAS # | Compound name | Molecular formula | Quality % | Chemical class | Biological activity | Reference |
|---|---|---|---|---|---|---|---|
| 1. | 91363 000128-37-0 | Butylated Hydroxytoluene | $C_{15}H_{24}O$ | 99 | Phenolic antioxidant | Antioxidant | Ghazawy *et al.,* 2025 |
| 2. | 144202 000112-39-0 | Methyl palmitate hexadecanoic acid, methyl ester | *C17H34O2* | 96 | Fatty acid ester | Larvacidal | Abdel-Motleb *et al.,* 2022 |
| 3. | 152342 000084-74-2 | Dibutyl phthalate | C16H22O4 | 96 | Phthalic acid esters | Insecticidal | Huang *et al.,* 2021 |
| 4. | 170190 1000336 -44-2 | Methyl 10-trans,12cisoctadecadienoate | C19H34O2 | 99 | Methyl linoleate | Insecticidal | Baz *et al.,* 2023 |
| 5. | 170211 002566-97-4 | 9,12-Octadecadienoic acid, methyl ester, (E,E) | C19H34O2 | 99 | Fatty acid methyl ester | Insecticidal | Khanday and Sharma, 2021 |
| 6. | 168087 000301-00-8 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) | C19H32O | 99 | Fatty acid methyl ester | Insecticidal | Balogun *et al.,* 2021 |
| 7. | 185335 1000336 -39-1 | Methyl 2-hydroxyoctadeca-9,12,15trienoate | C19H32O3 | 90 | Methyl hydroxylinolenate | Insecticidal | Shilaluke,. and . Moteetee, 2020 |
| 8. | 152478 017851-53-5 | 1,2-Benzenedicarboxyli c acid, butyl 2methylpropyl ester | C16H22O4 | 95 | Phthalate esters | Insecticidal and phytotoxic | Lanchana *et al.,* 2024 |
| 9. | 170209 000112-63-0 | Methyl 10-trans,12cisoctadecadienoate | C19 H34 O2 | 99 | Linoleic acid methyl ester | Insecticidal | Kifle *et al.,* 2025 |
| 10. | 172448 000150-86-7 | Phytol | C20H40O | 91 | Diterpenoi d | Anti-cancer, antimicrobial and anti – oxidant properties | Vandana, *et al.,* 2021 |

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|---|---|---|---|---|---|---|---|
| 11. | 185836 015307-78-5 | Diclofenac, methyl ester | C15H13Cl2NO 2 | 97 | Phenylaceti c acid | Antibacterial, antidiarrhoea l and analgesic | Hossain *et al.,* 2017 |
| 12. | 259469 000117-81-7 | Bis(2-ethylhexyl) phthalate | C24H38O4 | 91 | Diester of phthalic acid | Antibacterial and Larvacidal | Javed *et al.,* 2022 |
| 13. | 259636 074746-55-7 | 1,2-Benzenedicarboxyli c acid, bis(2ethylhexyl) ester | C24H38O | 90 | Benzoic acid | Antimicrobia l | Balogun *et al.,* 2022 |
| 14. | 285776 959085-66-6 | Heptadecyl heptafluorobutyrate | C21H35F7O2 | 90 | Fluorinated ester | Antimicrobia l | Kumari *et al.,* 2022 |
| 15. | 283580 1000314 -56-3 | Carbonic acid, octadecyl 2,2,2trichloroethyl ester | C21H39Cl3O3 | 91 | Carbonic acid esters | Insecticides | Khaled *et al.,* 2021 |
| 16. | 139725 018435-45-5 | 1-Nonadecene | C19 H38 | 89 | *Nitrogencontaining organic compound* | Antimicrobia l and Antioxidant | Adeyemo, 2024 |
| 17. | 270408 000111-02-4 | Squalene | C30H50 | 99 | Triterpenoid | Anti-oxidant | Vandana, 2021 |
| 18. | 270407 007683-64-9 | Supraene | *C30H50* | 97 | Triterpenoid | Insecticidal | Lin *et al.,*2021 |
| 19. | 279062 010191-41-0 | dl-.alpha.-Tocopherol | C29H50O2 | 90 | Triterpenoid | Anti-oxidant | Roopa, M., *et al.,* 2020 |
| 20. | 279055 000059-02-9 | Vitamin E | C29H50O2 | 86 | Resorcinol | Anti-oxidant | Shimizu et al.,2018 |

*Journal of Animal & Plant Sciences (J.Anim.Plant Sci. ISSN 2071-7024)*
*Vol.67(2) : 13289 -13309* *https://doi.org/10.35759/JAnmPlSci.v67.3.1*

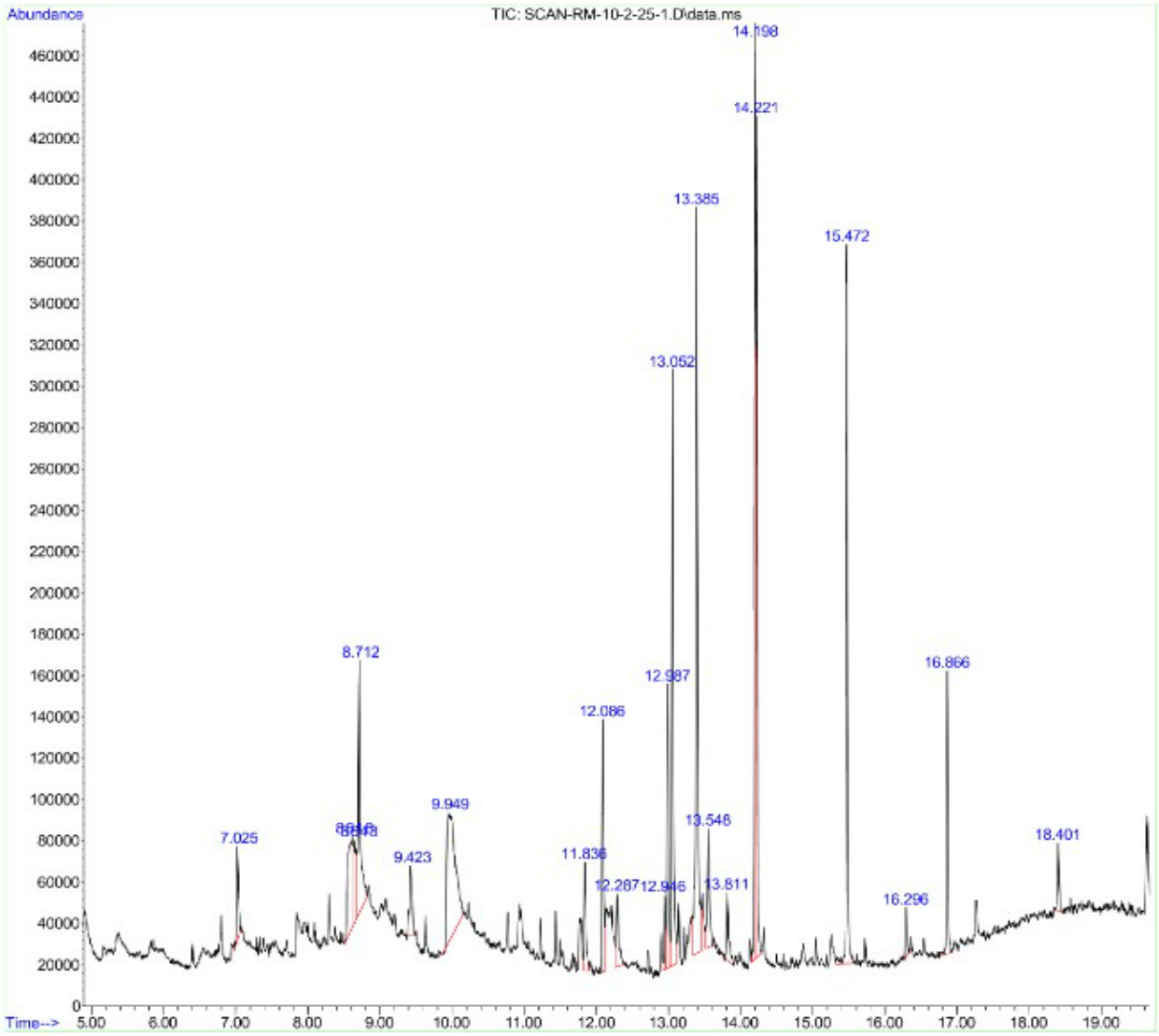


**Figure 1:** Chromatogram of ethanolic leaf extract of *Rosa moschata*

**Table 6:** GCMS profiling of bioactive phytoconstituents in 2 % ethanolic flower extract of *Rosa moschata*

| Sr. no. | CAS # | Compound name | Molecular formula | Quality % | Chemical class | Biological activity | Reference |
|---|---|---|---|---|---|---|---|
| 1. | 91363 000128-37-0 | Butylated Hydroxytoluene | $C_{15}H_{24}O$ | 99 | Phenolic antioxidant | Antioxidant | Ghazawy,2025 |
| 2. | 213382 000085-69-8 | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | C20H30O4 | 91 | phthalate ester | Antifungal | Qureshi *et al.,* 2018 |
| 3. | 152342 000084-74-2 | Dibutyl phthalate | C16H22O4 | 86 | Phthalic acid esters | Insecticidal | Huang *et al.,* 2021 |
| 4. | 252899 000593-49-7 | Heptacosane | C27H56 | 91 | Alkane | antibacterial, antioxidant, anticancer | Rangayasami,, 2020 |
| 5. | 296990 000630-06-8 | Hexatriacontane | *C36H74* | 91 | Alkane | antibacterial, antioxidant, anticancer | Rangayasami,, 2020 |
| 6. | 303840 007098-22-8 | Tetratetracontane | *C44H90* | 91 | Alkane | antibacterial, antioxidant, anticancer | Rangayasami,, 2020 |
| 7. | 262955 000084-71-9 | 1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester | C24H44O4 | 90 | phthalate ester | Larvicidal | Vasumathi, *et al.,* 2023 |
| 8. | 259469 000117-81-7 | Bis(2-ethylhexyl) phthalate | C24H38O4 | 91 | Diester of phthalic acid | Antibacterial and Larvacidal | Javed, 2022 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9. | 259636 074746-55-7 | 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester | C24H38O | 91 | Benzoic acid | Antimicrobial and indsecticidal activity | Balogun, 2022 |
| 10. | 259638 000137-89-3 | 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester | C24H38O4 | 94 | isophthalate | Larvicidal | Vasumathi, 2023 |

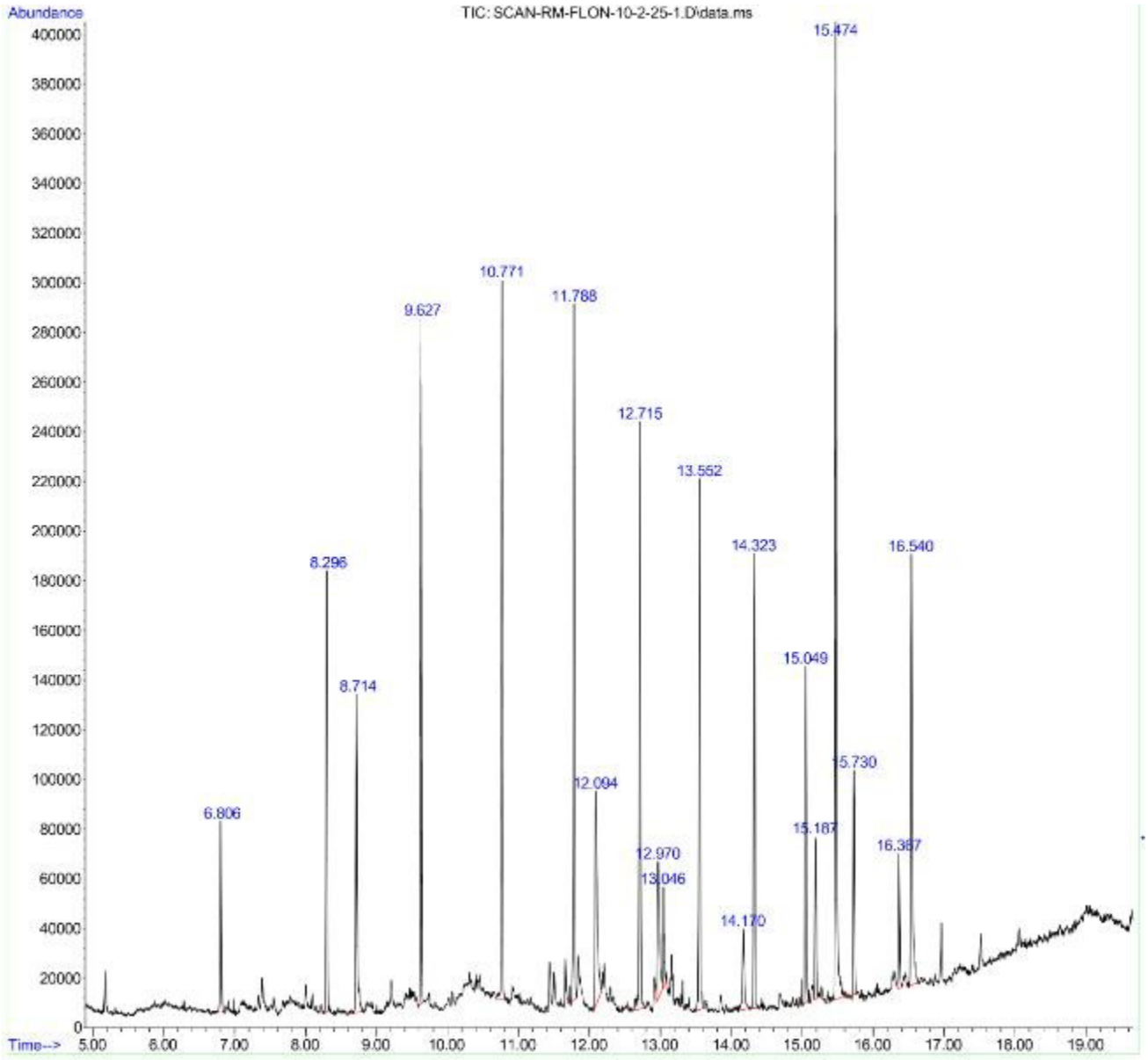


**Figure 2:** Chromatogram of ethanolic flower extract of *Rosa moschata*

## 5 DISCUSSION

Plants have evolved a rich arsenal of secondary metabolites terpenoids, phenolics, alkaloids, and fatty-acid derivatives that function as chemical defenses against herbivores and pathogens and are increasingly revisited as eco-compatible pest controls Building on this rationale, we screened ethanolic leaf and flower extracts of *Rosa moschata* against four representative pests (*Acyrthosiphon pisum*, *Drosophila melanogaster*, *Spodoptera exigua*, *Tribolium castaneum*) to gauge spectrum and selectivity of action as shown in Figure 3. Comparative bioassays revealed strong concentration-dependent aphicidal activity of *Rosa moschata* extracts against *Acyrthosiphon pisum* at 24 h. The 2% leaf extract was the most potent overall ($LC_{50}$ = 17 ppm; $LC_{90}$ = 47 ppm), followed by the 2% flower extract ($LC_{50}$ = 67 ppm; $LC_{90}$ = 156 ppm). Lower concentrations (1% and 0.5%) of both extracts showed markedly reduced efficacy, with $LC_{50}$ values exceeding 50–150 ppm for leaf and over 600 ppm for flower treatments. Thus, while both

extracts demonstrated dose-dependent toxicity, the leaf extract was approximately four times more potent than the flower extract, confirming it as the superior formulation for aphid control.

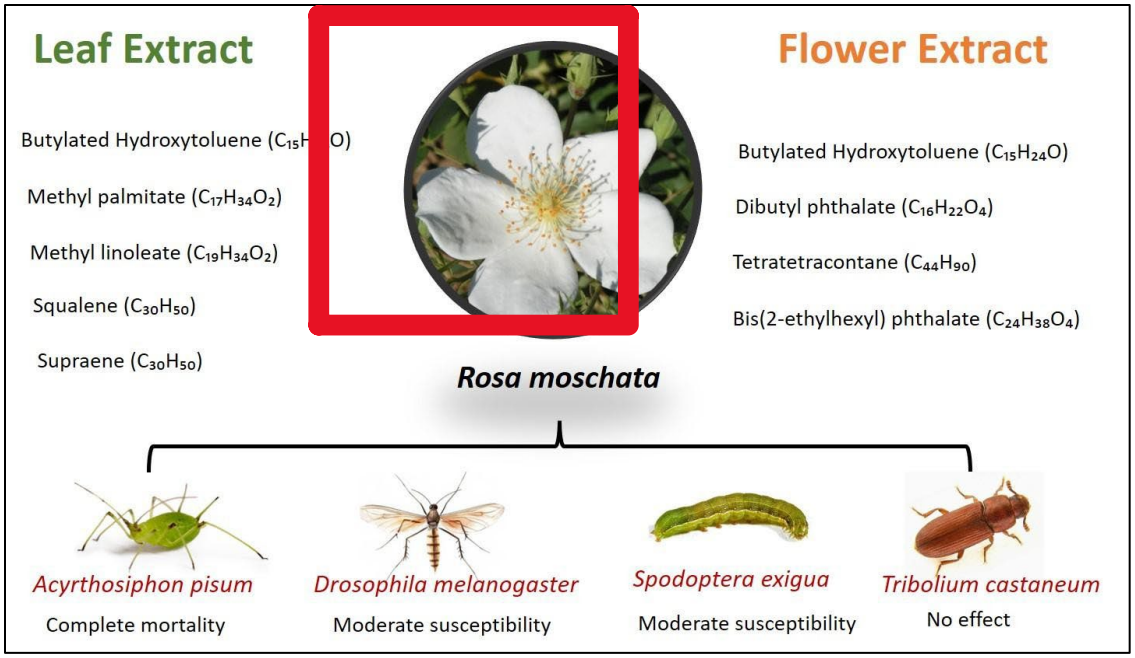


**Figure 3** shows the key compounds detected by GC–MS in leaf and flower ethanolic extracts and their relative insecticidal effects. Leaf extract (green) contained fatty-acid esters and triterpenoids such as methyl linoleate, squalene, and supraene, showing strong toxicity against *Acyrthosiphon pisum* (100% mortality) and moderate effects on *Drosophila melanogaster* and *Spodoptera exigua*. Flower extract (orange) was dominated by phthalate esters and long-chain alkanes, displaying weaker bioactivity and no effect on *Tribolium castaneum*. Source: Adapted from online sources, Trevor White Roses (Rosa moschata), available at: https://www.trevorwhiteroses.co.uk).

Screening at 2% (w/v) revealed a clear differential response: *A. pisum* was uniquely sensitive, showing 100% mortality within 24 h; *D. melanogaster* and *S. exigua* displayed only moderate, time-dependent effects; *T. castaneum* was refractory across all intervals. These order-specific outcomes are biologically plausible. First, the ingestion route used for aphids (liquid diet) likely enhanced internal exposure to polar and amphiphilic constituents relative to surface-coated solid diets used for *Drosophila* and *Spodoptera*, where uptake is constrained by feeding mode and gut processing. Second, taxon-level differences cuticle thickness/chemistry, midgut pH, and detoxification capacity (e.g., esterases, GSTs, P450s) routinely drive disparate susceptibility; coleopterans, including *T. castaneum*, frequently show higher tolerance to botanicals and essential oils than hemipterans and dipterans, consistent with the null response we observed. Finally, our dose–response work corroborates this selectivity: the leaf extract was markedly more aphicidal than the flower extract (e.g., leaf 2% ~$LC_{50}$ 17 ppm; flower 2% ~$LC_{50}$ 67 ppm at 24 h), and lower concentrations (1%, 0.5%) of either matrix were far less potent—patterns typical of concentration dependent botanical activity. This finding aligns with the results reported by Zewdu , 2020 who demonstrated the toxic effects of various solvent extracts of *Millettia ferruginea*, Birbira against the same aphid species. It was delineated that among all the solvent extracts tested, the deionised water extract demonstrated the highest level of toxicity, resulting in 98% mortality of the test subjects. This was followed by the acetic acid

extract, which caused 89% mortality. In contrast, the chloroform, toluene, and hexane extracts exhibited markedly lower toxicity, indicating a comparatively weaker bioactive potential in non-polar solvent systems. The ethanolic leaf extract at 2% concentration exhibited the highest toxicity against aphids, demonstrating greater bioactivity than flower-derived extracts. This was evidenced by the lowest $LC_{90}$ values recorded for the 2% ethanolic leaf extract. These findings suggest that the bioactivity of the ethanolic leaf extract is concentration-dependent, with optimal efficacy demonstrated at the 2% level. investigated ethanolic leaf extracts of *Ocimum gratissimum*, *Sida acuta*, *Telfaria occidentalis*, and *Vernonia amygdalina* for insecticidal activity against *Acanthscelides obtectus*. Mechanistically, the GC–MS profiles support the biological signal: fatty-acid esters (e.g., hexadecanoate/linoleate derivatives), diterpenoids (e.g., phytol), and triterpenoids (e.g., squalene/supraene) were among the detected constituents. Such chemotypes have been repeatedly implicated in aphid toxicity, antifeedancy, membrane perturbation, and enzyme inhibition (e.g., AChE and detox enzymes), which likely act additively or synergistically. The stronger activity of leaf versus flower extracts is therefore consistent with a higher load or more favorable ratios of these bioactives in leaf tissues. Overall, our results align with prior reports that (i) aphids can be especially susceptible to mixtures rich in fatty-acid derivatives and aromatics; (ii) dipterans and lepidopterans show moderate, sometimes delayed responses to botanicals under dietary exposure; and (iii) coleopterans often exhibit resilience without optimized contact formulations. Among the *Rosa moschata* matrices, the leaf extract at 2% (w/v) showed the greatest aphicidal potency (e.g., $LC_{50} \approx 17$ ppm at 24 h in our leaf assay), whereas flower extracts required far higher doses (e.g., 2% flower $LC_{50} \approx 67$ ppm) and fell off steeply at 1% and 0.5%, indicating that leaf tissue is the richer source of active metabolites. This concentration-dependent pattern mirrors prior phytopesticide work—for example, ethanolic leaf extracts of *Ocimum gratissimum* and *Sida acuta* displayed dose-dependent lethality against storage pests (and by extension support the principle that higher botanical doses often cross efficacy thresholds more reliably) [53]. Similar potency scaling is seen in aphid systems using different botanicals and delivery formats: supercritical $CO_2$ extracts of *Alcea nudiflora* produced very low LC values against *Macrosiphum euphorbiae* and nanoliposome formulations of plant extracts achieved $LC_{50} \approx 84$ mg $L^{-1}$ against *Acyrthosiphon pisum*, underscoring how both extract chemistry and formulation govern practical toxicity. Notably, high-concentration treatments can converge at similar $LC_{90}$ (overlapping 95% CIs) while remaining distinct at $LC_{50}$. This pattern suggests a mortality plateau beyond a threshold once enough targets are hit (or cuticular/gut barriers are overcome), incremental potency differences compress at the upper tail of the dose–response. Analogous saturation behavior is reported for essential-oil major constituents on cereal aphids and for botanical mixtures used on *A. pisum* under greenhouse conditions . GC–MS showed 20 compounds in leaf and 10 in flower extract with high match scores; prominent classes included fatty-acid esters (e.g., hexadecanoate/palmitate and octadecadienoate/linoleate derivatives), terpenoids (e.g., phytol), and aromatic acids. These chemotypes have repeatedly been implicated in aphid control: reviews and experimental studies identify linoleic (18:2), oleic (18:1), and palmitic (16:0) acids and their esters as antifeedant/toxic to aphids, while terpenoids (e.g., βcitronellol, carvacrol, linalool) reduce survival and fertility. Importantly, (9Z,12Z)octadecadienoic acid (linoleic acid) has shown stronger 24 h aphicidal activity than thiamethoxam in *Aphis craccivora*, supporting a causal link between leaf profile (rich in C16/C18 derivatives) and the very low LC values observed for *A. pisum*. Comparable fatty-acid-rich mixtures from other sources (e.g., pyrolysis bio-oils, *Eucalyptus* oils) also report substantial insecticidal/repellent effects, frequently listing

octadecadienoic and hexadecanoic acids among the dominant bioactives . Mechanistically, multi-component synergy is likely: fatty-acid esters and phenolics can disrupt membranes and respiration; terpenoids can inhibit neuroenzymes (e.g., AChE) and modulate octopaminergic signaling; and aromatics can impose oxidative stress together yielding greater toxicity than single constituents alone. Recent work on phytol-derived lactones showing deterrence toward *Myzus persicae* further supports a terpenoid contribution consistent with the results of our chromatography . Overall, the leaf>flower potency in our study aligns with its denser, more favorable phytochemical profile, while cross-study comparisons reinforce that (i) aphids are particularly susceptible to mixtures enriched in C16/C18 fatty acids/esters and select terpenoids; (ii) delivery/formulation strongly modulates efficacy; and (iii) dose–response plateaus can produce similar $LC_{90}$ despite divergent $LC_{50}$, precisely for high-dose treatments. There are a few limits to keep in mind. First, how we gave the extracts to the insects can affect the results. Aphids drank the extract in a liquid diet, which can make the extract seem stronger than when it is applied on surfaces or as a residue especially for insects that don't feed the same way. Second, the GC–MS results tell us likely compounds, but they are not final. We would need tests like LC–MS or NMR to confirm the exact structures, especially for look-alike (isomeric) compounds. Third, we only measured deaths at 24 hours, so slower effects like reduced growth, fewer offspring, or delayed deaths may not be captured. Finally, we have not tested real-world use yet. Factors like spray coverage on leaves, how long the extract lasts, sunlight (UV) breakdown, rain, and effects on non-target organisms still need to be checked in semi-field or field trials.

## 6 CONCLUSIONS AND OUTLOOK

In conclusion, ethanolic extracts of *Rosa moschata* especially the leaf extract showed strong aphicidal activity at 2% (w/v), yielding low 24-h LC values (e.g., $LC_{50}$ in the tens of ppm) in dietbased assays. These results position *R. moschata* as a credible plant-derived option for integrated pest management (IPM) against aphids, with the added advantage of a favorable perception due to its established medicinal use. To translate this potential into practice, next steps should prioritize fractionation (e.g., silica gel/column chromatography) to isolate the most active constituents and test them alone and in mixtures; mechanism studies using enzyme assays (AChE, GST, esterases); and evaluation of sublethal/behavioral endpoints (feeding deterrence, fecundity). Parallel formulation work (emulsifiable concentrates, nanoemulsions) is needed to improve stability, persistence, and delivery, followed by semi-field and field trials to assess performance under sunlight, rainfall, plant surface deposition, and potential effects on non-target organisms. Collectively, these efforts will establish the toxicological profile and operational fit of *R. moschata* extracts as aphid management tools.

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