Biological Control 201 (2025) 105701

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Efficacy of *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) and *Alternaria destruens* (Ascomycota: Pleosporaceae) for managing *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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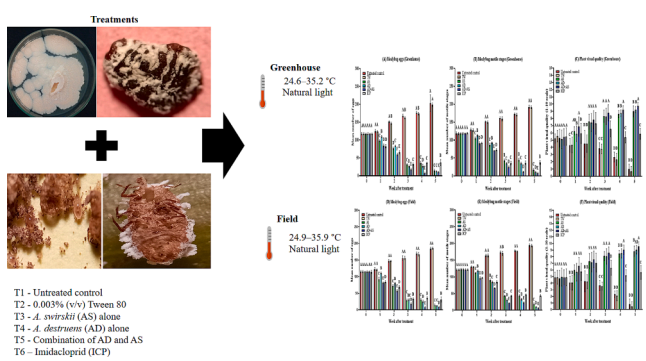
HIGHLIGHTS

- Amblyseius swirskii and Alternaria destruens reduced Phenacoccus solenopsis to 9.22 eggs/plant (field).
- Combined treatment reduced motile stages to 7.11/plant (field) by week 5 post treatment.
- A. destruens yielded 26.7 mummified mealybugs/plant (field) by week 5 post treatment.
- A. swirskii peaked at 34.8 individuals/plant (greenhouse) and 26.7 individuals/plant (field) by week 3 post treatment.
- AD + AS treatment maintained treated plants' visual quality score > 9.50 in both greenhouse and field trials.

GRAPHICAL ABSTRACT



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https://doi.org/10.1016/j.biocontrol.2025.105701
Received 8 November 2024; Received in revised form 10 January 2025; Accepted 11 January 2025
Available online 17 January 2025
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ARTICLE INFO

Keywords:
Integrated pest management
Greenhouse conditions
Mummified mealybugs
Treatment efficacy
Agricultural pests

ABSTRACT

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is a major pest of potatoes and other crops, highlighting the need for effective management strategies. This study evaluated the efficacy of a predatory mite and an entomopathogenic fungus, both individually and in combination, against *P. solenopsis* on potato plants under greenhouse (24.6–35.2 °C) and field conditions (24.9–35.9 °C). The treatments included: untreated control, Tween 80 (TW), the predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) (AS), the fungal pathogen *Alternaria destruens* (AD), AD + AS, and imidacloprid (ICP) (positive control). Treatment efficacy was evaluated weekly for five weeks, with predators released five days after fungal application. The AD + AS treatment significantly reduced *P. solenopsis* egg and motile stage counts compared to both initial levels and the individual treatments, reaching 9.88 eggs and 8.11 motile stages in greenhouse trials, and 9.22 eggs and 7.11 motile stages in field trials by week 5. The AD treatment alone caused the highest number of mummified mealybugs by week 5 (28.7 and 26.7 per plant in greenhouse and field trials, respectively), indicating strong pathogen-induced mortality. *Amblyseius swirskii* populations peaked at 34.8 and 26.7 mites per plant in the greenhouse and field, respectively, by week 3 in the AS alone treatment, with lower densities in the AD + AS treatment due to predator-fungus interactions. The AD + AS treatment effectively reduced *P. solenopsis* infestations while preserving the visual quality of treated plants (visual quality score > 9.50 by week 5), highlighting its potential for the management of *P. solenopsis*.

1. Introduction

The polyphagous insect pest Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae), native to North America (Fand and Suroshe, 2015), was first identified in Morocco in 2021 (El Aalaoui and Sbaghi, 2021) and has since been reported in over 50 countries worldwide (Fand and Suroshe, 2015). Although primarily a pest of cotton, this species can infest a more than 200 plant species across 60 botanical families, mainly in tropical and subtropical regions (Abbes et al., 2024). The pest affects vegetables, weeds, field crops, greenhouse plants, and ornamental plants, including cotton, and potato (Solanum tuberosum L.) (Chen et al., 2021; Abbes et al., 2024). In Africa, P. solenopsis has emerged as a significant pest, infesting economically important crops such as Solanum tuberosum L. (Solanaceae), Bougainvillea glabra Choisy (Nyctaginaceae), Lantana camara L. (Verbenaceae), Lycopersicon esculentum Mill. (Solanaceae), Capsicum annuum L. (Solanaceae), and Hibiscus mutabilis L. (Malvaceae) in Tunisia (Abbes et al., 2024), Opuntia spp. in Morocco (El Aalaoui and Sbaghi, 2021), and Citrus lemon (L.) Osbeck (Rutaceae) in Algeria (Aroua et al., 2020), causing substantial yield losses. This mealybug exhibits high reproductive capacity, reproducing both sexually and ovoviviparously, with females capable of laying between 200 and 600 eggs in a white, waxy sac (Abbas et al., 2010). It can produce 12 to 15 generations annually, facilitating its rapid proliferation and making control measures challenging (Arif et al., 2013).

The urgent threat posed by *P. solenopsis* to infested crop production has resulted in the widespread and often indiscriminate application of conventional insecticides for its control (Nagrare et al., 2020). This has led to the development of resistance among field populations of *P. solenopsis* to both traditional and newer insecticides (Afzal et al., 2018). Field populations of P. solenopsis demonstrated resistance to both profenofos and thiodicarb, yet they remained susceptible to imidacloprid (Shankarganesh et al., 2022). Additionally, the excessive use of these chemicals has negatively impacted the mealybug's natural enemies, contributing to its resurgence and triggering secondary pest outbreaks, along with raising environmental and human health concerns (Cloyd and Dickinson, 2006).

Biological control, utilizing predatory mites (Elhalawany et al., 2024) and entomopathogenic fungi (Gao et al., 2017), offers a viable alternative to reduce reliance on chemical insecticides within integrated pest management (IPM) strategies. The generalist predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) has been effective in managing key pests of ornamental and vegetable crops such as tomatoes, potatoes, sweet peppers, and cucumbers, including *P. solenopsis* (Calvo et al., 2015; Salwa and Helmy, 2023). This mite's ability to control *P. solenopsis* in diverse crop settings underscores its potential role in IPM programs. Furthermore, the effectiveness of entomopathogenic fungi against *P. solenopsis* has been confirmed in both controlled environment and field studies (Nagrare et al., 2011). *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), *Verticillium lecanii* (Zimm) (Hyphomycetes: Moniliales), and *Metarhizium anisopliae* (Metschn.) (Hypocreales: Clavicipitaceae) have shown efficacy against *P. solenopsis*, achieving 45–60 % mortality in laboratory settings (Nagrare et al., 2011). Additionally, *Alternaria* spp. are recognized for their entomopathogenic properties (Poitevin et al., 2018). *Alternaria alternata* (Fr.) Keissl (Pleosporales: Pleosporaceae) produces over 70 toxins, including destruxins and chitinases, which contribute to its entomopathogenic activity (Green et al., 2001). In a laboratory study, *Alternaria murispora* (PP264308) (Pleosporales: Pleosporaceae) caused 79.7 % mortality at 10^{10} conidia/mL after 14 days, while *Alternaria destruens* (PP264311) (Pleosporales: Pleosporaceae) resulted in 57.4 % mortality at the same concentration and duration, and greenhouse trials confirmed that combined treatments were more effective in reducing *P. solenopsis* population density than individual treatments (El Aaloaui et al., 2024a). These fungi infect insects by germinating on the cuticle, penetrating the body, and ultimately causing death (Vega et al., 2012).

In the present study, we evaluated the effect of combining the application of the entomopathogenic fungus *A. destruens* and the predatory mite *A. swirskii* for controlling *P. solenopsis* on potato under greenhouse and field conditions.

2. Materials and methods

2.1. P. solenopsis culture

The *Phenacoccus solenopsis* used in this study was sourced from a colony maintained on purslane (*Portulaca oleracea* L. (Portulacaceae)) at the insectarium of the National Institute of Agricultural Research (INRA) in Zemamra, Morocco (32°37′48″ N, 8°42′0″ W, Elevation 165 m). The insects were reared under controlled conditions of 25 ± 2°C, 60 ± 10 % relative humidity, and a 12:12 h L:D photoperiod. The original colony was established from mealybugs collected from an infested *P. oleracea* field in the Beni Mellal region, Morocco (32°20′ N, 6°21′ W) in 2020 (El Aalaoui and Sbaghi, 2021).

2.2. Mass-rearing of predatory mite

Different developmental stages of the predatory mite *A. swirskii* were collected from potato (*Solanum tuberosum* L. Var. Florice) fields infested with *P. solenopsis* in Zemamra, Casablanca-Settat region, Morocco (33°15′ N, 8°30′ W, elevation 165 m) during weekly field surveys in 2023. Infested potato leaves containing the mites were placed in plastic containers (11 cm length × 7 cm width × 3 cm height) and transported

to the INRA insectarium in Zemamra. The rearing process followed the method described by Ibrahim et al. (2010). *Amblyseius swirskii* was maintained on *P. solenopsis* as both a food source and oviposition support, in plastic containers (11 cm length × 7 cm width × 3 cm height) within an environmental chamber set to 25 ± 2°C, 65 ± 5 % relative humidity, and a 16:8h L:D photoperiod. Prior to release, the predatory mites were starved for 24 h. To prevent cross-contamination between treatment replicates, a minimum spacing of 2 m was maintained in the field (Fig. 1).

2.3. Fungus

The *Alternaria destruens* isolate (NCBI GenBank Acc. No: PP264311) used in this study was obtained from the INRA insectarium in Zemamra. It was originally isolated from sterilized cadavers of the *Opuntia* spp. false cochineal, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) (Moroccan biotype) and identified based on spore and colony morphology and confirmed through ITS regions sequencing (El Aalaoui et al., 2024b). Additionally, this isolate has shown effectiveness in controlling *D. opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) (El Aalaoui et al., 2024b), *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) (El Aalaoui et al., 2024c), and *P. solenopsis* (El Aalaoui et al., 2024a) in both controlled and field settings. To prepare the isolate, it was revived from potato dextrose agar (PDA) (Biokar Diagnostics, France) stored at −80 °C. The isolate was then grown on PDA in 90-mm Petri dishes for 20 days at an environmental chamber (24–29 °C) in darkness. After incubation, the conidia and mycelia were removed from the PDA using a sterile scalpel and transferred to a 50-mL sterile centrifuge tube containing 20 mL of 0.03 % (v/v) Tween 80 solution. The mixture was vortexed for 5 min to detach the conidia from the mycelia and then filtered through sterile muslin cloth to remove mycelial fragments. The concentration of conidia was measured with a hemocytometer (HGB, Germany) and adjusted to 1.0×10^8 conidia mL^{-1}, which is the recommended concentration for field applications (El Aalaoui et al., 2024b). Conidial viability was assessed before each experiment using the method described by Inglis et al. (2012), showing consistently high viability rates above 98 %. The conidial suspensions were kept at 4 °C and used within 12 h.

2.4. Insecticide

In this study, Imipower (35 % imidacloprid SC, Nanjing Red Sun Co. Ltd., China) was used as the positive control. It was applied at a rate of 0.75 cm3 per liter of tap water to cover an area of 42 m2, following the manufacturer's recommended field rate (El-Mageed et al., 2018).

2.5. Greenhouse experiments

Greenhouse experiments for managing P. solenopsis were conducted using two-month-old potato plants (Solanum tuberosum L.) grown in plastic pots (33 cm in diameter, 12 cm in height) filled with a mixture of two-thirds fine sand and one-third peat. These experiments took place from March to July in 2023 and from March to July in 2024 in a greenhouse (11 m in length, 7 m in width, and 3 m in height) at the Institute Technique Agricole, Zemamra, Morocco. The average temperature was 27.1 °C (ranging from 25.5 to 35.2 °C) during the first experiment and 26.5 °C (ranging from 24.6 to 35.0 °C) during the second experiment. Both experiments were conducted in the same greenhouse. Temperature data inside the greenhouse was recorded every hour using a thermohygrometer (Testo, Germany) placed 2 m above the ground, starting from the day of natural enemy release. Nighttime temperatures were determined by averaging the three lowest readings taken each day (Vinogradova and Reznik, 2013). The plants were watered as needed. Each plant was artificially infested with 100 adult P. solenopsis (approximately 50 females and 50 males) directly on the leaves using a fine hairbrush and allowed to establish and multiply for 21 days. At the start of each experiment, the plants were examined to confirm that they were free from cochineal and other pests.

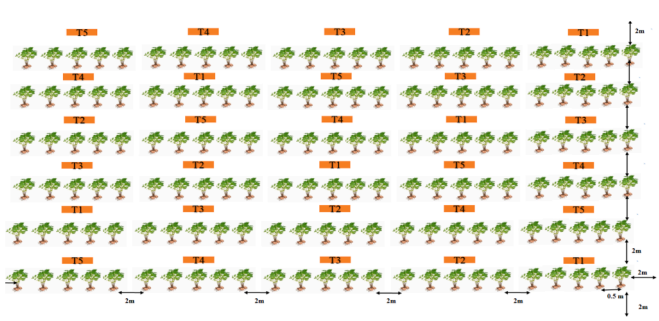

Fig. 1. Design of the potato field experiment plot.

2.6. Field experiments

Field experiments were conducted from March to July in both 2023 and 2024 on a carefully prepared 308 m^2 plot (22 m × 14 m) at the Institut Technique Agricole, Zemamra. Both experiments took place on the same plot, which has vertisol soil with an angular structure in the top 15 cm and extends to a depth of 1.5 m. This soil is difficult to work when dry but becomes easier to manage when its water content is high. It has an alkaline pH of 8.6. Potato seeds (*Solanum tuberosum* Var. Desiree), sourced from the local market, and were planted at a depth of 10 cm. Before planting, the soil's chemical composition was as follows: N (200 mg/kg), P_2O_5 (46 mg/kg), K_2O (203 mg/kg), Mo (1.5 mg/kg), and Ec (0.35). Irrigation was provided four times using a drip system: at planting, mid-growth (40 days after planting), during tuberization, and at the beginning of tuber swelling. Water requirements were estimated to be 3,000 to 4,000 L per hectare. One month before planting, the soil was thoroughly tilled, and base fertilizers were applied at 50 kg N/ha, 150 kg P_2O_5/ha, and 200 kg K_2O/ha. During mid-growth, cover fertilizers were applied at 30 kg N/ha and 30 kg K_2O/ha, with an additional 50 kg K_2O/ha applied at the start of tuber swelling. The average temperature recorded by an iMetos electronic weather station (iMetos AG/CP/DD 280, Pessl Instruments GmbH, Weiz, Austria) was 26.9 °C (ranging from 25.8 to 35.9 °C) during the first experiment and 26.5 °C (ranging from 24.9 to 35.6 °C) during the second experiment. Each experiment involved six rows of potato plants with 25 plants per row. The rows were spaced 2 m apart, and within each row, the plants were spaced 0.5 m apart. Additionally, every 5 plants within a row were spaced 2 m apart (Fig. 1). After two months of growth, each plant was artificially infested with 100 adult *P. solenopsis* of both sexes (approximately 50 females and 50 males) using a fine hairbrush to apply them directly to the leaves, and allowed to establish and multiply for 21 days. At the start of each experiment, the plants were examined to confirm that they were free from cochineal and other pests.

2.7. Treatments

In both greenhouse and field experiments, the treatments applied were as follows: T1 – untreated control, T2 – 0.003 % (v/v) Tween 80 (TW), T3 – *A. swirskii* alone (AS), T4 – *A. destruens* alone (AD), T5 – AD + AS, and T6 – imidacloprid (ICP) at 0.75 cm^3 per liter of tap water. In both the greenhouse and field experiments, ten ovipositing female mites were released per plant. Combining the two biological control agents with the chemical insecticide was not the focus of this study.

In the greenhouse experiments, *A. destruens* and imidacloprid were applied at a rate of 30 mL per plant using a 1.5 L Garden Pressure Spray Bottle (Mesto Spritzenfabrik Ernst Stockburger GmbH, Germany). To avoid cross-contamination, plants were treated outside the greenhouse and returned only after application. The treatments were arranged in a randomized complete block design with three replicates per treatment,

each replicate consisting of 10*P. solenopsis*-infested potato plants, resulting in a total of 150 plants per experiment. Predatory mites were introduced five days after fungus application to address any potential repellency effects observed in previous lab tests (El Aalaoui M, pers. obs.). Additionally, the greenhouse was partitioned into separate chambers using insect-proof netting, and treatments were randomly assigned to these chambers to prevent the movement of predatory mites among treatments. In the field, *A. destruens* and imidacloprid treatments were applied using a Matabi sprayer (Super Green 16 L; Goizper S.Coop., Gipuzkoa, Spain) at a rate of 1000 L/ha. Each treatment was applied to five plants per row (replicate), with six replicates per treatment (Fig. 1), in a randomized complete block design, resulting in a total of 30 plants per treatment and 150 plants per experiment. To minimize spray drift to neighboring rows, polythene sheets were used on the windward side to separate the rows. A 2-meter spacing between treatment plant groups was maintained to prevent the movement of predatory mites among treatments. The predatory mites were released five days after the fungal application to mitigate any potential repellency effects caused by the fungus.

2.8. Assessment of the effectiveness of treatments

In both greenhouse and field experiments, *P. solenopsis* populations were monitored two days before treatment application and then weekly for five consecutive weeks after treatment. In the greenhouse experiments, three plants per replicate were selected for each treatment. In the field experiments, two plants per row were selected for each treatment. From each plant, three leaves—one from the upper, middle, and lower sections—were collected. These leaves were placed individually in labeled paper bags and taken to the laboratory, where the mealybug density was assessed by directly counting under a dissecting microscope (Motic). The mealybug motile stages (nymphs, and adults), as well as eggs, were recorded. Additionally, the number of mummified *P. solenopsis* (both sporulated and non-sporulated) resulting from *A. destruens* infection was recorded. In both the greenhouse and field trials, the colony and spore characteristics of the fungal isolates from dead cadavers of treated insects matched those of the original culture. Each week after applying treatments, we assessed damage caused by *P. solenopsis* (=plan visual quality) using a numerical scale from 0 to 10, as detailed by Gettys et al. (2021). On this scale, 0 means the plants are dead, and 10 means they are in perfect condition. This assessment method has also been used to evaluate the effects of herbicides, salt stress, and other factors (Smith et al., 2014; Tootoonchi et al., 2020).

2.9. Predatory mite recovery

In both greenhouse and field experiments, predatory mites were sampled weekly following their introduction. Leaves that were examined for mealybug density were also checked for predatory mites under a dissecting microscope. The number of motile predatory mites and their eggs on these leaves was recorded.

2.10. Data analysis

For the statistical analyses, we assessed the density of *P. solenopsis* on three leaves from each potted potato plant—one from the upper, middle, and lower sections—since the total number of leaves per potato plant typically did not exceed 15. These specific leaves were selected due to their generally high density of *P. solenopsis*, providing a representative sample of insect infestation. Moreover, these leaves are frequently targeted by the predatory mite *A. swirskii* during its nocturnal movement from the plant apex, making them ideal for quantitatively assessing the predation effects of *A. swirskii* (Onzo et al., 2013). Data on the effects of different treatments on *P. solenopsis* and related metrics were first checked for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. In both greenhouse and field studies, we applied a single-factor analysis of variance (ANOVA) using R software version 4.3.2 to compare the effects of the natural enemy treatments and exposure time on *P. solenopsis* densities and plant damage, as well as the effect of exposure time on predatory mite density and the number of mummified mealybugs (i.e., *P. solenopsis* killed by *A. destruens*). When significant differences were detected by ANOVA, we conducted the Student-Newman-Keuls (SNK) multiple range test in R to separate the treatment means. A two-sample *t*-test was used to compare the number of mummified mealybugs between the *A. destruens* (AD) combined with *A. swirskii* (AS) treatment and the AD alone treatment, as well as to compare the predatory mite density between the AS alone and AD + AS treatments in both greenhouse and field environments. To address variance homogeneity, *P. solenopsis* count data were log-transformed ($\log_{10}(x + 1)$), and proportion data were transformed using the arcsine square root method before statistical analysis.

3. Results

3.1. 1. Efficacy of treatments on P. solenopsis eggs

In the greenhouse study, before the application of treatments, no significant difference was recorded among the treatments in egg counts ($F_{5,\ 102} = 0.476$, $P = 0.793$) (Fig. 2A). Post-treatment, significant reductions in egg counts were observed for all treatments. In week 1, AD + AS and ICP treatments showed significant reductions in egg counts ($F_{5,\ 102} = 451.0$, $P < 2.0 \times 10^{-16}$). By week 2, AD + AS treatment was the most effective (151.28 eggs/plant) ($F_{5,\ 102} = 2858.0$, $P < 2.0 \times 10^{-16}$). This trend continued in weeks 3 and 4 (Week 3 $F_{5,\ 102} = 8846.0$, $P < 2.0 \times 10^{-16}$; Week 4 $F_{5,\ 102} = 9151.0$, $P < 2.0 \times 10^{-16}$). By week 5, AD + AS, AD, and AS treatments were the most effective in reducing egg counts ($F_{5,\ 102} = 1472.0$, $P < 2.0 \times 10^{-16}$). Exposure time significantly affected mealybug egg counts ($P < 0.001$). The control group showed an increase from 117.50 to 203.72 eggs/plant by week 5 ($F_{1,\ 106} = 801.8$, $P < 2.0 \times 10^{-16}$). Similarly, TW treatment increased from 117.39 to 198.94 eggs/plant ($F_{1,\ 106} = 3260.0$, $P < 2.0 \times 10^{-16}$). In contrast, AS treatment reduced counts from 116.33 to 15.67 eggs/plant ($F_{1,\ 106} = 1513.0$, $P < 2.0 \times 10$^-16), and AD treatment decreased counts from 117.67 to 12.22 eggs/plant ($F_{1,\ 106} = 1117.0$, $P < 2.0 \times 10^{-16}$). AD + AS treatment led to a decrease from 117.17 to 8.33 eggs/plant by week 4 ($F_{1,\ 106} = 1060.0$, $P < 2.0 \times 10^{-16}$). ICP treatment reduced counts from 117.50 to 32.22 eggs/plant at week 3, but increased to 36.83 eggs/plant by week 5 ($F_{1,\ 106} = 458.0$, $P < 2.0 \times 10^{-16}$) (Fig. 2A).

In the field study, before the application of treatments, no significant difference was recorded among the treatments in *P. solenopsis* egg counts ($F_{5,\ 102} = 0.228$, $P = 0.95$) (Fig. 2D). In week 1 post-treatment, significant reductions in egg counts were observed with AD + AS and ICP treatments ($F_{5,\ 102} = 364.1$, $P < 2.0 \times 10^{-16}$). By week 2, AD + AS treatment was the most effective ($F_{5,\ 102} = 2372.0$, $P < 2.0 \times 10^{-16}$). This trend continued in weeks 3 and 4 (Week 3 $F_{5,\ 102} = 4278.0$, $P < 2.0 \times 10^{-16}$; Week 4 $F_{5,\ 102} = 6726.0$, $P < 2.0 \times 10^{-16}$). By week 5, AD + AS, AD, and AS treatments were identified as the most effective in reducing egg counts ($F_{5,\ 102} = 8704.0$, $P < 2.0 \times 10^{-16}$). Exposure time significantly affected mealybug egg counts ($P < 0.001$). The control group showed an increase from 114.67 to 184.61 eggs/plant by week 5 ($F_{1,\ 106} = 2013.0$, $P < 2.0 \times 10^{-16}$). Similarly, TW treatment increased from 114.94 to 185.50 eggs/plant ($F_{1,\ 106} = 2267.0$, $P < 2.0 \times 10^{-16}$). In contrast, AS treatment reduced counts from 114.17 to 13.83 eggs/plant ($F_{1,\ 106} = 1517.0$, $P < 2.0 \times 10^{-16}$), and AD treatment decreased counts from 114.89 to 12.17 eggs/plant ($F_{1,\ 106} = 1327.0$, $P < 2.0 \times 10^{-16}$). AD + AS treatment led to a decrease from 114.83 to 9.22 eggs/plant by week 4 ($F_{1,\ 106} = 1103.0$, $P < 2.0 \times 10^{-16}$). ICP treatment reduced counts from 114.17 to 31.61 eggs/plant at week 5 ($F_{1,\ 106} = 703.0$, $P < 2.0 \times 10^{-16}$) (Fig. 2D).

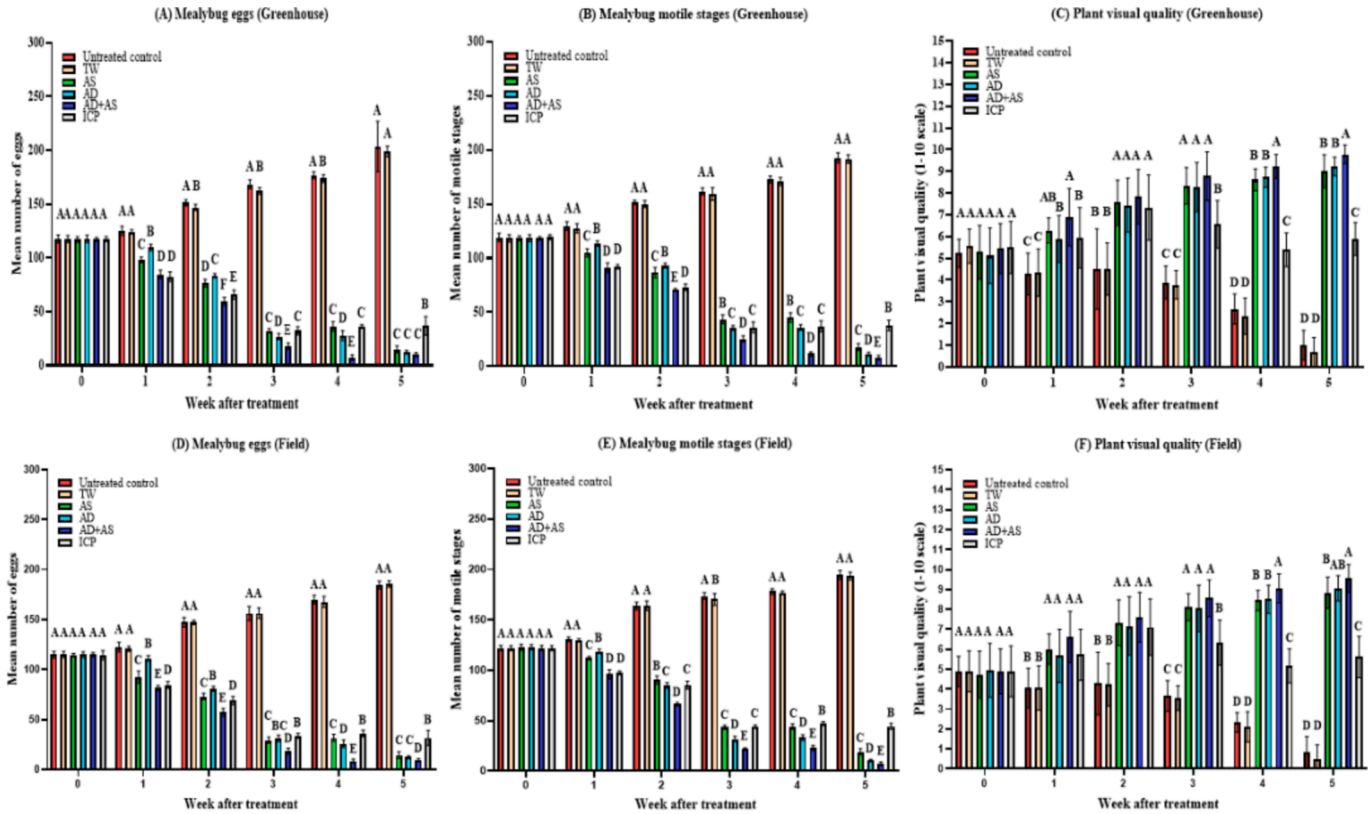

Fig. 2. Mean (± SE) active stages and egg densities of *P. solenopsis*, and treated potato plants' visual quality following spray application of *A. destruens* (AD) and imidacloprid (ICP), and release of predatory mite *A. swirskii* (AS) in the greenhouse (ABC) and field (DEF) trials. Different letters above bars indicate statistical differences (based on Student-Newman-Keuls test, α = 0.05).

3.2. Efficacy of treatments on P. solenopsis motile stages

In the greenhouse study, before the application of treatments, no significant effect was recorded on mealybug motile stages ($F_{5,\ 102}$ = 0.496, P = 0.778) (Fig. 2B). In week 1 post-treatment, the AD + AS and ICP treatments demonstrated significant reductions, while the control and TW treatments showed the highest counts ($F_{5,\ 102}$ = 393.6, P < 2.0 × 10^{-16}). By week 2, the AD, AS, ICP, and AD + AS treatments showed significant reductions ($F_{5,\ 102}$ = 2526, P < 2.0 × 10^{-16}). In weeks 3 and 4, the control and TW treatments continued to show high counts, while the AD + AS treatment demonstrated significantly lower counts (Week 3 $F_{5,\ 102}$ = 4721, P < 2.0 × 10^{-16}; Week 4 $F_{5,\ 102}$ = 7273.0, P < 2.0 × 10^{-16}). By week 5, the AD + AS, AD, and AS treatments were identified as the most effective in reducing motile stage counts ($F_{5,\ 102}$ = 11244.0, P < 2.0 × 10^{-16}). Exposure time significantly affected mealybug motile stage counts (P < 0.001). The control group showed an increase from 119.22 to 192.72 motile stages/plant by week 5 ($F_{1,\ 106}$ = 3329.0, P < 2.0 × 10^{-16}). Similarly, the TW treatment increased from 118.33 to 191.56 motile stages/plant ($F_{1,\ 106}$ = 2652.0, P < 2.0 × 10^{-16}). In contrast, the AS treatment reduced counts from 118.39 to 17.83 motile stages/plant ($F_{1,\ 106}$ = 1761.0, P < 2.0 × 10^{-16}), and the AD treatment decreased counts from 118.39 to 11.50 motile stages/plant ($F_{1,\ 106}$ = 1231.0, P < 2.0 × 10^{-16}). The AD + AS treatment led to a decrease from 118.39 to 8.11 motile stages/plant by week 5 ($F_{1,\ 106}$ = 1746.0, P < 2.0 × 10^{-16}). The ICP treatment reduced counts from 119.39 to 37.83 motile stages/plant by week 5 ($F_{1,\ 106}$ = 638.3, P < 2.0 × 10^{-16}) (Fig. 2B).

In the field study, before the application of treatments, no significant difference was recorded among the treatments in the counts of mealybug motile stages ($F_{5,\ 102}$ = 0.494, P = 0.78) (Fig. 2E). In Week 1 post-treatment, the AD + AS and ICP treatments showed significant reductions ($F_{5,\ 102}$ = 652.1, P < 2.0 × 10^{-16}). By Week 2, AD + AS treatment emerged as the most effective treatment ($F_{5,\ 102}$ = 2438, P < 2.0 × 10^{-16}). In weeks 3 and 4, the AD + AS treatment demonstrated the highest efficacy (Week 3 $F_{5,\ 102}$ = 7965, P < 2.0 × 10^{-16}; Week 4 $F_{5,\ 102}$ = 15335, P < 2.0 × 10^{-16}). By week 5, AD + AS, AD, and AS treatments were identified as the most effective in reducing mealybug motile stage counts ($F_{5,\ 102}$ = 15407, P < 2.0 × 10^{-16}). Overall, exposure time (5 weeks) significantly affected mealybug motile stage counts across all treatments (P < 0.001). The control group showed a marked increase from 122.22 motile stages/plant before treatment application to 194.61 motile stages/plant at week 5 ($F_{1,\ 106}$ = 1363, P < 2.0 × 10^{-16}). Similarly, the TW treatment showed an increased from 122.00 to 193.44 motile stages/plant ($F_{1,\ 106}$ = 1233, P < 2.0 × 10^{-16}), while the AS treatment showed a reduction from 122.78 to 19.22 motile stages/plant ($F_{1,\ 106}$ = 1758, P < 2.0 × 10^{-16}). The AD treatment showed a reduction from 123.00 to 10.33 motile stages/plant ($F_{1,\ 106}$ = 1327, P < 2.0 × 10^{-16}), and the AD + AS treatment showed a reduction from 122.00 to 7.11 motile stages/plant ($F_{1,\ 106}$ = 1476, P < 2.0 × 10^{-16}) (Fig. 2E).

3.3. Effect of treatments on treated plant visual quality

In the greenhouse study, no significant differences in visual quality were observed among treatments before application ($F_{5,\ 102}$ = 0.474, P = 0.795) (Fig. 2C). By week 1 post-treatment, significant differences were recorded ($F_{5,\ 102}$ = 16.84, P = 4.2 × 10^{-12}), with the AD + AS and AS treatments achieving the highest visual quality scores. In week 2, the significant effect ($F_{5,\ 102}$ = 23.81, P = 8.5 × 10^{-16}) indicated that AD + AS, AS, AD, and ICP treatments had the highest scores. Week 3 showed continued significant differences ($F_{5,\ 102}$ = 103.2, P < 2.0 × 10^{-16}), with AD + AS and AD treatments scoring highest. By weeks 4 and 5, the AD + AS treatment maintained its top performance with visual quality scores of 9.22 and 9.78, respectively (Week 4 $F_{5,\ 102}$ = 419.1, P < 2.0 × 10^{-16}; Week 5 $F_{5,\ 102}$ = 761.3, P < 2.0 × 10^{-16}). Overall, exposure time significantly affected plant visual quality across all treatments (P <

0.001), with the control group showing a decline from 5.22 before treatment application to 1.00 by week 5 ($F_{1, 106} = 142.7$, $P < 2.0 \times 10^{-16}$). The TW treatment similarly showed a decrease from 5.56 to 0.67 ($F_{1, 106} = 242.6$, $P < 2.0 \times 10^{-16}$), while the AS treatment showed an increase from 5.28 to 9.00 ($F_{1, 106} = 215.9$, $P < 2.0 \times 10^{-16}$), and the AD treatment showed an increase from 5.11 to 9.22 ($F_{1, 106} = 215.6$, $P < 2.0 \times 10^{-16}$). The AD + AS treatment showed a remarkable increase from 5.44 to 9.78 ($F_{1, 106} = 203.5$, $P < 2.0 \times 10^{-16}$) (Fig. 2C).

In the field study, before the application of treatments, no significant differences were recorded among the treatments in plant visual quality ($F_{5, 102} = 0.08$, $P = 0.995$) (Fig. 2F). In Weeks 1 and 2 post-treatment, the AD + AS, AS, ICP, and AD treatments recorded the highest mean quality scores (Week 1 $F_{5, 102} = 15.38$, $P < 3.0 \times 10^{-11}$; Week 2 $F_{5, 102} = 25.81$, $P < 2.0 \times 10^{-16}$). In Week 3, AD + AS, AS, and AD treatments demonstrated the highest efficacy ($F_{5, 102} = 113.7$, $P < 2.0 \times 10^{-16}$). Weeks 4 and 5 continued to highlight the AD + AS treatment as the most effective in enhancing plant visual quality (Week 4 $F_{5, 102} = 387.3$, $P < 2.0 \times 10^{-16}$; Week 5 $F_{5, 102} = 503.8$, $P < 2.0 \times 10^{-16}$). Overall, exposure time significantly affected plant visual quality across all treatments ($P < 0.001$). The control group showed a marked decline from 4.89 before treatment application to 0.83 by week 5 ($F_{1, 106} = 154.5$, $P < 2.0 \times 10^{-16}$). Similarly, the TW treatment showed a decrease from 4.89 to 0.50 ($F_{1, 106} = 195.8$, $P < 2.0 \times 10^{-16}$), while the AS treatment showed an increase from 4.72 to 8.83 ($F_{1, 106} = 237.2$, $P < 2.0 \times 10^{-16}$). The AD treatment visual quality score improved from 4.94 to 9.06 ($F_{1, 106} = 169$, $P < 2.0 \times 10^{-16}$), and the AD + AS treatment showed a substantial increase from 4.89 to 9.56 ($F_{1, 106} = 221.2$, $P < 2.0 \times 10^{-16}$) (Fig. 2F).

3.4. The effect of single and combined release of A. destruens on counts of mummified P. solenopsis

In the greenhouse study (Table 1), by week 1, there was a significant treatment effect on the number of mummies, with the AD treatment showing higher counts than the AD + AS treatment. In week 2, the treatment effect became highly significant, with the AD treatment again surpassing the AD + AS treatment. The effect was not significant in week 3. In week 4, the non-significant treatment effect continued. By week 5, there was a significant treatment effect again, with AD yielding 28.7 mummies compared to 25.7 mummies in the AD + AS treatment. Exposure time significantly affected the number of mummies across all treatments. For the AD treatment, the effect was highly significant ($F_{1, 106} = 165.3$, $P < 2.0 \times 10^{-16}$), with the number of mummies peaked at week 3 (40.1 mummies). For the AD + AS treatment, the effect was also highly significant ($F_{1, 105} = 156.2$, $P < 2.0 \times 10^{-16}$), with the highest counts occurred at week 3 (39.8 mummies).

By week 1, there was a significant treatment effect on the number of mummies under field conditions, with the AD treatment showing higher counts than the AD + AS treatment (Table 2). In week 2, the treatment effect became highly significant, with the AD treatment again surpassing the AD + AS treatment. In week 3, the effect was not significant, with the counts being similar. By week 4, a significant treatment effect re-emerged. By week 5, the AD treatment continued to show a significant effect, with 26.7 mummies compared to 21.8 mummies in the AD + AS treatment. Exposure time significantly influenced the number of mummies across all treatments. For the AD treatment, the effect was highly significant ($F_{1, 106} = 170.1$, $P < 2.0 \times 10^{-16}$), with the number of mummies peaking at week 3 (34.4 mummies). For the AD + AS treatment, the effect was also highly significant ($F_{1, 106} = 156.8$, $P < 2.0 \times 10^{-16}$), with the highest counts occurring at week 3 (32.9 mummies) (Table 2).

3.5. Variation in A. swirskii (AS) densities following A. destruens (AD) application

In the greenhouse study (Table 3), by week 1, there was a significant treatment effect on the density of the predatory mite *A. swirskii*, with the AS treatment showing higher counts than the AD + AS treatment. In week 2, the treatment effect became highly significant, with the AS treatment again surpassing the AD + AS treatment. By week 3, the effect remained highly significant, with the AS treatment maintaining a notable advantage. In week 4, the significant treatment effect continued, and by week 5, AS treatment still showed a significant difference, with 12.9 mites compared to 9.7 mites in the AD + AS treatment. Exposure time significantly influenced the density of *A. swirskii* across all treatments. For the AS treatment, the effect was highly significant ($F_{1, 106} = 25.02$, $P = 2.3 \times 10^{-6}$), with the number of predatory mites peaking at week 3 (34.8 mites). For the AD + AS treatment, the effect was also highly significant ($F_{1, 106} = 30.18$, $P = 2.8 \times 10^{-7}$), with the highest counts occurring at week 3 (22.4 mites).

In the field conditions (Table 4), by week 1, there was a significant treatment effect on *A. swirskii* density, with the AS treatment again showing higher counts than the AD + AS treatment. In week 2, the treatment effect remained highly significant, with the AS treatment continuing to outnumber the AD + AS treatment. By week 3, the effect was not significant, with similar counts observed between treatments. In week 4, a significant treatment effect was re-established, and by week 5, the AS treatment continued to show a significant difference, with 15.9 mites compared to 11.8 mites in the AD + AS treatment. Exposure time

Table 1
Impact of *A. destruens* (AD) alone, and *A. destruens* (AD) + *Amblyseius swirskii* (AS) treatments released for controlling *P. solenopsis* on the number of mummies (i.e., *P. solenopsis* killed by *A. destruens*) under greenhouse conditions.

Week after treatment	AD treatment (Mean ± SE)	AD + AS treatment (Mean ± SE)	t	df	P value	Significance
1	14.3 ± 0.7	10.7 ± 0.4	4.3	26.0	2.4×10^{-4}	***
2	27.8 ± 0.8	9.7 ± 0.7	18.2	33.4	6.4×10^{-19}	***
3	40.1 ± 0.8	39.8 ± 0.6	0.3	31.6	7.9×10^{-1}	ns
4	37.2 ± 0.7	36.3 ± 0.6	1.0	33.3	3.3×10^{-1}	ns
5	28.7 ± 0.6	25.7 ± 0.5	4.1	32.4	2.6×10^{-4}	***

The table shows the results of t-tests performed to compare the mean number of mummies (i.e., *P. solenopsis* killed by *A. destruens*) between treatments AD and AD + AS at different weeks after treatment. The significance levels are denoted as follows: *** for $p < 0.001$, and ns for non-significant p-values.

Table 2
Impact of *A. destruens* (AD) alone, and *A. destruens* (AD) + *Amblyseius swirskii* (AS) treatments released for controlling *P. solenopsis* on the number of mummies (i.e., *P. solenopsis* killed by *A. destruens*) under field conditions.

Week after treatment	AD treatment (Mean ± SE)	AD + AS treatment (Mean ± SE)	t	df	P value	Significance
1	13.6 ± 0.7	10.1 ± 0.6	3.9	32.3	5.1×10^{-4}	***
2	24.6 ± 1.0	11.3 ± 0.6	11.1	28.8	6.8×10^{-12}	***
3	34.4 ± 1.1	32.9 ± 0.9	1.1	32.3	3.0×10^{-1}	ns
4	31.0 ± 0.8	26.5 ± 0.7	4.3	33.6	1.3×10^{-4}	***
5	26.7 ± 0.8	21.8 ± 0.5	5.2	27.2	1.7×10^{-5}	***

The table shows the results of t-tests performed to compare the mean number of mummies (i.e., *P. solenopsis* killed by *A. destruens*) between treatments AD and AD + AS at different weeks after treatment. The significance levels are denoted as follows: *** for $p < 0.001$, and ns for non-significant p-values.

Table 3
Impact of *A. destruens* (AD) spray application on predatory mite *A. swirskii* (AS) density released for controlling *P. solenopsis* under greenhouse conditions.

Week after treatment	AS treatment (Mean ± SE)	AD + AS treatment (Mean ± SE)	t	df	P value	Significance
1	9.9 ± 0.4	7.4 ± 0.4	4.2	34.0	1.8×10^{-4}	***
2	30.2 ± 1.1	15.2 ± 0.6	11.6	25.5	1.0×10^{-11}	***
3	34.8 ± 0.8	22.4 ± 0.5	14.0	28.5	2.7×10^{-14}	***
4	23.9 ± 0.6	13.2 ± 0.5	13.1	33.6	8.7×10^{-15}	***
5	12.9 ± 0.6	9.7 ± 0.6	3.9	34.0	4.3×10^{-4}	***

The table shows the results of t-tests performed to compare the mean number of predatory mite *A. swirskii* between treatments AS and AD + AS at different weeks after treatment. The significance levels are denoted as follows: *** for $p < 0.001$, and ns for non-significant p-values.

Table 4
Impact of *A. destruens* (AD) spray application on predatory mite *A. swirskii* (AS) density released for controlling *P. solenopsis* under field conditions.

Week after treatment	AS treatment (Mean ± SE)	AD + AS treatment (Mean ± SE)	t	df	P value	Significance
1	7.1 ± 0.5	5.6 ± 0.4	2.3	34.0	3.0×10^{-2}	*
2	22.7 ± 0.8	18.2 ± 0.5	8.2	30.6	3.3×10^{-9}	***
3	26.7 ± 1.1	24.5 ± 1.0	1.5	33.9	1.5×10^{-1}	ns
4	22.1 ± 0.7	16.4 ± 0.9	5.1	33.2	1.5×10^{-5}	***
5	15.9 ± 0.9	11.8 ± 0.5	3.9	24.4	6.3×10^{-4}	***

The table shows the results of t-tests performed to compare the mean number of predatory mite *A. swirskii* between treatments AS and AD + AS at different weeks after treatment. 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.

significantly influenced A. swirskii density across all treatments. For the AS treatment, the effect was highly significant ($F_{1, 106} = 57.61$, $P = 1.3 \times 10^{-11}$), with the number of predatory mites peaking at week 3 (26.7 mites). For the AD + AS treatment, the effect was also highly significant ($F_{1, 106} = 47.88$, $P = 3.6 \times 10^{-10}$), with the highest counts occurring at week 3 (24.5 mites).

4. Discussion

This study shows that the AD + AS treatment significantly reduces egg and motile stages of *P. solenopsis* in greenhouse and field settings. Its effectiveness, especially in week 3, highlights the importance of prompt application for optimal results. In contrast, control and Tween 80 treatments increased pest counts, while AD and AS consistently lowered them, indicating their potential for integrated pest management. The effectiveness of *A. swirskii* (AS) in reducing *P. solenopsis* populations has also been reported in a previous study under controlled conditions in Egypt (Salwa and Helmy, 2023). Amblyseius swirskii is a vital biocontrol agent used in greenhouses across over 50 countries (Knapp et al., 2018). To reduce rearing costs, storage mites, such as *Carpoglyphus lactis* (Linnaeus) (Acari: Astigmata), are used in mass rearing of *A. swirskii*, though they can pose health risks to workers (Elshazly, 2022). Amblyseius swirskii can develop on spider mites Tetranychus urticae Koch (Acari: Tetranychidae) (Fahim and El-Saiedy, 2021), eriophyid, tenuipalpid, and tarsonemid mites (Abou-Awad et al., 2014), small insects (Medd and Greatrex, 2014), and plant pollen (Nemati et al., 2019). Predatory mites can be as effective as many insecticidal sprays (Elhalawany et al., 2024). Abou-Haidar et al. (2021) found it maintained whitefly and thrips populations below economic thresholds. Barghout et al. (2022) reported its effectiveness against Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) and Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae). Amblyseius swirskii can be released as a preventive treatment during flowering and remains effective throughout the growing season (Elhalawany et al., 2024).

In both greenhouse and field experiments, the number of mummified *P. solenopsis*—an indicator of the effectiveness of *A. destruens*—reached its highest level at three weeks for all treatments involving the pathogen. This finding implies that the fungal pathogen effectively reduces pest populations through mummification, contributing to long-term pest management (El Aalaoui et al., 2024b). This aligns with previous reports by Kaur et al. (2019) on the insecticidal properties of this fungus. Additionally, Kaur et al. (2019) found that larvae of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) fed protein fractions from *A. destruens* AKL-3 (Fr.) experienced a 31.96–53.94 % reduction in relative growth rate and a 19.24–72.93 % decrease in food consumption, negatively affecting their food conversion efficiency. The entomopathogenic effects of various *Alternaria* species, especially *A. alternata*, have also been documented against multiple pest species (Sharma and Sharma, 2014). Furthermore, both *Ulocladium* and *Alternaria* species have resulted in significant mortality rates of *D. opuntiae* nymphs, achieving mortality rates between 70 % and 90 % in laboratory conditions (Ouguas et al., 2022).

In both greenhouse and field studies, the predatory mite *Amblyseius swirskii* initially reduced egg and motile stage counts of *P. solenopsis* more effectively than *A. destruens* during the first three weeks post-treatment. However, by week three, *A. destruens* began to show greater effectiveness. This pattern is due to the behavior of hungry predatory mites, which rapidly consume prey until satiated, leading to a quick decline in their numbers, after which their feeding becomes less intense (Omkar et al., 2004). In contrast, *A. destruens* controls pest populations through fungal infections, a process that requires time for spores to contact and infect their hosts (Hajek, 2001). Although *A. swirskii* may provide quick results, its effectiveness for sustained long-term pest control may be less reliable. This highlights the importance of releasing a large number of predators for effective control. While predatory mite offer immediate but short-lived results, *A. destruens* proves to be more suitable for long-term pest management strategies. Additionally, the conditions of our experiments likely favored the effectiveness of *A. destruens*, creating optimal environments for spore transmission and host infection, thus enhancing its long-term pest management potential.

The present study demonstrates that the AD + AS combination significantly reduced pest populations, indicating its potential to disrupt reproduction and control existing infestations. Similarly, combining *A. murispora* (PP264308) with *Chilocorus bipustulatus* (Linnaeus) and *Exochomus nigripennis* (Erichson) (Coleoptera: Coccinellidae) effectively reduced *D. echinocacti* infestations on *Opuntia ficus-indica* (L.) Mill without harming treated-plants quality (El Aalaoui et al., 2024c). Additionally, integrating *Typhlodromalus aripo* De Leon (Acari: Phytoseiidae) with *Neozygites tanajoae* Delalibera (Zygomycetes: Entomophthorales) effectively controls the cassava green mite, *Mononychellus tanajoa* Bondar (Acari: Tetranychidae), but may negatively impact fungal populations (Onzo et al., 2013). Integrating different biological agents can enhance pest control, but careful management of fungal dosage and timing is essential to maintain their effectiveness and compatibility (Aqueel and Leather, 2013). Furthermore, the notable improvements in visual quality, especially within the AD + AS group, indicate that these treatments may enhance plant vigor. In line with this, Maniania et al. (2016) observed that combining *M. anisopliae* with the predatory mite *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) resulted

in significantly less tomato leaf damage while controlling *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) compared to untreated plants.

The effectiveness of *A. destruens*, indicated by the number of mummified mealybugs, depends on environmental conditions and its interaction with *A. swirskii*. The similar mummification rates between the AD and AD + AS treatments in weeks 3 and 4 in the greenhouse and in week 3 in the field suggest that *A. swirskii* may help spread *A. destruens*, improving its impact over time and providing more consistent pest control. Similarly, *Cheilomenes lunata* Fabricius (Coleoptera: Coccinellidae) has been shown to enhance the dispersal of *Metarhizium anisopliae* (Metschn.) (Hypocreales: Clavicipitaceae) conidia, increasing aphid mortality through combined predation and fungal infection (Bayissa et al., 2016).

Under greenhouse conditions, the population density of *A. swirskii* was higher when released alone compared to its combination with *A. destruens*. This suggests that *A. swirskii* may face competition from *A. destruens*, affecting its establishment and effectiveness. This aligns with research showing that some predators, like *Hippodamia convergens* Guérin-Méneville and *Adalia bipunctata* L. (Coleoptera: Coccinellidae), avoid fungus-infected prey (Pell and Vandenberg, 2002; Mohammed, 2018). However, fungal pathogens like *B. bassiana* and *M. anisopliae* are generally compatible with predators like *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), which use volatiles to detect and avoid infected prey (Rizwan et al., 2021). The significant increase in predatory mite densities observed around week 3 for both treatments in greenhouse and field conditions suggests an optimal period for *A. swirskii* activity, underscoring the importance of timing in the release of biological control agents.

Pest management strategies for *P. solenopsis* vary in effectiveness depending on the treatment method employed. The predatory mite *A. swirskii* provides rapid control through predation, while *A. destruens* offers a long-term solution via fungal infection. Combining these two biocontrol agents enhances overall control by meeting both immediate and sustained needs, thereby improving plant health. These findings highlight the complexity of integrated pest management, where the interactions among different biological control agents can significantly affect overall efficacy. Future research should focus on optimizing release strategies and timing for both *A. destruens* and *A. swirskii* in managing *P. solenopsis* populations, as well as exploring the potential synergistic effects of their combined use.

5. Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

CRediT authorship contribution statement

Mohamed El Aalaoui: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Said Rammali:** Visualization, Software. **Fatima Zahra Kamal:** Visualization, Formal analysis. **Alin Ciobică:** Visualization, Validation, Investigation, Formal analysis. **Bouchaib Bencharki:** Visualization, Validation, Investigation, Formal analysis. **Abdellatif Rahim:** Visualization, Software. **Luminita Diana Hritcu:** Visualization, Validation, Investigation, Formal analysis. **Laura Romila:** Validation, Software. **Vasile Burlui:** Visualization, Validation, Investigation, Formal analysis. **Mohamed Sbaghi:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization.

Funding

The research of this study was supported by the National Institute of Agricultural Research (INRA), Morocco.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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