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RESEARCH ARTICLE

Development of bioinsecticide based on *Streptomyces griseoflavus* PAL114 for control of black bean aphids *Aphis fabae*

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ABSTRACT

This research highlights the efficiency of a bioinsecticide based on *Streptomyces griseoflavus* PAL114 in controlling black bean aphids and its effect on fava beans. Three actinobacterial strains were tested *in vitro* for their aphicidal activity. The PAL114 strain was then formulated in talc powder and tested again. Formulation processes were performed with four spore suspension densities (10^2, 10^4, 10^6 and 10^8 spore ml^{-1}) at a rate of 25 ml per 100 g of talc powder. Furthermore, the *in vivo* effect of bioinsecticide on fava bean plants was studied in pot experiments using two application methods: spray and spray + soil amendment. The results showed that only the PAL114 strain had a highly significant effect on mortality ($p = .001$). Their high-density formulations at 10^6 and 10^8 spore ml^{-1} induced very highly significant activity ($p < .001$) for both, with no influence difference exceeding 90% after 58 h. In contrast, the other low-density formulations at 10^2 and 10^4 spore ml^{-1} had no significant effect ($p = .322$ and $p = 1.000$, respectively). There was no adverse effect of the bioinsecticide on fava bean; instead, there was an improvement in growth, especially when the spray application was combined with soil amendment. The present study opens up prospects for field studies on the biocontrol of this crop pest.

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Introduction

The black aphid *Aphis fabae* is one of the most damaging insect pests of fava bean crops, posing a serious threat worldwide and particularly in Algeria (Bennour et al., 2021; Bouabida et al., 2020). It causes major economic losses, with yield losses of up to 50% in addition to insecticide treatment costs (Dedryver et al., 2010; Hansen et al., 2008). This polyphagous insect threatens more than 200 species of host plants other than fava bean (Shannag & Ababneh, 2007). Crop damage is induced directly by phloem feeding, sucking sap and honeydew excretion, or by

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deformation of plant organs and gall formation. In contrast, indirect damage is due to the transmission of over 30 plant viruses (Bennour et al., 2021; Dedryver et al., 2010). Control methods against this pest are mainly chemical, using a variety of insecticides such as pyrethroid, neonicotinoids, indoxacarb and imidacloprid. However, these techniques have limitations linked to treatment costs, development of insect resistance to their active compounds, effectiveness against direct damage but not indirect damage, and risks to human health and the environment by targeting other organisms such as pollinators and natural enemies of aphids (Bennour et al., 2021; Dedryver et al., 2010; Stoddard et al., 2010). Other cultural strategies, such as site selection, crop rotation, row spacing, weed control and stubble retention, can reduce black aphid populations but not eradicate them (Stoddard et al., 2010). Biological alternatives therefore represent an ecofriendly, low cost and safer approach that offers high action levels to control black aphid populations (Bennour et al., 2021; Dedryver et al., 2010). They involve the use of natural enemies and microorganisms displaying interesting antagonistic activities, of which actinobacteria are well known.

Actinobacteria are one of the most dominant microbial communities in soil. They are highly valuable prokaryotes that play an important role in protecting crops and maintaining the sustainability of agriculture and the environment (Aggarwal et al., 2016; Devi et al., 2022; Shivlata & Satyanarayana, 2017). Used as bioformulations, these bacteria enhance soil fertility and promote plant growth through nitrogen fixation, nutrient availability, phytohormone production and organic matter decomposition (Mitra et al., 2022). They are also used as effective biocontrol agents against a multitude of fungal diseases; due to their antagonistic and resistance-inducing potential in plants (Aggarwal et al., 2016; Devi & Manhas, 2023; Silva G da et al., 2022). The insecticidal activity of actinobacteria has been reported in several studies, especially the *Streptomyces* genus (Atif et al., 2023; Boykova et al., 2023; Devi et al., 2023). In fact, their production of insecticide metabolites in addition to extracellular enzymes is at the origin of their insecticidal activity. These unique properties led us to choose this research topic by exploiting the antagonistic potential of native actinobacterial strains originating from the Algerian arid zones.

In the context of microbial control of *Aphis fabae* black aphid pest, most subsequent research has focused on entomopathogenic fungi (Bensaci et al., 2015; Boni et al., 2021; Mousavi et al., 2022; Saruhan, 2018). However, actinobacteria are rarely studied for this type of pest, and this is what characterises this research, which will focus for the first time on the ability of *Streptomyces griseoflavus* strain PAL114 to control black aphids (Benbelkhir et al., 2023). The objective of the study is to demonstrate the potential of a bioinsecticide formulated from this strain against *Aphis fabae*, and to evaluate its effect on fava bean plants. We aim to develop an eco-friendly bioinsecticide that is cost-effective, easy to produce, and apply. The formulation process should result in a powdery product that can be dissolved in water for spraying. In this case, talc powder has been chosen as the carrier for the actinobacterial spores.

Materials and methods

Actinobacterial strains

Three strains of actinobacteria, namely *Streptomyces rochei* PT2, *Nocardiopsis dassonvillei* B22, and *Streptomyces griseoflavus* PAL114 were exploited for their ability to control

Table 1. Actinobacterial strains and their origin of isolation.

Strain	Isolation	References
PAL114 *Streptomyces griseoflavus*	Soil from Beni Isguen, Ghardaïa, Algeria	(Aouiche et al., 2013)
B22 *Nocardiopsis dassonvillei*	Soil from palm grove in Ghardaïa, Algeria	(Mokrane et al. 2013)
PT2 *Streptomyces rochei*	Roots of *Panicum turgidum* from Hassi R'Mel, Laghouat, Algeria	(Goudjal et al. 2013)

black aphids. These strains are part of the collection of the LBSM research laboratory (Laboratoire de Biologie des Systèmes Microbiens, ENS-Kouba, Algiers, Algeria). The 16S rRNA sequences for PT2 and B22 are deposited in Genbank under accession numbers KC414013 and KJ470134, respectively, and the whole genome shotgun sequence for PAL114 is deposited under accession number JAWDJQ010000951. The latter is the subject of a whole-genome sequencing project currently being conducted by Tata S and Belaouni H (2023).

The strains PAL114 and B22 are soil bacteria isolated from different desert zones, while PT2 is an endophyte isolated from the roots of the native desert plant *Panicum turgidum* (Table 1). They were selected on the basis of their strong chitinolytic activities, for which chitin is one of the major components of insect exoskeleton. These activities have been previously demonstrated by our research team's studies (Allali et al., 2019; Aouiche et al., 2013; Zamoum et al., 2017).

Black aphid

Adult black aphids were collected in March 2023 from an infested fava bean field located in Laghouat city (33,86288° N, 2,87350° E), Algeria (Fig. S1). The sampling consisted of collecting plant parts containing black aphid colonies, placing them in rearing cages and transferring them to the laboratory for immediate testing. They were identified as *Aphis fabae* according to the key of Martin (1983).

Spore suspensions

Preparation of the actinobacterial spore suspensions was carried out using a modified method of Zamoum et al. (2017). Actinobacteria were cultivated on ISP2 medium and incubated at 28°C for 10 days until sporulation. Spores were then recovered with a Tween-80 (0.05% v/v) solution and adjusted to $\approx 10^6$ spore ml^{-1} using a Malassez counting cell.

Screening for insecticidal activity

For the *in vitro* insecticidal activity, a ventilated chamber simulation was adopted as used by Bensaci et al. (2015) with a few modifications. Petri plates were transformed into ventilated chambers by perforating their lids and covering them with muslin; and then lining them with moistened paper to ensure humidity. Detached fava bean leaves were placed on paper to serve as carriers and feeds for black aphids. They were covered at their excision points with sterile cotton soaked in a nourishing solution to prevent senescence (Table S1). The black aphids were then detached from their host plant as described

above using a fine natural bristle brush and transferred to bioassay plates (Bensaci et al., 2015, 2022; Karthiba et al., 2010).

Black aphid treatment consisted of spraying separately actinobacteria spore suspensions previously prepared at a rate of 5 ml per treatment. The activity of the three strains PAL114, PT2 and B22 was tested with a negative control in which sterile distilled water was applied instead of spore suspension. For each treatment, 6 replicates were performed with 10 black aphids per plate. The plates were incubated at 25°C (12 h photoperiod), and a second spray was applied in the same way after 34 h. Mortality rate was measured after the first 10 h and then every 24 h up to 58 h (Bensaci et al., 2022). The experiment was conducted three times in order to maintain reproducibility.

Enzymatic activity

After analysing the results of screening for insecticidal activity, the strain PAL114 was selected to proceed with the subsequent study steps. To elucidate the mode of action of PAL114 against black aphids, its enzymatic activity was explored. The exoskeleton composition of black aphids is predominantly chitinic and proteic, so while the chitinase activity of this strain has already been proven as mentioned above, this test was only concerned with its protease activity. It was assessed by PAL114 culture on skim milk-based medium according to the method of Wei et al. (2010). Fifty millilitres of skim milk and fifty millilitres of 4% water agar were sterilised separately and then mixed in sterile conditions at 55°C. The strain was then cultured on medium plates and incubated at 28°C for 5 days. The presence of a transparent halo surrounding the colony revealed protease activity where halo diameter was calculated as an average of three replicates.

Bioinsecticide formulation

The strain PAL114 was formulated in talc powder at four spore suspension densities, $\approx 10^2$, $\approx 10^4$, $\approx 10^6$ and $\approx 10^8$ spore ml^{-1}. For each formulation, a mixture of 100 g of talc powder and 1.5 g of $CaCO_3$ was autoclaved at 121°C for 30 min (El_Komy et al., 2020; Nandakumar et al., 2001). To this mixture, 25 ml of spore suspension was inoculated under sterile conditions and thoroughly mixed to ensure good spore dispersion (Zamoum et al., 2017). The mixture was then dried under a flow laminar hood for 2 days. The produced formulations were then stored in sterile plastic boxes at 6°C for subsequent tests.

Spore viability and bioinsecticide purity

The viability of spores from different PAL114 formulations was checked by culturing on ISP2 plates. For this, a series of decimal dilutions was performed after preparing the initial suspension by dissolving 0.2 g of formulation powder in 4 ml of sterile distilled water. A 0.1 ml inoculum was seeded onto ISP2 plates and incubated at 28°C for 5 days. The density of viable spores was determined using the plate count method with three replicates for each formulation. Culture plates were checked for any contaminants that could affect formulation purity (Zamoum et al., 2022).

Biocontrol assays

The bioinsecticides based on PAL114 spores were applied by spray using hand-held sprayers. The spray suspensions were prepared from formulation powder at a concentration of 50 g l^{-1} sterile distilled water (Zamoum et al., 2022).

In vitro biocontrol trials were carried out in the same way as the screening test for insecticidal activity. Five treatments were performed: bioinsecticide at a density of $\approx 10^2$ spore ml^{-1}, $\approx 10^4$ spore ml^{-1}, $\approx 10^6$ spore ml^{-1}, $\approx 10^8$ spore ml^{-1} and negative control. The density indicated in this case was that of the sporal suspension with which the bioinsecticide was formulated; and not the density of viable spores estimated after formulation. The assay conduct, number of black aphids and replicates, volume and number of sprays in addition to incubation conditions were all the same as the screening test described above, except for mortality measurement, which was carried out after the first 10 h and then every 24 h up to 178 h. To ensure the reliability of the results, the trials were replicated three times.

Effect of bioinsecticide on Vicia faba bean

To assess the safe use of our bioinsecticide based on PAL114 spores, their *in vivo* effects on fava beans have been studied in greenhouse pot experiments. The semi-early local variety *Aguadulce* was chosen because it is the most widely grown in Algeria, and has high productivity (9 seeds per pod). Analysis of biocontrol assay results led to the selection of the bioinsecticide at $\approx 10^6$ spore ml^{-1} density for this study.

The experimental design was a randomised block design that tested two methods of bioinsecticide application with a negative control. Each method of application consisted of five replications of the experimental unit, where the unit was represented by a single pot. The pot was rectangular, measuring 13 cm in diameter and 5.5 cm in height. It was filled with 200 g of soil and sown with four seeds (Boukaya, 2020; Mbazia et al., 2016).

The soil used in these experiments was a mixture of 1/3 clay soil collected from Dhaya (33,77906° N, 2,87477° E) and 2/3 sandy soil collected from Nebket Bendjeddou (33,78874° N, 2,87052° E) in Laghouat city, Algeria. It was treated by drying, sieving, homogenising and then sterilising two times by autoclaving at 121°C for 20 min with an interval of 24 h (Noumavo et al., 2015). Fava bean seeds were disinfected superficially by soaking in 1% NaClO solution for 10 min, and then rinsed 3 times with sterile distilled water (Taffa et al., 2013).

The bioassays comprised two treatments with a negative control. The first treatment Spray consisted of sowing disinfected seeds in the soil and placing the pots in a growth chamber at 25°C with a photoperiod of 12 h with daily watering with 20 ml tap water. After 14 days, the plantlets were sprayed by hand sprayers with 20 ml of bioinsecticide suspension (50 g l^{-1} sterile distilled water) in each pot. Treated plantlets were then covered with plastic bags for 24 h to ensure actinobacterial spore development (Taffa et al., 2013; Zamoum et al., 2017). In the second treatment, Spray + soil amendment, the soil was first mixed with bioinsecticide powder at a rate of 20 g kg^{-1} soil (Novinscak & Filion, 2020). Subsequent sowing and spraying steps were carried out in the same way and under the same conditions as the first treatment. The negative control was also performed using the same procedure and conditions as the

treatments, except that the soil was not mixed with bioinsecticide and the spray was applied with sterile distilled water instead of bioinsecticide suspension. The germination rate, fresh and dry weight, and plant and root length were evaluated after 15 days of spraying (Saber et al., 2009).

Statistical analyses

Data were analysed using IBM Statistics software version 22.0, in addition to ARTool.Exe version 2.1.2 for Windows. Microsoft Excel version 16.0 was also used to create the files needed by ARTool and for graphical presentation of the results.

To study different treatment effects, ANOVA was performed, followed by Tukey's and Dunnett's (2-sided) post hoc comparisons. The $p < .05$ value was considered statistically significant. The ANOVA conditions of homoscedasticity and normality were first verified by Levene's and Shapiro Wilk tests, respectively.

The data from the first part of this study on PAL114, PT2 and B22 insecticidal activity met ANOVA conditions, so they were subjected to multifactorial ANOVA with time and actinobacterial treatment as factors; and different periods and bacteria as levels. Similarly, the conditions were met by the data from the third part of bioinsecticide effect on fava beans, and they were analysed by one-factor ANOVA, where bioinsecticide treatment presented the factor and different application methods presented the levels.

Unlike the others, data from the second part of biocontrol assays on black aphids did not meet ANOVA conditions. They showed heteroscedasticity with an abnormal distribution, which needed a nonparametric multifactorial analysis. According to Mangiafico (2016) and Wobbrock et al. (2022), the ART Aligned Rank Transform approach is the best tool for this case (Fig. S2.) The data therefore underwent an aligned rank transformation step using ARTool for Windows, followed by multifactorial ANOVA using SPSS. The ANOVA in this case was performed individually for each factor (bioinsecticide density, time and interaction). For post hoc comparisons in this approach, the p-value must undergo Holm's sequential Bonferroni correction, which yielded a $p < .005$ value considered statistically significant (Elkin et al., 2021).

Results

Screening for insecticidal activity

Actinobacterial treatment had a highly significant effect on black aphid mortality ($p = .003$). Similarly, time had a very highly significant effect ($p < .001$), while the interaction between the factors had no significant effect ($p = .762$) (see Table S2).

The results of the three bacterial activities are shown in Figure 1. The strain PAL114 increased mortality rate in a highly significant manner compared to the negative control (Dunnett's test $p = .001$). The recorded rate in the first 10 h was (34%), more than double that of the control (13%). The rise in mortality in this treatment was therefore clearly remarkable from the first 10 h (Figure 1(a)). Likewise, it reached a very high percentage (91%) at the 58th hour.

In contrast to PAL114, treatment with PT2 and B22 did not significantly influence the mortality rate compared to the negative control (Dunnett's test $p = .294$ and $p = .223$,

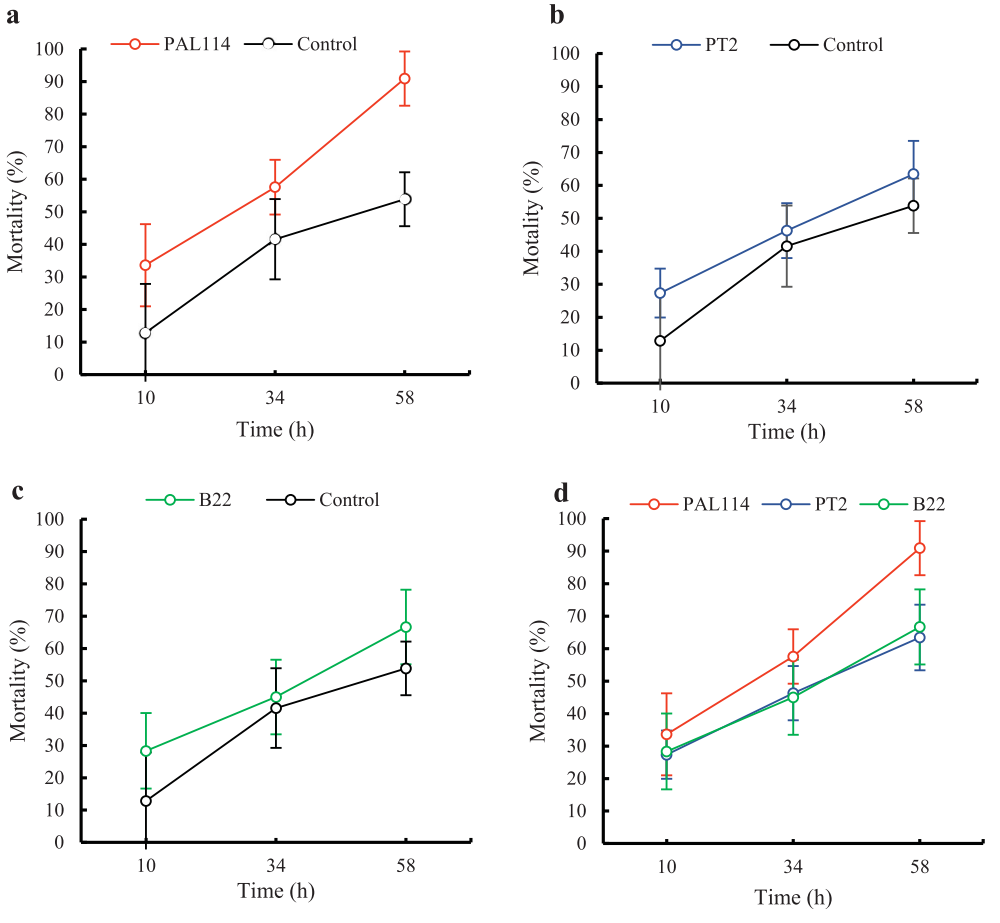

Figure. 1. Mortality of *Aphis fabae* induced by actinobacterial strains. a. *Streptomyces griseoflavus* PAL114. b. *Streptomyces rochei* PT2. c. *Nocardiopsis dassonvillei* B22. d. Comparison between the three strains. Error bars present the standard deviation of 6 replicate means.

respectively). Although the mortality recorded after 10 h of PT2 (27%) and B22 (28%) treatment was more than double that of the control, its progress was not considerable after 34 h and even after the second spray (Figure 1(b,c)). A comparison of the three actinobacteria revealed that the mortality caused by PT2 and B22 was very close (approximately the same), while that caused by PAL114 was clearly different and higher than that of the negative control (Figure 1(d)). The appearance of *Aphis fabae* mortality induced by PAL114, PT2 and B22 strains is illustrated in Figure 2(a, b and c) respectively.

Enzymatic activity

The protease activity results are shown in Figure 3. After 5 days of incubation, strain PAL114 formed a clearly visible transparent halo with a large diameter estimated at (24.25 ± 0.35 mm) (Figure 3(a)). This confirms the existence of potent proteolysis due to proteases. The bacterial growth in skim milk-based medium is shown in Figure 3(b,c), where the aspect is illustrated after 4 days of incubation. It forms a

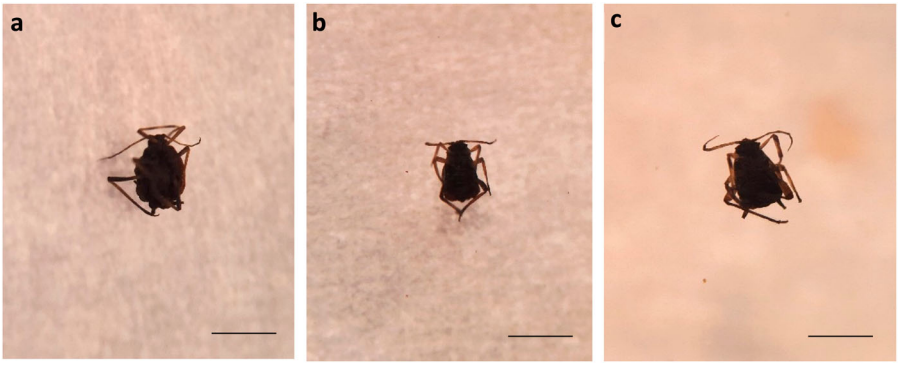

Figure. 2. Appearance of dead *Aphis fabae* adults after treatment with actinobacterial strains. a. *Streptomyces griseoflavus* PAL114. b. *Streptomyces rochei* PT2. c. *Nocardiopsis dassonvillei* B22. Bars present 1000 µm.

grayish-white aerial mycelium with yellowish stranded substrate mycelium, which is similar to their aspect on ISP2 but without transparent halos surrounding the colonies, as in this case.

Spore viability and bioinsecticide purity

The purity of PAL114 formulations was checked before the biocontrol assays. The colonies formed on ISP2 plates had a unique, homogeneous appearance, with no other contaminants having a different appearance. The viable spore density for formulations at $\approx 10^2$, $\approx 10^4$, $\approx 10^6$ and $\approx 10^8$ spore ml^{-1} was estimated at $\approx 4 \times 10^2$ CFU g^{-1}, $\approx 1.80 \times 10^4$ CFU g^{-1}, $\approx 1.12 \times 10^6$ CFU g^{-1} and $\approx 8.06 \times 10^5$ CFU g^{-1} respectively.

Biocontrol assays

Analysis of biocontrol assays revealed that both bioinsecticide density and time had a very highly significant effect on the mortality rate ($p < .001$) for both. Moreover, the

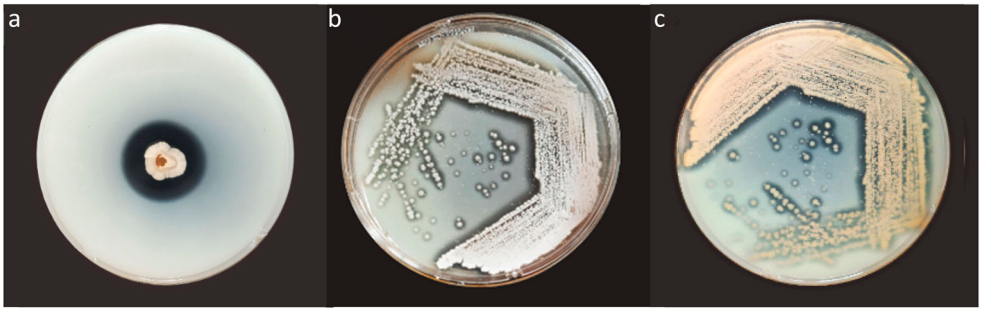

Figure. 3. Protease activity of *Streptomyces griseoflavus* PAL114 on skim milk-based medium. a. Formation of transparent halos surrounding the bacterial disc after 5 days of incubation at 28°C. b. Macroscopic aspect of aerial mycelium on skim milk agar after 4 days of incubation at 28° C. c. Macroscopic aspect of substrate mycelium on skim milk agar after 4 days of incubation at 28°C.

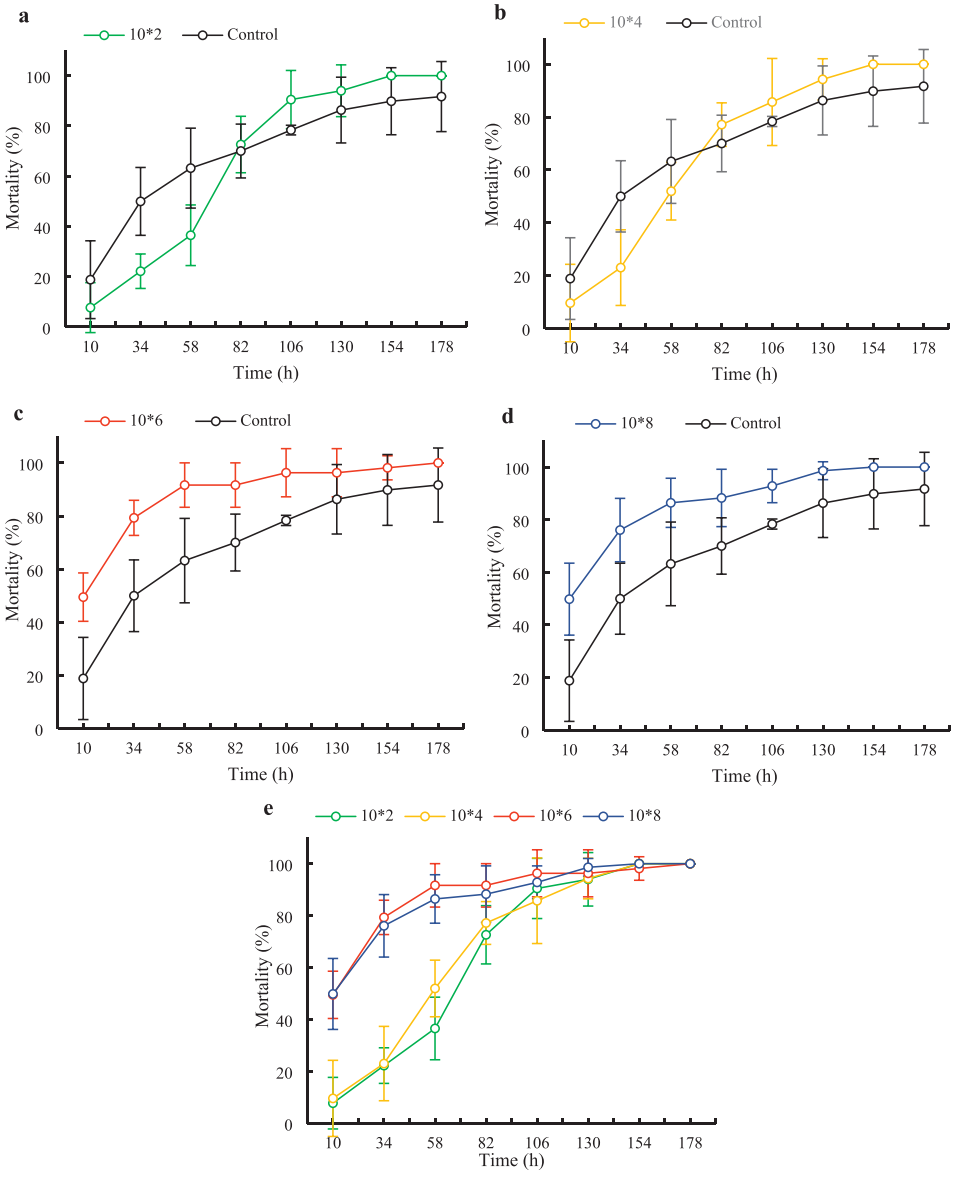

Figure. 4. Evolution of *Aphis fabae* mortality after treatment with a bioinsecticide formulated from *Streptomyces griseoflavus* PAL114 at different spore densities. a. density of $\approx 10^2$ spore ml^{-1}. b. density of $\approx 10^4$ spore ml^{-1}. c. density of $\approx 10^6$ spore ml^{-1}. d. density of $\approx 10^8$ spore ml^{-1}. e. Comparison between the four bioinsecticides. Error bars present the standard deviation of 6 replicate means.

interaction between the two factors also had a very highly significant effect ($p < .001$) (Table S3 a b c).

The evolution of mortality over time is shown in Figure 4. Treatment of black aphids with bioinsecticides at low densities of $\approx 10^2$ and $\approx 10^4$ spore ml^{-1} did not have a significant effect compared to the control, whose p-value was (Dunnett's test $p = .322$ and $p =$

1.000) respectively. In fact, the mortality induced by both bioformulations was lower than that of the control from the first 10 h of treatment until 58 h and even after the second spray. Thereafter, there was a gradual increase in mortality due to the interaction effect with time, but still close to that of the control (Figure 4(a,b)).

In contrast to the others, the bioinsecticides at high densities $\approx 10^6$ and $\approx 10^8$ spore ml^{-1} caused a very highly significant increase in the mortality rate compared to the control with a p-value of (Dunnett's test $p < .001$) for both. The rate started from the first 10 h by (49%) and (50%) for $\approx 10^6$ and $\approx 10^8$ respectively, which was more than double that of the control (19%). Mortality progression in both treatments is still high compared to the control from 10 h up to 106 h; after this period, control mortality begins to slightly approach that of the densities (Figure 4(c,d)).

A comparison between the four bioinsecticides showed that there is no significant difference either between the one at low densities $\approx 10^2$ and $\approx 10^4$ spore ml^{-1} (Tukey test $p = .598$) nor between the one at high densities $\approx 10^6$ and $\approx 10^8$ spore ml^{-1} (Tukey test $p = .967$). However, a very highly significant difference was found between the two types of formulations at high and low densities, with a p-value (Tukey test $p < .001$). Indeed, this can be clearly seen in the graph in Figure 4(e). Consequently, the bioinsecticide formulated at $\approx 10^6$ spore ml^{-1} was selected for testing on fava beans because its activity is almost the same as that formulated at $\approx 10^8$ spore ml^{-1} and it is practically the easiest to obtain and adjust.

Effect of bioinsecticide on Vicia faba *bean*

As shown in Figures 5 and 6 and in Table S4, the treatment of fava beans with bioinsecticide based on PAL114 spores increased the germination rate to (100%), compared with (95%) for the control (Figure 5(a)). On the other hand, the fresh and dry weights of treated plantlets did not differ significantly from those of the control, with ($p = .114$) for fresh weight and ($p = .090$) for dry weight (Figure 5(b,c)). Unlike weight, plant and root length were significantly influenced by bioinsecticide application, with p values of ($p = .012$) and ($p = .019$), respectively. As shown in Figure 6(a) and Figure 5(d,e), Spray resulted in a plant length of (46.63 ± 6.30 cm), which did not significantly differ from that of the control (47.15 ± 6.74 cm), but it significantly increased the root length to (14.73 ± 3.01 cm) compared to that of the control (12.09 ± 3.02 cm). Furthermore, the Spray + soil amendment still significantly increased plant and root lengths to (52.75 ± 7.58 cm and 14.51 ± 2.72 cm), respectively (Figure 6(b) and Figure 5(e)). The two treatment methods differed in plant length but not in root length, as can be observed in Figure 6(c) and Figure 5(d,e).

Discussion

Among the three strains screened for insecticidal activity, PAL114 was the only one that showed a highly significant effect compared to the control. It exhibited good activity against black aphids, achieving a mortality rate of (91%) after 58 h. Therefore, this strain was selected for formulation and subsequent trials. In our screening test, the actinobacteria were applied directly in the form of spores to elucidate their development and antagonistic capacity against insects. This method shares the same principle of application as that of Xu and Feng (2002), who studied the effect of fungus *Pandora delphacis* spores

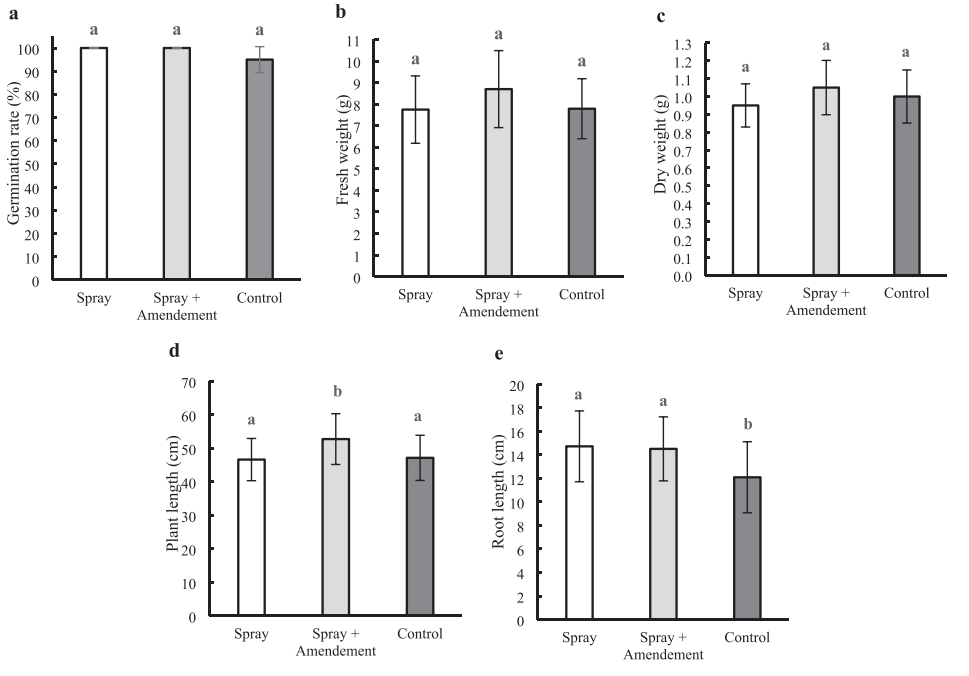

Figure. 5. *In vivo* bioinsecticide effect on fava beans after 30 days under greenhouse conditions. a. Germination rate. b. Fresh weight. c. Dry weight. d. Plant length. e. Root length. Each bar shows the average of 20 plants per treatment. Bars labelled with the same letter do not differ significantly at ($p<.05$) according to Tukey's test. Error bars present the standard deviation of 5 replicate means.

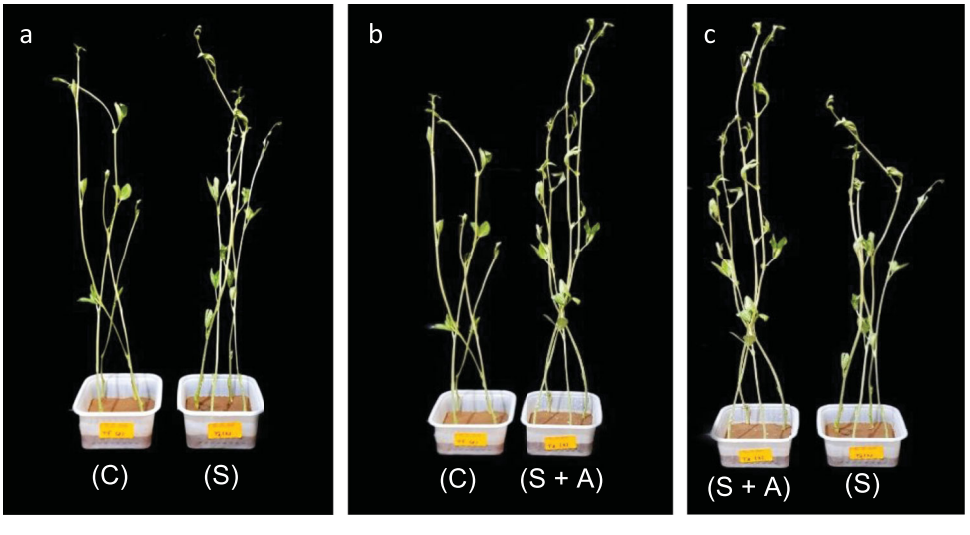

Figure. 6. *In vivo* effect of bioinsecticide application methods on plant length in pot experiments. a. Control (C) vs. Spray treatment (S). b. Control (C) vs. Spray + soil amendment treatment (S + A). c. Spray + soil amendment treatment (S + A) vs. Spray treatment (S).

against *Myzus persicae*. Most of the trials carried out on the *Streptomyces* genus for insecticidal activity have tested the activity of metabolic extracts against various insect species of agricultural and medical importance (Amelia-Yap et al., 2023; Devi et al., 2023). Some others, such as Samri et al. (2017), have used this bacteria as broth culture.

Basically, the modes of action of microbial agents against insects can be elucidated through the biochemical composition of their exoskeleton and the ability of microorganisms to degrade one or more of its compounds. The insect exoskeleton that forms a protective barrier is mainly composed of proteins and chitin. The latter are therefore the target of chitinase and protease enzymes possessed by microorganisms. The activities of these enzymes have been widely studied in researches on entomopathogenic fungi and bacteria (Bahar et al., 2012; Bensaci et al., 2015). Indeed, actinobacteria in general and *Streptomyces* in particular are known to produce a multitude of lytic enzymes, including chitinase and protease (Rashad et al., 2017; Rifaat et al., 2006). In our study, we also showed that the S. *griseoflavus* strain PAL114 produces both enzymes, which can explain its insecticidal activity against black aphids. This same species has previously been found to produce protease in the study of Hosseini et al. (2016) and chitinase in the study of Tang-um and Niamsup (2012).

After formulation, the viable spore density calculated for the bioinsecticide produced at $\approx 10^2$, $\approx 10^4$ and $\approx 10^6$ spore ml^{-1} remained $\approx 4 \times 10^2$ CFU g^{-1}, $\approx 1.80 \times 10^4$ CFU g^{-1}, $\approx 1.12 \times 10^6$ CFU g^{-1}, respectively; while that produced at $\approx 10^8$ spore ml^{-1} decreased to $\approx 8.06 \times 10^5$ CFU g^{-1}. In fact, the phenomenon of a decrease in viable spore numbers is known in the bioformulation processes of biocontrol agents and fertilisers, whether for talc or other carriers (Fatmawati et al., 2023; Meftah Kadmiri et al., 2021; Wong et al., 2019). Our results corroborate those of numerous comparative studies on different bioformulation carriers. Talc powder was found to be a very good carrier with more stable viability and higher efficacy for the *Streptomyces* genus (Fatmawati et al., 2023; Zamoum et al., 2022), and for other bacterial genera (Novinscak & Filion, 2020; Singh et al., 2020).

Among the bioinsecticides formulated with different densities of PAL114 spores, the one formulated at density of $\approx 10^6$ spore ml^{-1} showed the most promising results. It showed highly significant activity compared to the control and had a similar effect to the bioinsecticide formulated with $\approx 10^8$ spore ml^{-1}. In contrast, formulations with lower densities of $\approx 10^2$ and $\approx 10^4$ spore ml^{-1} had no effect. These results demonstrate the effectiveness of the $\approx 10^6$ spore ml^{-1} density and its stability for the bioformulation process of PAL114 strain. Additionally, the bioinsecticide-induced mortality rates similar to those induced by the spore suspension of the same density initially tested in the screening. This confirms that talc did not affect the insecticidal efficacy of PAL114, hence the interest in using it as an actinobacterial spore carrier.

In comparison with other trials for microbial control of black aphids, the bioinsecticide based on PAL114 spores showed interesting activity. It gave a very high mortality compared with that given by liquid bioinsecticide based on *Cladosporum oxysporum* despite being applied in a similar way as described by Bensaci et al. (2015). At the 106th hour of treatment, *Cladosporium oxysporum* gave a rate between (30% and 50%), whereas the rate given by PAL114 was between (93% and 96%). In the same context, another study of the effect of entomopathogenic fungi *Akanthomyces lecanii* and *Akanthomyces muscarius* showed a very low mortality rate (15% on the 7th day), even when they were used in combination (Soltani et al., 2022). These large differences

in mortality indicate that the *S. griseoflavus* strain PAL114 has greater insecticidal power against black aphids than entomopathogenic fungi. The strain also induced a mortality rate of (70%) after 24 h, which is somewhat closer to that of the chemical insecticide Fevantrale (82%) (Purhematy et al., 2013).

Bioformulations intended for insect biocontrol are usually produced on the basis of bacterial or fungal broth cultures, as in (Karthiba et al., 2010), or on the basis of fungal spores, as in (Bensaci et al., 2015, 2022; Mohammed, 2018). However, in our trial, the formulations were based on actinobacterial spores, as this type of formulation has already proven its effectiveness in the biocontrol of plant diseases and in growth promotion (Allali et al., 2022; Zamoum et al., 2022). Furthermore, their production is easier, low-cost, and does not require many conditions or large quantities of growth media if large-scale manufacturing is considered.

Regarding its *in vivo* effect on fava beans, the bioinsecticide based on PAL114 spores had no negative influence on the plant in either mode of application. There was no decrease in any of the parameters tested. In contrast, growth was promoted by an increase in germination rate and plant and root length, especially when delivered as Spray + soil amendment. Our results concur with those of other studies on the fertilising effect of *S.griseoflavus*. This species has displayed properties of nitrogen fixation, nodulation, increased nutrient uptake and yield in plants of the Fabaceae family when coinoculated with other bacteria (Soe & Yamakawa, 2013b, Soe & Yamakawa 2013a; Htwe et al., 2018, 2019).

The application of bioinsecticide by Spray + soil amendment had a more marked effect than that by Spray. The latter is known to be effective in controlling foliar diseases (Bora et al., 2021; Udhayakumar et al., 2019), while soil amendment is effective mainly for fertilisation (El_Komy et al., 2020; Parveen et al., 2012). This shows why the combination of the two that we used was more efficient in terms of growth promotion.

Conclusion

In conclusion, the present study highlighted for the first time the insecticidal activity of a formulated actinobacteria *Streptomyces griseoflavus* strain PAL114 and its potential to control black aphid (*Aphis fabae)*. The formulated bioinsecticide showed good efficacy in pest management, significantly increasing the mortality rate. This efficacy was due to the presence of lytic enzymes whose role was to degrade the insect exoskeleton, inducing its death. *In vivo* application of bioinsecticide on fava beans also showed a positive influence on growth, increasing germination rates and plant and root length. Treatment with Spray + soil amendment had a more remarkable influence than Spray alone. As a safer pesticide, the bioinsecticide based on strain PAL114 presents an ecofriendly and effective alternative for the control of black aphids. Our findings open up prospects for further field studies in actinobacterial control of crop pests and emphasise the interest in exploiting their insecticidal potential.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Availability of data

The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research, supporting data are not available.

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