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Synergistic control of *Aphis fabae* Scop. (Hemiptera: Aphididae) on faba bean using botanical extracts and predatory ladybirds

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Introduction: *Aphis fabae* Scop. (Hemiptera: Aphididae) poses a major threat to *Vicia faba* L. crops, demanding sustainable control strategies.
Methods: We assessed the single and combined efficacy of two predatory ladybirds—*Coccinella septempunctata* (CS) and *Hippodamia convergens* (HC)—with two 10% botanical extracts—*Nicotiana glauca* (NG) and *Ricinus communis* (RC) (Solanaceae)—against *A. fabae* under screenhouse conditions (25 ± 1°C; 60 ± 10% RH). Ten treatments, including imidacloprid (ICP, 20 mL hL^{-1}) as a positive control, were tested in a randomized complete-block design (3 replicates × 7 plants). Ladybirds were released one-week post-treatment.
Results and discussion: Combined treatments were most effective in reducing *A. fabae* populations. By week 5, egg densities dropped from ~95 to 2.0 (RC + CS treatment) and 3.9 (RC + HC treatment), compared to 15.0 with imidacloprid (ICP). Motile stages declined to 10 (RC + CS treatment) and 15 (RC + HC treatment), versus 45 with ICP treatment. NG-based combinations showed moderate efficacy, while single treatments were less consistent. Ladybird establishment was not affected by extracts. *C. septempunctata* reached 10.2 motile stages per three leaves per plant in RC + CS treatment by week 5, compared to 8.7 in CS treatment. *H. convergens* reached 10.0 motile stages per three leaves per plant in RC + HC treatment. Plant visual scores peaked at 9.67 (RC + CS treatment) and 9.35 (RC + HC treatment), outperforming imidacloprid (visual score: 8.25). The synergy between *R. communis* or *N. glauca* extracts and ladybird predators offers an effective alternative to imidacloprid for *A. fabae* control. Field trials and timing optimization are recommended to integrate these tactics into faba-bean IPM programs.

KEYWORDS

biocompatibility, secondary metabolites, polyphagous predators, trophic interactions, functional response, sustainability assessment

1 Introduction

The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is a major pest of over 200 cultivated and ornamental plant species across Europe, Western Asia, South America, and Africa, including faba bean (*Vicia faba* L.) (Meradsi and Laamari, 2018). It causes direct damage by phloem sap extraction and indirect damage via honeydew, which promotes sooty mold and reduces photosynthesis (Rashedi et al., 2019; Nordey et al., 2021). It is also a key virus vector, with aphids transmitting 275 of approximately 600 known plant viruses (Blackman and Eastop, 2007; Dedryver et al., 2010). In faba bean, yield losses can exceed 50% under severe infestations (Béji et al., 2015). This species prefers young plant tips, causing stunted growth and leaf distortion (Bennour et al., 2021), with damage severity depending on infestation timing and density (Stoddard et al., 2010; Almogdad and Semaškienė, 2021). Rapid population growth results from its short life cycle and telescoping generations (Singh and Singh, 2020). It follows a holocyclic life cycle, overwintering as eggs, with spring migration of winged adults to host plants (Raymond et al., 2001). Populations fluctuate with environmental and biological factors (Hansen et al., 2008). The economic threshold is around 5% infestation (Way et al., 1977), though modern cultivars may be more tolerant (Parker and Biddle, 1998). A*phis fabae* undergoes five stages: egg, four nymphal instars, and adult (Saruhan et al., 2015). It alternates between sexual and asexual reproduction. In autumn, winged females lay overwintering eggs on primary hosts after mating; in spring, these hatch into wingless parthenogenetic females (Fericean et al., 2012). Typically, four generations occur per growing season (Cichocka et al., 2002). Population growth is influenced by initial infestation, temperature, light intensity, natural enemies, and intraspecific competition (Way, 1967; Way and Banks, 1967). Climate plays a key role: in cold regions, the species alternates between sexual and asexual phases; in mild regions, it reproduces only by parthenogenesis (Blackman and Eastop, 2007; Béji et al., 2015). The optimal temperature range for population growth on faba bean is 16–24°C (Rochelyn and Satar, 2025). At 15°C, the aphid has the longest development time (11.45 days), greatest longevity (45.68 days), and highest fecundity (89.35 offspring). Development is fastest at 25°C, while at 30°C, lifespan and reproduction drop sharply (Tora et al., 2024). Broad-spectrum insecticides are widely used to control *A. fabae*. Pyrethroids, such as fenvalerate, are the main insecticides applied as foliar sprays to manage aphid populations (Johnson et al., 2009). Both pyrethroids and neonicotinoids, have proven highly effective against *A. fabae* under laboratory conditions (Purhematy et al., 2013). According to Ayala et al. (1996), chlorpyrifos methyl combined with cypermethrin, oxydemeton methyl, imidacloprid, and thiometon are among the most efficient insecticides. However, resistance has developed in *A. fabae* to some chemicals, including partial resistance to pirimicarb and strong resistance to methamidophos (Ioannidis, 2000). Beyond resistance concerns, chemical insecticides pose risks to human health, non-target organisms, and the environment. Aligned with global Integrated Pest Management (IPM) strategies, newer, more selective insecticides—such as mineral oil and spinosad—and biological control agents have been introduced (Stoddard et al., 2010). These selective insecticides reduce harm to beneficial insects and help prevent secondary pest outbreaks (Wilson et al., 2007). Biological control methods are cost-effective and safe for both humans and the environment (Mwanauta et al., 2015). They include natural enemies (predators and parasitoids), botanical insecticides, resistant plant varieties, and cultural practices. Aphid predators comprise 54 species across six insect orders: Coleoptera, Diptera, Hemiptera, Neuroptera, Thysanoptera, and Dermaptera (Bennour et al., 2021). The main insect predators include coccinellids like *Hippodamia convergens* Guérin-Méneville, *Coccinella novemnotata* Herbst, and *Coccinella septempunctata* L., along with chrysopids such as *Chrysopa oculata* Say and *Chrysopa nigricornis* (Burmeister) (Bennour et al., 2021). In addition to insect predators, spiders such as *Pardosa amentata* (Clerck) (Araneae: Lycosidae) and *Erigone* species (Araneae: Linyphiidae) (Traugott and Symondson, 2008), as well as birds like *Apus apus* (Linnaeus) (Apodiformes: Apodidae) (Bennour et al., 2021), also prey on *A. fabae*.

Plant secondary metabolites are important sources of biopesticides and offer an environmentally friendly and safer alternative to chemical insecticides (Isman, 2006). Various plants from different families, including *Citrus aurantium* L. (Rutaceae), *Eucalyptus* spp. (Myrtaceae), *Euphorbia* spp. (Euphorbiaceae), *Melia azedarach* (Meliaceae), and *Sonchus oleraceus* (Asteraceae), have been used to control *A. fabae* (Bennour et al., 2021). Different plant parts—especially leaves, stems, and peels—are utilized, with aqueous and alcoholic extracts being the most common forms (Bennour et al., 2021). Additionally, *Ricinus communis* (Castor bean) (Euphorbiaceae) and *Nicotiana glauca* (Tree Tobacco) (Solanaceae) have demonstrated high efficacy against various insect pests worldwide and are readily available in Morocco (El Aalaoui and Sbaghi, 2025). The primary bioactive compounds responsible for their insecticidal effects include ricin (a toxic protein) and ricinine (an alkaloid) in *R. communis* (Elijah and Somadina, 2020), capsaicin and dihydrocapsaicin in *Capsicum annuum*, and pyridine alkaloids such as nicotine and anabasine in *N. glauca* (Alghamdi, 2021a; Alghamdi, 2021b). Crucially, for successful Integrated Pest Management (IPM) programs, the effects of these bio-insecticides on natural enemies must be considered. Recent research highlights that many plant-derived insecticides are selective and less harmful to beneficial predators and parasitoids compared to synthetic pesticides, thus supporting their conservation and enhancing pest control (Regnault-Roger et al., 2020; Desneux et al., 2021). However, compatibility depends on the bioactive compounds, dose, and application methods, requiring localized assessment to optimize IPM outcomes (Sanchez-Bayo and Tennekes, 2020). Combining predators with plant-derived insecticides can improve control of *A. fabae* by increasing efficiency, reducing the frequency of applications, and minimizing environmental impacts (Abdel-Rahman et al., 2019).

Although research on IPM strategies combining biological and botanical control agents is still limited, existing studies suggest that such combinations can improve insecticidal efficacy and support more sustainable pest control (Abdel-Rahman et al., 2019; El Sayed and Ibrahim, 2020). Despite these findings, IPM in faba bean (*Vicia faba*) cultivation continues to rely largely on broad-spectrum insecticides. Most previous studies have evaluated control strategies independently, with few exploring their combined effects to fully realize their synergistic potential (Stoddard et al., 2010). In this context, the present study aims to investigate the efficacy of two predatory ladybird species, *H. convergens* and *C. septempunctata*, against the black bean aphid *Aphis fabae*. Additionally, it evaluates the insecticidal activity of plant extracts from *R. communis* and *N. glauca*, both individually and in combination with the predators. The goal is to assess their potential as biological control agents and to contribute to the development of more

environmentally sound IPM strategies for managing *A. fabae* in faba bean systems.

2 Materials and methods

2.1 *Aphis fabae* colony

A colony of *A. fabae* was established from infested broad bean (*Vicia faba* L., cv. 'Aguadulce') plants collected in fields at Zemamra, Morocco (32°37′48″N, 8°42′0″W; elevation 165 m). The aphids were continuously reared for several generations on healthy *V. faba* plants (aged 1 month) grown in plastic pots (33 cm diameter × 12 cm height) filled with a substrate mixture of two-thirds fine sand and one-third peat. The colony was maintained in entomological cages (80 cm × 80 cm × 80 cm) constructed with wooden frames and covered with mesh fabric to ensure adequate ventilation. Rearing was conducted under controlled laboratory conditions (26 ± 2°C, 60 ± 10% relative humidity, and a 16:8 h light:dark photoperiod). To sustain the colony, uninfected *V. faba* plants were regularly introduced when the existing plants showed signs of damage or deterioration. This procedure ensured a continuous and healthy supply of *A. fabae* individuals for use in experimental trials.

2.2 Predatory ladybirds mass rearing

Individuals of *C. septempunctata* and *H. convergens* used in this study were obtained from a colony maintained at the National Institute of Agricultural Research (INRA) insectarium in Zemamra. These predators were kept in entomological cages (80 × 80 × 80 cm) constructed with aluminum frames and covered with mesh fabric to provide proper ventilation. The cages were maintained at 26 ± 2°C, 60 ± 10% relative humidity, and an 8-h light:16-h dark photoperiod. The ladybirds were fed with *A. fabae*-infested *V. faba* plants (aged 1 month) (El Aalaoui and Sbaghi, 2023). To ensure sufficient prey for mass rearing, broad bean (*V. faba* L., cv. 'Aguadulce') plants were continuously grown in plastic pots (33 cm diameter × 12 cm height) filled with a mixture of two-thirds fine sand and one-third peat. These plants were cultivated in a glasshouse (11 m × 7 m × 3 m) maintained at 25 ± 4°C and 60 ± 10% relative humidity under natural light at the Regional Center for Agricultural Research of Settat (INRA), Settat, Morocco (32.9538° N, 7.6259° W; elevation 370 m).

2.3 Plant extracts

Leaf extracts from the local species *N. glauca* and *R. communis* were prepared following the method described by Ndereyimana et al. (2020). Leaves were collected from fields in Zemamra, washed, and dried in the shade for 2 weeks. After drying, they were ground into a fine powder using an electric grinder and stored in biodegradable plastic bags. To prepare the extract for field use, 100 grams of powder was mixed directly with 1 L of water and left to steep at room temperature for 24 h. The mixture was then filtered through muslin cloth and diluted with cold water to reach a final volume of 1 L, resulting in a 10% w/v concentration. This concentration corresponds to the recommended field dose for controlling various insect pests in multiple crops (Abdelgader and Elawad, 2022; El Aalaoui and Sbaghi, 2025).

2.4 Insecticide

In this study, Imipower (imidacloprid 35% SC; Nanjing Red Sun Co. Ltd., China) was used as a positive control at a concentration of 20 mL/hL. This concentration was selected based on Saruhan (2018), who demonstrated that 20 mL hL^{-1} effectively controls black bean aphid populations. Imidacloprid, a neonicotinoid insecticide, is known for its strong systemic activity and high efficacy against *A. fabae* (Saruhan, 2018; Almogdad and Semaškienė, 2021). Moreover, previous research has shown that imidacloprid not only effectively reduces aphid infestations but also leads to higher crop yields compared to other insecticides such as carbofuran and untreated plants (Al-Naser and Ezz Al-Dden, 2011). Therefore, imidacloprid serves as a reliable benchmark to evaluate alternative control methods in this study.

2.5 Study site and host tissue

The study was conducted in a screenhouse (11 m × 7 m × 3 m), maintained at 25 ± 4°C and 60 ± 10% relative humidity under natural light at the Regional Center for Agricultural Research of Settat (INRA), Settat, Morocco. Prior to the experiments, *V. faba* plants were grown in plastic pots (33 cm diameter × 12 cm height) filled with a substrate mixture of two-thirds fine sand and one-third peat. These plants were cultivated in the same screenhouse and allowed to develop for one month. The plants were watered as needed throughout the growing period. Each plant was then infested with 100 s-instar *A. fabae* nymphs (approximately 60 females and 40 males) from the laboratory strain colony. The nymphs were transferred using a fine brush and allowed to establish for 20 days.

2.6 Treatments

The experimental treatments were as follows: T1 – untreated control, T2 – *C. septempunctata* (CS) alone, T3 –*H. convergens* alone (HC), T4 –*N. glauca* at 10% (NG), T5 – *R. communis* at 10% (RC), T6 – NG + CS, T7 – NG + HC, T8 – RC + CS, T9 – RC + HC, and T10 – Imidacloprid 35% (ICP) at 20 mL/hL % concentration prepared with tap water. The combination of the two predatory ladybirds was not tested. Prior to release, ladybirds were starved for 24 h, and for each plant, ten ovipositing adult females were introduced. Botanical extracts and Imidacloprid were applied at 20 mL per plant using a 1.5 L garden pressure sprayer (Mesto Spritzenfabrik Ernst Stockburger GmbH, Germany). To prevent cross-contamination, each treated plant was enclosed in a ventilated cylindrical cage made of sealed transparent plastic film, with the top covered by coarse mesh organdy fabric to allow airflow. Treatments were arranged in a randomized complete block design with three replicates per treatment, each replicate consisting of 7 *Aphis fabae*-infested *Vicia faba* plants, spaced 20 cm apart in a row with 40 cm between rows, totaling 210 plants per experiment. Predatory ladybirds were introduced 1 week after botanical insecticide application to minimize any repellency effects observed in previous

study (El Aalaoui and Sbaghi, 2025). The experiment was repeated twice.

2.7 Data collection

Twenty days after infestation and before applying any treatments, three leaves were collected from each treated *V. faba* plant—one from the top, one from the middle, and one from the bottom. Leaves were cut using scissors, placed individually in paper bags, and transported to the laboratory. These leaves were chosen because they typically have high densities of *A. fabae*, providing a representative sample of infestation levels. Additionally, these leaf positions are commonly targeted by predatory ladybirds during their nocturnal movements from the plant apex, making them suitable for accurately assessing predation by *C. septempunctata* and *H. convergens* (Onzo et al., 2013). Both eggs and motile stages (nymphs and adults) of aphids were counted under a dissecting microscope (Motic).

Monitoring of treatment effectiveness began 1 week after the release of the predatory ladybirds and continued weekly for 5 weeks. Each week, three leaves per treated plant were randomly selected and examined following the same procedure described above. Counts included eggs and motile stages (larvae, nymphs, and adults) of both *A. fabae* and the predatory ladybirds. All collected ladybird specimens were identified to species level (Zare Khormizi et al., 2013). In addition, weekly assessments of aphid damage were made using a visual rating scale from 0 to 10, where 0 indicates a dead plant and 10 indicates a healthy plant, as described by Gettys et al. (2021). This scale has been widely used to evaluate plant health under various stresses, including herbicide exposure and salinity (Smith et al., 2014; Tootoonchi et al., 2020).

2.8 Statistical analysis

Data were analyzed using R software version 4.3.2. Prior to analysis, normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively. To evaluate the effects of treatments and sampling time (week) on *A. fabae* density, predator abundance, and plant damage scores, a two-way ANOVA was performed, considering treatment and time as fixed factors. When significant differences were detected, means were compared using the Student–Newman–Keuls (SNK) multiple comparison test. Plant damage scores (0–10 scale) were analyzed similarly. A two-sample t-test was used to compare predator densities (eggs and motile stages) between the two ladybird species under the following treatment pairs: T2 –CS vs. T3 – HC; T6 – NG + CS vs. T7 – NG + HC; and T8 – RC + CS vs. T9 – RC + HC. Data were transformed using log10(x + 1) when necessary to satisfy the assumptions of normality and equal variances. Results were visualized to illustrate treatment and time effects on pest densities, predator populations, and plant health.

3 Results

3.1 Efficacy of treatments on *Aphis fabae* eggs

Before treatment application, there was no significant difference in *A. fabae* egg density among treatments ($F_{9,200} = 1.1$, $p = 0.352$), confirming homogeneity of initial infestations (Figure 1A). However, from Week 1 onwards, treatment effects were highly significant (Week 1: $F_{9,200} = 728.2$, $p < 2.2 \times 10^{-16}$; Week 2: $F_{9,200} = 1694.2$, $p < 2.2 \times 10^{-16}$; Week 3: $F_{9,200} = 3077.1$, $p < 2.2 \times 10^{-16}$; Week 4: $F_{9,200} = 4931.5$, $p < 2.2 \times 10^{-16}$; Week 5: $F_{9,200} = 6992.3$, $p < 2.2 \times 10^{-16}$), with all treatments significantly reducing egg densities compared to the

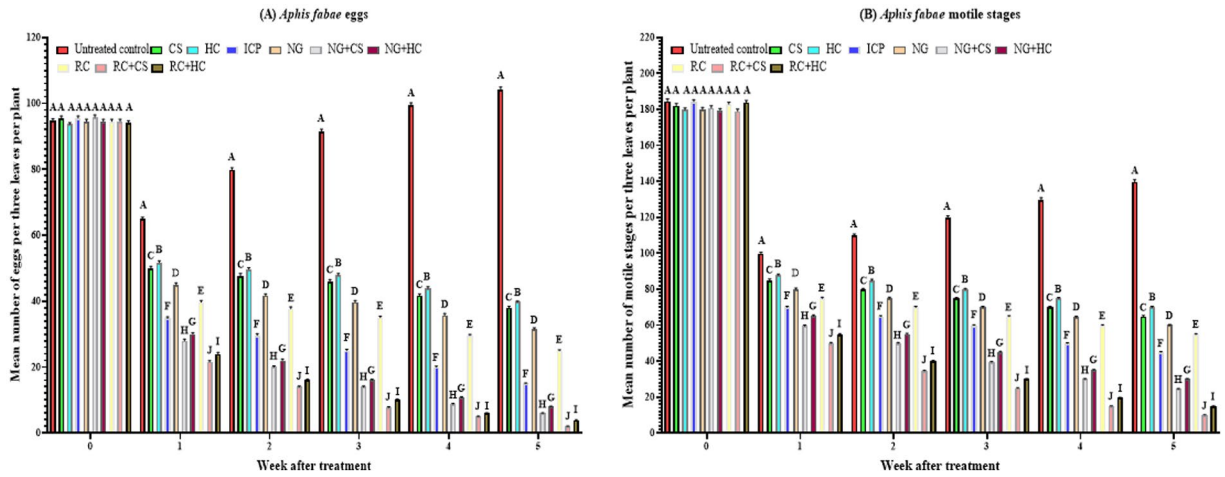

FIGURE 1
Mean (±SE) densities of eggs **(A)** and motile stages **(B)** of *Aphis fabae* following spray application of *Nicotiana glauca* and *Ricinus communis*, and Imidacloprid, and release of predatory ladybirds *Coccinella septempunctata* and *Hippodamia convergens* under screenhouse conditions. CS = *C. septempunctata* alone, HC = *H. convergens* alone, NG = *N. glauca* at 10%, RC = *R. communis* at 10%, and ICP = Imidacloprid 35% at 20 mL/hL % concentration prepared with tap water. Different letters above bars indicate statistical differences (based on the Student–Newman–Keuls test, $\alpha = 0.05$).

control. By week 1, RC + CS (21.62 eggs per plant) and RC + HC (24.00 eggs per 3 leaves per plant) treatments achieved the greatest reductions, followed by NG + CS (28.00 eggs) and NG + HC (30.00 eggs) treatments. This pattern continued through week 5, where RC + CS (2.00 eggs per 3 leaves per plant) and RC + HC (3.90 eggs per 3 leaves per plant) treatments showed the strongest suppression of aphid eggs, while the control treatment remained significantly higher (104.14 eggs per 3 leaves per plant). Intermediate effectiveness was observed for ICP (15.00 eggs per 3 leaves per plant), NG + CS (5.95 eggs per 3 leaves per plant), and NG + HC (8.00 eggs per 3 leaves per plant) treatments. Additionally, prolonged exposure significantly enhanced treatment efficacy in reducing *A. fabae* egg counts over time. The CS treatment reduced egg density from 95.52 to 38.00 eggs per 3 leaves per plant ($F_{1,120} = 1284.0$, $p < 2.2 \times 10^{-16}$), while HC treatment showed a reduction from 93.67 to 39.76 eggs ($F_{1,120} = 1722.6$, $p < 2.2 \times 10^{-16}$). Botanical treatments alone also resulted in significant reductions in egg counts: in the NG treatment, egg numbers decreased from 94.52 to 31.57 ($F_{1,120} = 1893.7$, $p < 2.2 \times 10^{-16}$), and in the RC treatment, from 94.52 to 25.00 eggs ($F_{1,120} = 2877.4$, $p < 2.2 \times 10^{-16}$). Combined treatments were the most effective: NG + CS dropped eggs density from 95.81 to 5.95 eggs ($F_{1,120} = 7361.2$, $p < 2.2 \times 10^{-16}$), NG + HC from 94.52 to 8.00 eggs ($F_{1,120} = 6517.2$, $p < 2.2 \times 10^{-16}$), RC + CS from 94.57 to 2.00 eggs ($F_{1,120} = 10520.0$, $p < 2.2 \times 10^{-16}$), and RC + HC from 94.19 to 3.90 eggs ($F_{1,120} = 8740.9$, $p < 2.2 \times 10^{-16}$). The chemical treatment ICP also resulted in a significant reduction from 95.52 to 15.00 eggs ($F_{1,120} = 4159.7$, $p < 2.2 \times 10^{-16}$). In summary, all treatments significantly reduced *A. fabae* egg densities over time, with combined treatments of *R. communis* extracts and predators showing the greatest and fastest reductions. Treatment effectiveness increased with longer exposure, confirming the cumulative impact of the applied strategies.

3.2 Efficacy of treatments on *Aphis fabae* motile stages

Before treatment application, there was no significant difference in *A. fabae* motile stage density among treatments (Figure 1B). However, from week 1 onwards, treatment effects were highly significant (Week 1: $F_{9,200} = 640.9$, $p < 2.2 \times 10^{-16}$; Week 2: $F_{9,200} = 1469.4$, $p < 2.2 \times 10^{-16}$; Week 3: $F_{9,200} = 2670.6$, $p < 2.2 \times 10^{-16}$; Week 4: $F_{9,200} = 3750.2$, $p < 2.2 \times 10^{-16}$; Week 5: $F_{9,200} = 5075.4$, $p < 2.2 \times 10^{-16}$), with all treatments significantly reducing densities compared to the control. By week 1, RC + CS (49.90 individuals) and RC + HC (54.90 individuals per 3 leaves per plant) treatments achieved the greatest reductions, followed by NG + CS (59.52 per 3 leaves per plant) and NG + HC (65.09 per 3 leaves per plant) treatments. This trend persisted through week 5, where RC + CS (10.00 individuals per 3 leaves per plant) and RC + HC (15.00 individuals per 3 leaves per plant) treatments maintained the lowest densities, while the control remained significantly higher (139.71 individuals per 3 leaves per plant). Moderate reductions were observed for ICP (45.00 individuals per 3 leaves per plant), NG + CS (24.52 individuals per 3 leaves per plant), and NG + HC (30.00 individuals per 3 leaves per plant) treatments. Additionally, prolonged exposure significantly enhanced treatment efficacy in reducing *A. fabae* motile stages over time. CS treatment reduced density from 182.05 to 64.81 individuals per 3 leaves per plant ($F_{1,120} = 3646.4$, $p < 2.2 \times 10^{-16}$), while HC showed a reduction from 179.81 to 69.86 ($F_{1,120} = 3394.7$, $p < 2.2 \times 10^{-16}$). Botanical treatments also led to substantial declines: NG from 179.90 to 60.00 ($F_{1,120} = 4045.1$, $p < 2.2 \times 10^{-16}$) and RC from 182.90 to 54.81 ($F_{1,120} = 5124.6$, $p < 2.2 \times 10^{-16}$). In NG + CS treatment number of motile stages dropped from 180.81 to 24.52 ($F_{1,120} = 9610.8$, $p < 2.2 \times 10^{-16}$), in NG + HC treatment dropped from 179.48 to 30.00 ($F_{1,120} = 9931.0$, $p < 2.2 \times 10^{-16}$), RC + CS from 178.95 to 10.00 ($F_{1,120} = 12656.0$, $p < 2.2 \times 10^{-16}$), and RC + HC from 183.81 to 15.00 ($F_{1,120} = 12460.0$, $p < 2.2 \times 10^{-16}$). The chemical treatment ICP also caused a significant reduction from 184.33 to 45.00 ($F_{1,120} = 6827.7$, $p < 2.2 \times 10^{-16}$). Overall, all treatments significantly reduced *A. fabae* motile stage densities compared to the control, with combined treatments of *R. communis* extracts and predators (RC + CS, RC + HC) showing the greatest and most sustained reductions. Treatment effectiveness improved over time, demonstrating cumulative suppression of motile stages.

3.3 Effect of treatments on *Coccinella septempunctata* egg densities

From week 1 onwards, treatment effects were highly significant (week 1: $F_{2,60} = 21.2$, $p = 1.10 \times 10^{-7}$; week 2: $F_{2,60} = 7.2$, $p = 0.002$; week 3: $F_{2,60} = 5.6$, $p = 0.006$; week 4: $F_{2,60} = 4.9$, $p = 0.011$; week 5: $F_{2,60} = 8.0$, $p = 8.42 \times 10^{-4}$), with the RC + CS treatment consistently maintaining the highest egg densities per 3 leaves per plant, followed by NG + CS and CS treatments (Figure 2A). By week 1, RC + CS treatment (7.52 eggs per 3 leaves per plant) had the highest egg density, significantly higher than CS (6.67 eggs per 3 leaves per plant) and NG + CS (6.67 eggs per 3 leaves per plant) treatments. This pattern persisted throughout the study period, with RC + CS treatment showing 6.76 eggs per 3 leaves per plant at week 5, compared to NG + CS (6.33 eggs per 3 leaves per plant) and CS (6.24 eggs per 3 leaves per plant) treatments. At weeks 2 and 4, no significant difference was recorded between RC + CS and NG + CS treatments. For all treatments, egg numbers peaked at week 2, with 7.00 eggs per 3 leaves per plant for CS, 7.30 eggs per 3 leaves per plant for NG + CS, and 7.67 eggs per 3 leaves per plant for RC + CS treatment. Additionally, prolonged exposure revealed a slight but significant decline in egg densities across treatments over time. In the CS treatment, egg counts decreased from 6.67 at week 1 to 6.24 eggs per 3 leaves per plant by week 5 ($F_{4,100} = 8.2$, $p = 9.8 \times 10^{-6}$); in the NG + CS treatment, from 6.67 to 6.33 eggs ($F_{4,100} = 12.6$, $p = 2.4 \times 10^{-8}$); and in the RC + CS treatment, from 7.52 to 6.76 eggs per 3 leaves per plant ($F_{4,100} = 18.6$, $p = 1.9 \times 10^{-11}$). Overall, the RC + CS treatment maintained the highest *C. septempunctata* egg densities throughout the study, followed by NG + CS and CS treatments. Egg densities peaked at week 2 for all treatments and then gradually declined over time, indicating a slight but significant reduction in egg numbers with prolonged exposure.

3.4 Effect of treatments on *Coccinella septempunctata* motile stage densities

From week 1 onwards, treatment effects on motile stage densities were significant for the CS and RC + CS treatments, but not for the NG + CS treatment (Figure 2B). In the CS treatment, weekly differences were highly significant ($F_{4,100} = 10.4$, $p = 4.6 \times 10^{-7}$), with motile stage density peaking at week 2 (10.00 individuals per 3 leaves per plant) and declining to 8.67 individuals per 3 leaves per plant by week 5. The

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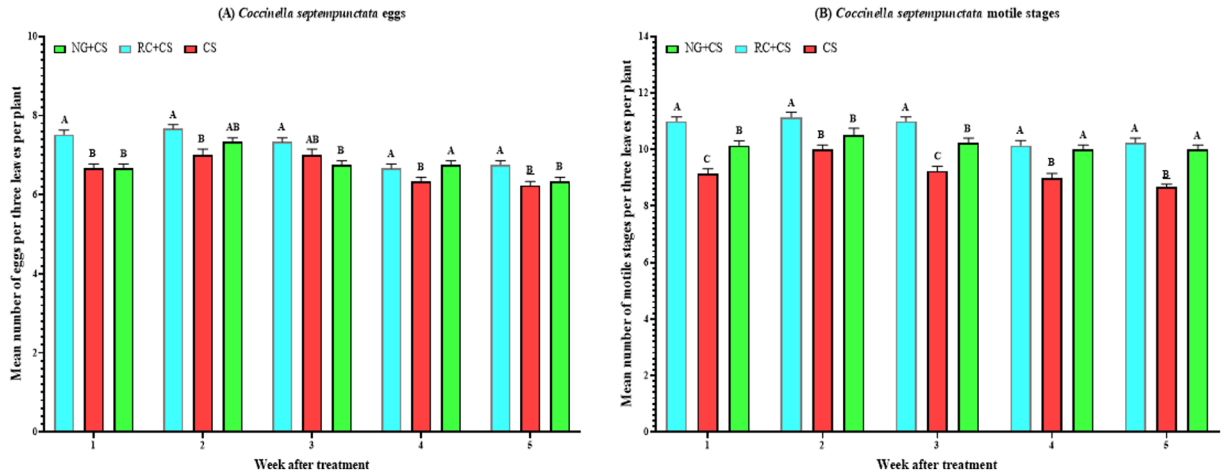

FIGURE 2
Mean (±SE) densities of eggs **(A)** and motile stages **(B)** of *Coccinella septempunctata* following spray application of *Nicotiana glauca* and *Ricinus communis*, and release of predatory ladybird *Coccinella septempunctata* under screenhouse conditions. CS = *C. septempunctata* alone, NG = *N. glauca* at 10%, and RC = *R. communis* at 10%, prepared with tap water. Different letters above bars indicate statistical differences (based on the Student–Newman–Keuls test, $\alpha = 0.05$).

RC + CS treatment also exhibited significant weekly variation ($F_{4,100} = 8.3$, $p = 8.08 \times 10^{-6}$), maintaining the highest overall motile stage densities. Densities remained stable from weeks 1 to 3 (~11.00 individuals) before slightly declining to 10.24 individuals by week 5. In contrast, the NG + CS treatment showed no significant differences across weeks ($F_{4,100} = 1.6$, $p = 0.190$), with densities remaining relatively constant. When comparing treatments within each week, the RC + CS treatment consistently recorded the highest motile stage densities, followed by NG + CS treatment and then CS treatment. At week 1, RC + CS treatment had significantly higher densities (11.00 individuals per 3 leaves per plant) compared to NG + CS (10.14 individuals per 3 leaves per plant) and CS (9.14 individuals per 3 leaves per plant) treatments ($F_{2,60} = 32.9$, $p = 2.3 \times 10^{-10}$). This trend persisted through week 5, where RC + CS (10.24 individuals per 3 leaves per plant) and NG + CS (10.00 individuals per 3 leaves per plant) treatments maintained significantly higher densities than CS treatment (8.67 individuals per 3 leaves per plant) ($F_{2,60} = 34.1$, $p = 1.3 \times 10^{-10}$). In weeks 2 and 3, RC + CS treatment showed significantly higher densities than both CS and NG + CS treatments (week 2: $F_{2,60} = 9.4$, $p = 2.8 \times 10^{-4}$; week 3: $F_{2,60} = 29.3$, $p = 1.3 \times 10^{-9}$). However, at week 4, no significant difference was observed between RC + CS and NG + CS treatments ($F_{2,60} = 15.5$, $p = 5.2 \times 10^{-6}$). Overall, motile stage densities peaked at week 2 for all treatments: CS (10.00 individuals per 3 leaves per plant), NG + CS (10.52 individuals per 3 leaves per plant), and RC + CS (11.14 individuals per 3 leaves per plant). Overall, the RC + CS treatment consistently maintained the highest motile stage densities throughout the study, followed by NG + CS and CS treatments. Motile stage densities peaked at week 2 for all treatments and then generally declined or stabilized over time.

3.5 Effect of treatments on *Hippodamia convergens* eggs density

From week 1 onwards, treatment effects on *H. convergens* egg densities varied across treatments and weeks (Figure 3A). In the HC treatment alone, weekly differences in egg density were significant ($F_{4,100} = 6.3$, $p = 1.6 \times 10^{-4}$), with egg density peaking at week 2 (6.67 eggs per 3 leaves per plant) and then stabilizing around 6.00–6.33 eggs per 3 leaves per plant through weeks 3 to 5. For the NG + HC treatment, significant but weaker weekly variation was observed ($F_{4,100} = 3.0$, $p = 0.022$), with densities stable at approximately 6.67 eggs per 3 leaves per plant from weeks 1 to 3, then slightly declining from week 4 onward. The RC + HC treatment showed highly significant weekly variation ($F_{4,100} = 5.3$, $p = 0.001$), with the highest egg density at week 2 (7.24 eggs per 3 leaves per plant), followed by a modest decline and stabilization (~6.52–6.67 eggs per 3 leaves per plant) in subsequent weeks. When comparing treatments within each week, egg densities differed significantly on most weeks. At week 1, NG + HC and RC + HC treatments had significantly higher densities (6.67 eggs per 3 leaves per plant each) than HC alone treatment (6.33 eggs) ($F_{2,60} = 3.3$, $p = 0.042$). In week 2, RC + HC treatment had the highest density (7.24 eggs per 3 leaves per plant), significantly exceeding both HC and NG + HC treatments (each 6.67 eggs per 3 leaves per plant) ($F_{2,60} = 6.5$, $p = 0.003$). In week 3, densities were similar between NG + HC and RC + HC treatments (each 6.67 eggs per 3 leaves per plant), both generally higher than HC treatment (6.33 eggs per 3 leaves per plant) ($F_{2,60} = 3.3$, $p = 0.042$). In week 4, RC + HC treatment had the highest density (6.67 eggs per 3 leaves per plant), significantly exceeding both HC and NG + HC treatments (each 6.33 eggs per 3 leaves per plant) ($F_{2,60} = 3.3$, $p = 0.042$). By week 5, RC + HC and NG + HC treatments maintained higher egg densities (6.52 and 6.33 eggs per 3 leaves per plant, respectively) compared to HC alone treatment (6.00 eggs) ($F_{2,60} = 8.9$, $p = 0.000$). In summary, the RC + HC treatment generally maintained the highest *H. convergens* egg densities throughout the study, with egg densities peaking at week 2 across treatments and stabilizing or slightly declining thereafter. NG + HC treatment showed intermediate egg densities, while the HC treatment alone maintained the lowest egg densities over time.

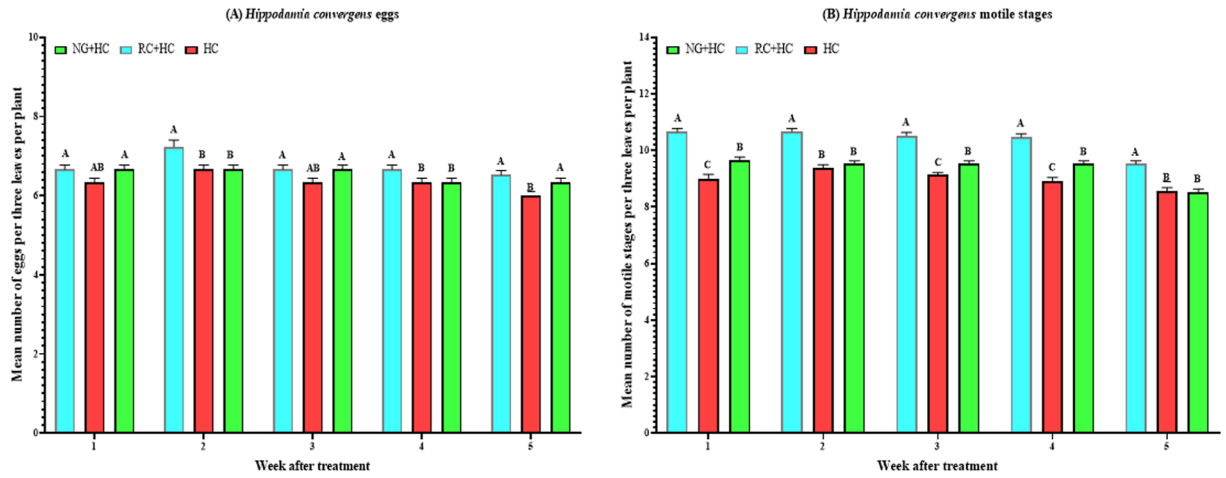

FIGURE 3
Mean (±SE) densities of eggs **(A)** and motile stages **(B)** of *Hippodamia convergens* following spray application of *Nicotiana glauca* and *Ricinus communis*, and release of predatory ladybird *Hippodamia convergens* under screenhouse conditions. HC = *Hippodamia convergens* alone, NG = *N. glauca* at 10%, and RC = *R. communis* at 10%, prepared with tap water. Different letters above bars indicate statistical differences (based on the Student–Newman–Keuls test, $\alpha = 0.05$).

3.6 Effect of treatments on *Hippodamia convergens* motile stages density

From week 1 onwards, treatment effects on *H. convergens* motile stage densities varied significantly across treatments and weeks (Figure 3B). In the HC treatment alone, weekly differences were significant ($F_{4,100} = 6.2$, $p = 1.8 \times 10^{-4}$), with density peaking at week 2 (9.38 individuals per 3 leaves per plant) and gradually declining to 8.57 individuals by week 5. The NG + HC treatment showed highly significant weekly variation ($F_{4,100} = 17.9$, $p = 4.1 \times 10^{-11}$), with densities stable at approximately 9.52 individuals from weeks 1 to 4, then decreasing to 8.52 individuals at week 5. The RC + HC treatment also exhibited highly significant weekly differences ($F_{4,100} = 19.4$, $p = 7.5 \times 10^{-12}$), with the highest densities recorded during weeks 1 and 2 (10.67 individuals per 3 leaves per plant), followed by a slight decline to 9.52 individuals at week 5. When comparing treatments within each week, motile stage densities differed significantly in all weeks. At week 1, the RC + HC treatment had the highest density (10.67 individuals per 3 leaves per plant), significantly exceeding NG + HC (9.67 individuals per 3 leaves per plant) and HC alone (9.00 individuals per 3 leaves per plant) treatments ($F_{2,60} = 45.9$, $p = 8.2 \times 10^{-13}$). This pattern persisted through week 5, with RC + HC treatment consistently showing the highest densities. Specifically, at week 2, the density of motile stages in the RC + HC treatment (10.67 individuals per 3 leaves per plant) was significantly higher than in the NG + HC (9.52 individuals per 3 leaves per plant) and HC (9.38 individuals per 3 leaves per plant) treatments ($F_{2,60} = 42.1$, $p = 3.7 \times 10^{-12}$). At week 3, densities remained highest in RC + HC (10.52 individuals per 3 leaves per plant), followed by NG + HC (9.52 individuals per 3 leaves per plant) and HC (9.14 individuals per 3 leaves per plant) treatments ($F_{2,60} = 49.1$, $p = 2.3 \times 10^{-13}$). At week 4, RC + HC treatment again had the highest density (10.48 individuals per 3 leaves per plant), significantly greater than NG + HC (9.52 individuals per 3 leaves per plant) and HC (8.90 individuals per 3 leaves per plant) treatments ($F_{2,60} = 43.2$, $p = 2.4 \times 10^{-12}$). By week 5, RC + HC treatment maintained the highest density (9.52 individuals per 3 leaves per plant), significantly higher than HC (8.57 individuals per 3 leaves per plant) and NG + HC (8.52 individuals per 3 leaves per plant) treatments ($F_{2,60} = 25.7$, $p = 8.8 \times 10^{-9}$). In summary, motile stage densities of *H. convergens* peaked at week 2 for all treatments, with RC + HC treatment consistently supporting the highest densities across all weeks. NG + HC treatment showed intermediate densities, while HC treatment alone maintained the lowest densities over time.

3.7 Effect of treatments on treated plant visual quality

By week 1, the treatment effect became highly significant ($F_{9,200} = 498.2$, $p < 2.2 \times 10^{-16}$), with RC + CS (visual quality score: 8.80) and RC + HC (8.68) treatments being the most effective treatments, followed by NG + CS (8.20) and NG + HC (7.87) treatments. In contrast, the control (5.50), HC (6.10), and CS (6.20) treatments showed the lowest scores. This pattern was further reinforced in week 2 ($F_{9,200} = 628.7$, $p < 2.2 \times 10^{-16}$), where RC + CS (9.10), RC + HC (8.89), and NG + CS (8.49) treatments significantly outperformed all other treatments, while the control remained the least effective (5.68). In week 3, the same top-performing treatments maintained their superiority ($F_{9,200} = 650.5$, $p < 2.2 \times 10^{-16}$), with RC + CS, RC + HC, and NG + CS treatments achieving visual quality scores of 9.29, 9.02, and 8.73, respectively. The control (6.10), HC (6.30), and CS (6.58) treatments continued to score significantly lower. Week 4 analysis ($F_{9,200} = 694.5$, $p < 2.2 \times 10^{-16}$) confirmed that RC + CS (9.50) and RC + HC (9.27) treatments remained the most effective, followed by NG + CS (8.85) and NG + HC (8.52) treatments, while the control (6.20) and HC (6.51) were among the least effective. By week 5, the treatment effect peaked ($F_{9,200} = 708.5$, $p < 2.2 \times 10^{-16}$), with RC + CS (9.67), RC + HC (9.35), NG + CS (8.87), and NG + HC (8.73) treatments continuing to lead. The control remained the lowest (6.51), significantly different from all other treatments. Across weeks,

all treatments exhibited significant improvement in plant visual quality scores over time. In the CS treatment, the score rose from 5.15 before treatment application to 6.69 in week 5 ($F_{5,120} = 120.8$, $p < 2.2 \times 10^{-16}$), and in the HC treatment from 5.25 to 6.58 ($F_{5,120} = 74.3$, $p < 2.2 \times 10^{-16}$). The NG treatment showed a steady increase from 5.27 to 7.31 ($F_{5,120} = 193.7$, $p < 2.2 \times 10^{-16}$), while NG + CS rose from 5.59 to 8.87 ($F_{5,120} = 383.8$, $p < 2.2 \times 10^{-16}$), and NG + HC from 5.46 to 8.73 ($F_{5,120} = 370.4$, $p < 2.2 \times 10^{-16}$). In the ICP treatment, the score improved from 5.59 to 8.25 ($F_{5,120} = 255.8$, $p < 2.2 \times 10^{-16}$); in the RC treatment from 5.38 to 7.74 ($F_{5,120} = 250.3$, $p < 2.2 \times 10^{-16}$); in the RC + CS treatment from 5.27 to 9.67 ($F_{5,120} = 332.4$, $p < 2.2 \times 10^{-16}$); and in the RC + HC treatment from 5.34 to 9.35 ($F_{5,120} = 563.5$, $p < 2.2 \times 10^{-16}$). These results clearly demonstrate that combined treatments involving RC and NG, particularly when paired with CS or HC, provided the most consistent and effective improvement in plant visual quality over time, significantly outperforming the control and single-component treatments. In summary, plant visual quality improved significantly across all treatments over time, with RC + CS and RC + HC treatments consistently achieving the highest scores. Treatments combining RC or NG with CS or HC were notably more effective than single-component and control treatments in maintaining superior plant appearance.

3.8 Comparison of *Coccinella septempunctata* and *Hippodamia convergens* densities across treatments

The densities of *C. septempunctata* (CS) and *H. convergens* (HC) were compared under three different treatments: CS alone versus HC alone, NG + CS versus NG + HC (where NG = *N. glauca* at 10%), and RC + CS versus RC + HC (where RC = *R. communis* at 10%). When comparing CS alone to HC alone, CS had higher egg densities at weeks 1 (6.67 vs. 6.30), 3 (7.00 vs. 6.30), and 5 (6.24 vs. 6.00), while motile stage densities were mostly similar except at week 2 where CS was higher (10.00 vs. 9.38) (Table 1). In the NG treatments, NG + CS showed greater egg densities than NG + HC at weeks 2 (7.33 vs. 6.70) and 4 (6.76 vs. 6.30). Motile stages were also consistently more abundant in NG + CS across weeks 1 to 5, with a notable difference at week 5 (10.00 vs. 8.50) (Table 2). For the RC treatments, RC + CS had higher egg densities than RC + HC during the first 3 weeks, such as at week 1 (7.52 vs. 6.67). Motile stage densities were higher in RC + CS compared to RC + HC at weeks 2, 3, and 5 (for example, week 5: 10.24 vs. 9.50) (Table 3). These results indicate that *C. septempunctata* generally exhibits higher egg and motile stage densities than *H. convergens*, when combined with *N. glauca* or *R. communis* (Figure 4).

4 Discussion

This study evaluated the individual and combined efficacy of two predatory ladybird species, *C. septempunctata* (CS) and *H. convergens* (HC), botanical insecticides from *N. glauca* (NG) and *R. communis* (RC), and the chemical insecticide imidacloprid (ICP) in controlling *A. fabae* populations on *V. faba* plants. Our findings demonstrate that all treatments significantly reduced aphid densities and improved plant health compared to untreated controls, with combined treatments—particularly those pairing predatory ladybirds with botanical extracts—producing the most substantial and consistent suppression of aphid populations and enhancement of plant visual quality over time. The results confirm the effectiveness of *C. septempunctata* and *H. convergens* against *A. fabae*, aligning with previous studies (Shannag and Obeidat, 2008).

Across all 5 weeks of observation, *C. septempunctata* consistently outperformed *H. convergens* in reducing both aphid egg and motile stage densities, as well as in maintaining higher predator reproduction and motile stage populations. This was evident not only in the CS versus HC treatments, but also in the combined treatments (NG + CS vs. NG + HC; RC + CS vs. RC + HC). These findings suggest that CS possesses superior predation efficiency, reproductive output, and possibly better adaptation to local environmental conditions. The superior performance of CS aligns with earlier studies that have documented its higher fecundity, voracity, and broader prey spectrum compared to other coccinellid species (Hodek and Honek, 1996). Furthermore, the relatively lower performance of *H. convergens* may be attributed to its smaller body size, which can limit its predation

TABLE 1 Comparison of *Coccinella septempunctata* (CS) and *Hippodamia convergens* (HC) densities (eggs and motile stages per 3 leaves per plant) between treatment T2 (*C. septempunctata* alone, CS) and treatment T3 (*H. convergens* alone, HC).

Predator stage	Week after treatment	CS treatment (Mean ± SE)	HC treatment (Mean ± SE)	*t*	df	*p-value*	Significance
Eggs	1	6.67 ± 0.11	6.30 ± 0.11	2.46	39	0.0183	*
	2	7.00 ± 0.15	6.70 ± 0.11	1.61	35	0.117	ns
	3	7.00 ± 0.15	6.30 ± 0.11	3.75	35	6.4×10^{-4}	***
	4	6.33 ± 0.11	6.30 ± 0.11	0.22	39	0.824	ns
	5	6.24 ± 0.10	6.00 ± 0.00	2.50	20	0.0212	*
Motile stages	1	9.14 ± 0.17	9.00 ± 0.15	0.62	39.5	0.541	ns
	2	10.00 ± 0.15	9.38 ± 0.11	3.28	35.9	2.3×10^{-3}	**
	3	9.24 ± 0.17	9.14 ± 0.08	0.51	28.3	0.611	ns
	5	9.00 ± 0.15	8.90 ± 0.14	0.46	39.4	0.646	ns
	5	8.67 ± 0.11	8.57 ± 0.11	0.62	39.9	0.537	ns

The significance levels are denoted as follows: *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, and ns for non-significant *p*-values.

TABLE 2 Comparison of *Coccinella septempunctata* (CS) and *Hippodamia convergens* (HC) densities (eggs and motile stages per 3 leaves per plant) between treatment T6 – NG + CS and treatment T7 – NG + HC.

Predator stage	Week after treatment	NG + CS treatment (Mean ± SE)	NG + HC treatment (Mean ± SE)	t	df	*p-value*	Significance
Eggs	1	6.67 ± 0.11	6.70 ± 0.11	−0.22	39	0.824	ns
	2	7.33 ± 0.11	6.70 ± 0.11	4.25	39	1.3×10^{-4}	***
	3	6.76 ± 0.10	6.70 ± 0.11	0.44	38.4	0.665	ns
	4	6.76 ± 0.10	6.30 ± 0.11	3.26	38.4	2.4×10^{-3}	**
	5	6.33 ± 0.11	6.30 ± 0.11	0.22	39	0.824	ns
Motile stages	1	10.14 ± 0.16	9.70 ± 0.11	2.33	34.4	0.0260	*
	2	10.52 ± 0.22	9.50 ± 0.11	4.06	29.6	3.3×10^{-4}	***
	3	10.24 ± 0.17	9.50 ± 0.11	3.63	35.0	8.9×10^{-4}	***
	5	10.00 ± 0.15	9.50 ± 0.11	2.6	36.5	0.0134	*
	5	10.00 ± 0.15	8.50 ± 0.11	7.8	36.5	2.7×10^{-9}	***

The significance levels are denoted as follows: *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, and ns for non-significant *p*-values. NG = *Nicotiana glauca* at 10%.

TABLE 3 Comparison of *Coccinella septempunctata* (CS) and *Hippodamia convergens* (HC) densities (eggs and motile stages per 3 leaves per plant) between treatment T8 – RC + CS and T9 – RC + HC.

Predator stage	Week after treatment	RC + CS treatment (Mean ±SE)	RC + HC treatment (Mean ±SE)	t	df	*P-value*	Significance
Eggs	1	7.52 ± 0.11	6.67 ± 0.11	5.58	39.9	1.9×10^{-6}	***
	2	7.67 ± 0.11	7.24 ± 0.17	2.16	33.7	0.0377	*
	3	7.33 ± 0.11	6.67 ± 0.11	4.47	40.0	6.3×10^{-5}	***
	4	6.67 ± 0.11	6.67 ± 0.11	0.00	40.0	1.0	ns
	5	6.76 ± 0.10	6.52 ± 0.11	1.62	39.0	0.113	ns
Motile stages	1	11.00 ± 0.15	10.70 ± 0.11	1.61	35.0	0.117	ns
	2	11.14 ± 0.17	10.70 ± 0.11	2.19	32.8	0.0359	*
	3	11.00 ± 0.15	10.50 ± 0.11	2.60	36.5	0.0134	*
	5	10.14 ± 0.17	10.50 ± 0.11	−1.72	34.4	0.0943	ns
	5	10.24 ± 0.17	9.50 ± 0.11	3.63	35.0	8.9×10^{-4}	***

The significance levels are denoted as follows: *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, and ns for non-significant *p*-values. RC = *Ricinus communis* at 10%.

efficiency. As noted by Blum (1981), in the absence of prey defenses, predator effectiveness is largely shaped by prey specificity and the relative size of the predator to its prey.

Furthermore, *R. communis* consistently showed higher efficacy than *N. glauca* in reducing populations of *A. fabae* and improving the visual quality of *V. faba* plants. This trend was evident both when applied alone and in combination with predatory ladybirds. The superior performance of *R. communis* is likely related to its rich content of toxic secondary metabolites, including ricin, ricinine, alkaloids, and flavonoids, which exhibit strong insecticidal and repellent properties (Chouhan et al., 2021). Previous studies have demonstrated that *R. communis* effectively controls pests such as *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae) (da Silva Santos et al., 2016), *Aedes aegypti* Linnaeus (Diptera: Culicidae) (Neves et al., 2014), and *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) (Ramos-López et al., 2010). The toxicity of *R. communis* seeds is mainly due to ricin, a potent protein toxin (Chouhan et al., 2021). Additionally, flavonoids and other secondary metabolites contribute by damaging insect cells and disrupting DNA, RNA, and protein synthesis (Randhir et al., 2004). *Nicotiana glauca* also showed insecticidal effects but was less effective across all parameters measured. It has been reported to affect pests such as *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) (Alghamdi, 2021a; Alghamdi, 2021b), *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Abdelgader and Elawad, 2022), and *D. opuntiae* (Zim et al., 2025). The relatively lower efficacy of *N. glauca* in this study may be due to lower concentrations of active compounds, differences in the mode of action, environmental factors during application, or pest susceptibility. Moreover, toxicity of botanical extracts varies according to the plant parts used, since concentrations of bioactive chemicals differ among plant tissues (Santiago et al., 2008).

Taken together, the ranking of treatment efficacy based on *A. fabae* suppression and plant health improvement followed the order: *C. septempunctata* > *H. convergens* > *R. communis* > *N. glauca*. This consistent hierarchy throughout the experimental period highlights the superior and sustained predation by ladybird species, which actively locate and consume aphids across all developmental stages. In contrast, the botanical extracts demonstrated lower efficacy, likely due to their limited persistence and rapid degradation under field conditions, as reflected in the less pronounced aphid suppression observed. These findings suggest that while botanical insecticides offer environmentally friendly options, their practical effectiveness may

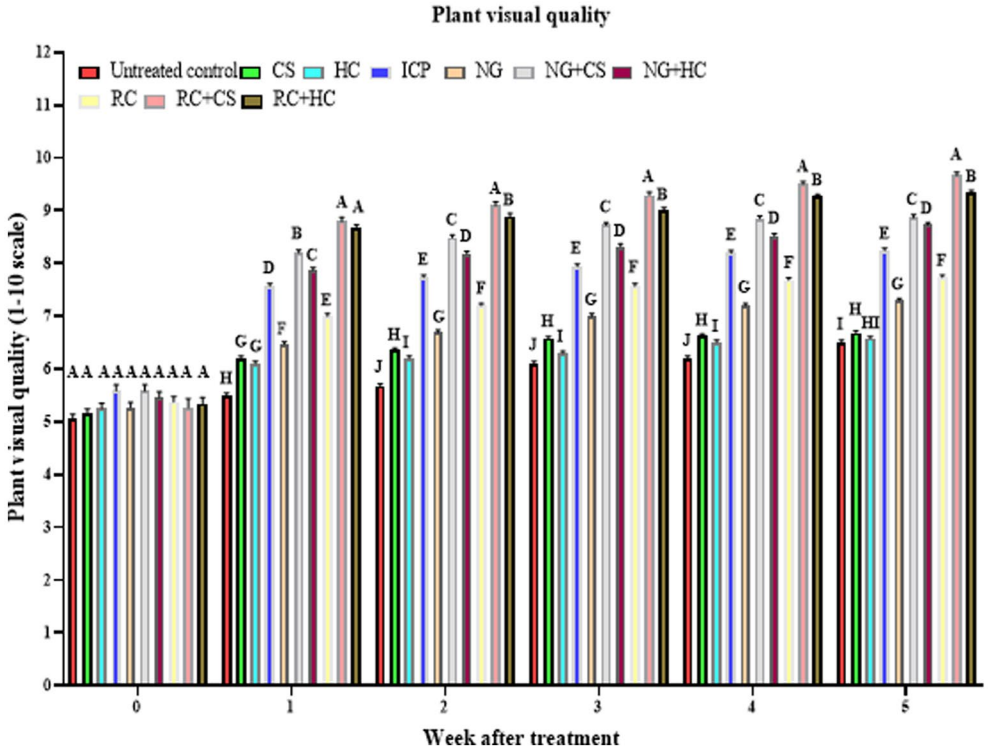

FIGURE 4
Visual quality of *Vicia faba* L. plants after spray application of *Nicotiana glauca* and *Ricinus communis*, and Imidacloprid, and release of predatory ladybirds *Coccinella septempunctata* and *Hippodamia convergens* under screenhouse conditions. CS = *C. septempunctata* alone, HC = *H. convergens* alone, NG = *N. glauca* at 10%, RC = *R. communis* at 10%, and ICP = Imidacloprid 35% at 20 mL/hL % concentration prepared with tap water. Visual quality is rated on a scale from 0 to 10, where 0 represents dead; 5 denotes fair quality with acceptable form and color, minimal chlorosis or necrosis; and 10 indicates excellent quality, healthy and robust with optimal color and form. Different letters above bars indicate statistical differences (based on the Student–Newman–Keuls test, $\alpha = 0.05$).

be constrained by factors such as reduced residual activity and slower action, which were evident in our study. Future work should focus on optimizing formulations to improve stability and efficacy, thereby enhancing their role in integrated pest management strategies.

The results of this study revealed a clear additive or synergistic effect when predatory ladybirds were combined with botanical extracts, particularly *R. communis* combined with *C. septempunctata* and *H. convergens*. These combined treatments consistently achieved the greatest reductions in both *A. fabae* eggs and motile stages, surpassing the efficacy of single treatments and the chemical insecticide imidacloprid throughout the monitoring period. The improved efficacy likely results from complementary modes of action: direct predation by ladybirds reduces aphid populations, while botanical extracts exert repellent, toxic, or anti-feeding effects on the pest (Acheuk et al., 2017; Kayange et al., 2019). These findings align with many studies demonstrating that botanical insecticides enhance biological control by reducing pest pressure with minimal impact on beneficial natural enemies. *Azadirachta indica* A. Juss. (Meliaceae) exhibits systemic action within plants, rapid environmental degradation, and low toxicity to predators (Kunbhar et al., 2018). In this study, predator abundance was higher in botanical treatments than in the chemical pesticide treatment, indicating acute safety for natural enemies. Similarly, *Melia azedarach* L. (Meliaceae) extracts did not show significant toxicity to insect pests or their natural enemies. These observations are consistent with Kraiss and Cullen (2008), who reported that azadirachtin is safer for adults of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Patel et al. (2009) documented increased activity of coccinellid beetles in mustard crops treated with neem oil formulations. Smitha and Giraddi (2006) also concluded that botanical pesticides are generally safer for predatory coccinellids. Ma et al. (2000) found that biorational pesticides, including azadirachtin and bifenthrin, were effective against *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae) while being safer for coccinellids and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), unlike synthetic chemicals which were highly toxic. Additionally, Qi et al. (2001) reported that azadirachtin at concentrations of 50–200 ppm did not affect predation rates of coccinellid beetles. Several studies have confirmed the minimal adverse effects of botanical insecticides on non-target natural enemies under laboratory and field conditions (Chaudhary et al., 2017; Kunbhar et al., 2018). These combined findings support the integration of predatory ladybirds with botanical extracts as an effective, environmentally safe approach for aphid management.

Both *C. septempunctata* and *H. convergens* demonstrated significant predation activity, leading to marked reductions in aphid populations. However, *C. septempunctata* generally exhibited higher egg and motile stage densities than HC across treatments, particularly when combined with botanical extracts, suggesting a greater reproductive potential or better adaptation under the experimental conditions. This observation is consistent with prior research indicating that *C. septempunctata* may have

higher fecundity and more robust establishment on aphid-infested plants (Hodek and Honek, 1996; Obrycki et al., 2000). Interestingly, while both predator species effectively reduced aphid populations, the differences in their population dynamics and abundance suggest potential niche differentiation or variable responses to botanical extracts. For instance, NG + CS treatments maintained higher predator densities than NG + HC, which may reflect differential sensitivity or preference to chemical cues from *N. glauca* extracts. Similarly, in RC + CS treatments, *C. septempunctata* densities were significantly higher than in RC + HC at several time points, including a highly significant difference in egg density during week 1. These consistent patterns across treatments support the superior reproductive performance and adaptability of *C. septempunctata* in the presence of botanical insecticides. Such findings have important implications for biocontrol strategies, as they suggest that *C. septempunctata* may be more effective than *H. convergens* in maintaining stable populations when combined with plant-based products. Therefore, integrating this predator with compatible botanicals can enhance the sustainability and efficacy of IPM programs. This highlights the importance of carefully selecting predator-plant extract combinations in integrated pest management (IPM) programs to maximize predator efficacy and survival (Poderoso et al., 2016; Vikas, 2024; Hyder et al., 2025).

Although imidacloprid reduced aphid densities, its effectiveness was generally lower than that of combined botanical and biological treatments, especially those based on *R. communis*. This could potentially be due to sublethal effects on predatory ladybirds or aphid resistance to neonicotinoids (Matsuura and Nakamura, 2014; Bass et al., 2015). Additionally, imidacloprid did not improve plant visual quality as much as combined treatments, highlighting the benefits of integrating biocontrol agents with botanical insecticides. These results support IPM approaches that effectively control pests while reducing environmental and non-target risks linked to synthetic chemicals (Isman, 2020).

Treatment efficacy improved over time, with the strongest aphid control and plant health observed in later weeks, especially in RC + CS and RC + HC treatments. Delaying predator release by one week likely prevented negative effects from botanical residues, supporting effective establishment. The stable predator populations indicate that the botanical extracts used were safe at applied doses, supporting their use in IPM programs (Snyder, 2019). In the present study, treated plant visual quality scores closely followed aphid suppression patterns, with combined treatments achieving the highest ratings. This demonstrates the practical effectiveness of RC + CS and RC + HC in maintaining plant health and reducing pest damage, supporting their potential for sustainable agricultural use.

While the study provides robust evidence for the efficacy of combining botanical extracts with predatory ladybirds, it did not test combinations involving both ladybird species simultaneously. Future work should explore whether synergistic interactions between *C. septempunctata* and *H. convergens* further enhance aphid suppression, as mixed predator assemblages may exploit complementary hunting strategies and reduce prey escape (Lundgren, 2009; Sethuraman and Obrycki, 2024). Additionally, field trials are necessary to validate these findings under variable environmental conditions and pest pressure. Long-term assessments of predator survival, botanical extract persistence, and potential non-target effects will be essential to optimize application protocols and ensure sustainability.

5 Conclusion

This study demonstrates that combining predatory ladybirds (*C. septempunctata* and *H. convergens*) with botanical extracts from *R. communis* and *N. glauca* significantly reduces *A. fabae* populations and improves plant health better than using either method alone or imidacloprid. Among treatments, *R. communis* combined with predators was the most effective, and *C. septempunctata* generally showed higher predator numbers than *H. convergens*. These results support using integrated biological and botanical control as a safer, more sustainable alternative to chemical pesticides. Future work should validate these findings in field conditions, test combined use of both ladybird species, and refine application methods to maximize pest control and crop protection.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ME: Writing – original draft. FK: Writing – review & editing. SR: Writing – review & editing. AC: Writing – review & editing. CA: Writing – review & editing. VB: Writing – review & editing. MS: Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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