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Insecticidal and antioxidant potential of volatile compounds and nanoparticles from *Tulbaghia violacea* Harv. inoculated with endophytic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill

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ABSTRACT

This study aimed to investigate the effect of the entomopathogenic fungus *Beauveria bassiana* on the growth and ability of *Tulbaghia violacea* to synthesise nanoparticles and volatile compounds. *Tulbaghia violacea* was inoculated by drenching with different concentrations of *B. bassiana* conidial concentrations (0 mL^{-1}, 1×10^6 mL^{-1}, 1×10^7, and 1×10^8 conidia mL^{-1}) and the colonization of plant tissue in the different fungal treatments was evaluated. A UV–VIS spectrometer (SPECTROstar nano) was used to determine the presence of nanoparticles while volatile constituents in the plants were analysed with chromatography-mass spectrometry. Polyphenols, alkaloids, flavonoids and antioxidant contents were determined using referenced methods. At least 57 % of plants were successfully colonized at the highest concentration (1×10^8 conidia mL^{-1}). The results showed that treating *B. bassiana* with *T. violacea* had no significant effect ($P>0.05$) on the growth parameters (height, number of leaves, aerial part fresh weight, aerial part dry weight, root fresh weight, and root dry weight) of *T. violacea* after four weeks post-treatment. The aqueous extracts of *T. violacea* from both control and *B. bassiana*-treated plants successfully synthesized gold nanoparticles. Furthermore, the aqueous extracts of plants and gold nanoparticles from both fungus and control treatments were equally toxic to mealybug and induced high mortality. The Gas chromatography–mass spectrometry (GC–MS) results revealed that the number of compounds detected in plants subjected to the different concentrations of fungal inoculum and control did not vary significantly ($P>0.05$) in leaves and roots. The polyphenol contents in the leaves were not significantly different ($P>0.05$) among treatments. Similarly, the antioxidant activities were not significantly influenced ($P>0.05$) by fungal treatment on both leaves and roots. However, *T. violacea* extracts reduced aqueous gold to gold nanoparticles in both control and fungus-treated plants.
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1. Introduction

Synthetic pesticides are used worldwide to control grapevine pests (Sabatier et al., 2014). However, many pests have developed resistance over time towards synthetic chemical control (Marrone 2019). Over the years, pest resistance to well-known pesticides, including synthetic insecticides, has been increasing at an alarming rate (Auteri et al., 2018). Additionally, chemical pesticides are expensive and toxic to the environment, making many consumers, weary of synthetic insecticides and prefer organically cultivated produce (Park et al., 2018). Thus, the search for biological approaches to help slow down the resistance rate is intensifying. One of these approaches is the use of plant growth-promoting rhizobacteria (PGPR) which can directly activate and modulate plant genetic and molecular pathways, leading to an increase in plant growth and induction of plant resistance and tolerance (Rosier et al., 2018). The ecological importance, coupled with the continual metabolic interactions between the fungus and the plant seems to serve as a strong growing pressure for the endophytes to synthesize secondary metabolites (Vigneshwari et al., 2019) which may benefit the host plant as they play an important role in a plant's interaction with the environment and defence (Narayanan and Glick 2022).

Endophytic fungi present an excellent opportunity to optimize the biosynthesis of secondary metabolites, which could serve as a precursor for the production of nanoparticles (Ding et al. 2018). Many fungi can be manipulated to colonize plants systemically during cultivation and synthesize successfully, biologically active insecticidal nanoparticles from plant extracts and volatile compounds which have comparable effects to synthetic chemicals (Mondal et al., 2022). Endophytic fungi can be easily handled in the laboratory and have good prospects

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as nanoparticle synthesizers as they could provide a sustainable source of pesticides, produce substantial amounts of enzymes, enhance plant growth and influence plant nutrient uptake (Verma et al., 2010).

Beauveria bassiana (Cordycipitaceae) is an endophyte that colonizes the internal tissues of plants in a mutually beneficial relationship without causing any harm to the host (Tuli et al., 2014). Many studies have demonstrated that these endophytes can enhance growth and control diseases in plants (Zinniel et al., 2002). Tefera and Vidal (2009) reported that fungal endophytes, such as *B. bassiana,* are active against phytophagous insects. For these reasons, they are used in agriculture to improve crop performance (Atugala and Deshappriya 2015). Nevertheless, results from recent studies showed that plants inoculated with *B. bassiana* have variable physiological responses depending on the species and fungal strain (Parsa et al., 2013). This was substantiated by inconsistencies observed in the growth patterns of plants inoculated with entomopathogenic fungi (Jaber and Enkerli 2017) although *B. bassiana* was reported to have had a positive impact on the survival, growth, length and dry weight of cabbage (Dara et al., 2017).

Tulbaghia violacea (Amaryllidaceae) is a well-known medicinal plant species, particularly in the Eastern Cape and KwaZulu-Natal regions. The species occurs across South Africa due to cultivation in gardens and commercial medicinal plant farms (Van Wyk and Gericke, 2000). Leaves of *T. violacea* are used to repel ticks, mosquitoes and other pests when crushed (Staffa et al., 2020). It is expected that fungal treatment of plant growth medium could enhance the green synthesis of insecticidal nanoparticles and volatile chemicals. In the present study, the species *T. violacea* known to have insecticidal properties was selected. This could contribute towards developing a novel alternative insect control strategy for mealybugs (*Planococcus ficus*) which are difficult to control with synthetic insecticides due to their cryptic lifestyle (Ji et al., 2020). Continuous use of synthetic chemicals to control mealybug could lead to resistance (Cocco et al., 2021), hence, the application of natural insecticides with novel modes of action and long-lasting effectiveness combined with eco-friendly semichemical-based materials could be a sustainable approach.

2. Materials and methods

2.1. Plant materials used

Six-week-old seedlings of *T. violacea* were purchased from Stodels Garden Centre, Cape Town. Large clumps of the seedlings were divided and gently washed under a running tap for 4–5 min. After that, they were transplanted into 15 cm pots with a substrate mix of peat, silica sand, perlite and vermiculite (1:1:1:1 in volume). Each pot was then irrigated twice a week with 200 mL of deionized water containing nutrient solution (Nutrifeed® from Stodels Pty Ltd), which consists of the following ingredients: N (65 mg kg^{-1}), P (27 mg kg^{-1}), K (130 mg kg^{-1}); Ca (70 mg kg^{-1}), Cu (20 mg kg^{-1}), Fe (1500 mg kg^{-1}), Mo (10 mg kg^{-1}), Mg (22 mg kg^{-1}), Mn (240 mg kg^{-1}), S (75 mg kg^{-1}), B (240 mg kg^{-1}), and Zn (240 mg kg^{-1}).

2.2. Preparation of fungal culture

Entomopathogenic fungal culture of *B. bassiana* strain (SM3) was obtained from existing cultures in the Department of Horticultural Sciences Research Laboratory, Cape Peninsula University of Technology, Bellville 7535 (S33 55.927; E18 38.379). The fungus was originally isolated from a soil sample collected in the Cape Winelands (Strain: SM3) by (Moloinyane and Nchu 2019). The fungus was cultured on potato dextrose agar (half-strength) supplemented with 0.02 g L^{-1} of ampicillin (Sigma-Aldrich, Cape Town, South Africa), and 0.04 g L^{-1} streptomycin (Sigma-Aldrich, Cape Town, South Africa). Uncontaminated fungal sub-cultures on agar were prepared in 9 cm diameter Petri dishes, and followed by incubation at 25 °C. Three to four weeks cultures of *B. bassiana* conidia (Fig. 1) were scrapped using a sterile spatula and suspended into 500 mL bottles of sterile distilled water containing sterile 0.01 % Tween 80 v/v. To ensure the separation of spores, the suspension was vortexed for 5 min. The conidial concentrations were determined using an improved Neubauer hemocytometer (Merck KGaA, Darmstadt, Germany) and the suspensions were adjusted to 1×10^6 conidia mL^{-1} in sterile distilled water.

2.3. Assessment of B. bassiana colony

The colonization of *B. bassiana* on leaves and roots of *T. violacea* was assessed after four weeks of inoculation following the method described by (Posada and Vega 2006). One plant was used from each treatment: T1 (1×10^6 conidia/mL), T2 (1×10^7 conidia/mL), and T3 (1×10^8 conidia/mL). Each plant was then washed gently with tap water, roots and leaves were separated. They were then transferred to the laboratory soaked in distilled water. Leaf sections of 1–2 mm^2 were cut under a laminar flow hood. The sections were then surface-sterilised by dipping them in 0.5 % sodium hypochlorite for 2 min, followed by 70 % ethanol for 2 min and rinsed twice in sterile distilled water and placed on the selective media (19.5 g Potato Dextrose Agar [PDA], 0.02 g/L of ampicillin (Sigma-Aldrich), and 0.04 g/L streptomycin [Sigma-Aldrich]) Petri dishes. They were incubated in the dark at 25 °C for daily observation, after which the presence of fungal growth was observed (Fig. 2). Positive colonisation was scored by counting the number of plants showing outgrowth of mycelia from at least one leaf section. The data were expressed as percentage colonisation ([number of plant replicates colonised/ number of plants replicates excised] × 100). Only the plants in the fungus treatment that showed successful *B. bassiana* colonization were used.

2.4. Greenhouse experiment

The experiment was conducted in an environmentally controlled research greenhouse at Cape Peninsula University of Technology. Plant roots were washed thoroughly to remove soil debris before being transplanted to 15 cm pots, filled with the substrate mix (peat, perlite, vermiculite and silica sand). Plants were allocated randomly to one of four treatment groups (control and fungus treatments (1×10^6, 1×10^7 and 1×10^8 conidia/mL). Each treatment was randomly allocated 25 plants, which makes 100 the number of total

Fig. 1. Cultures of *B. bassiana* conidia.

Fig. 2. Mycelia outgrowth from the leaf section of *T. violacea*.

plants required for this research. Each plant was grown separately in a 15 cm pot containing the substrate mixes mentioned above. One hundred millilitres of fungal conidial suspension of *B. bassiana* (1×10^6,1×10^7 and 1×10^8 conidia/mL) was added separately in the test treatments using the soil drenching method. Plants in the control treatment were not exposed to fungal spores (Moloinyane and Nchu 2019). Conidial inoculation of potted plants was repeated after three weeks. They were irrigated twice a week with 200 mL of deionized water containing the nutrient solution (Nutrifeed® from Stodels Pty Ltd, Bellville, Cape Town). The fungal suspension was added twice for two weeks apart. To determine plant growth in response to combinations of substrates, parameters such as the number of shoots, and plant height were measured weekly. The experiment was allowed to run for four weeks, after which aerial and root fresh and dry weights were recorded. The following ambient conditions were maintained: temperature (25 ± 2 °C) and relative humidity (70 %). The experiment was laid out in a randomized complete block design.

2.5. *Growth parameters in inoculated* T. violacea

The following parameters were measured after the experiment: Number of leaves, root length (cm), plant height (cm) and plant total fresh weight (g). Leaves were counted for each plant, and new shoots were also counted per plant. Plant height was measured by setting a ruler from the surface of the medium where the plant is coming out to the tip of the longest leaf of the plant (Nkcukankcuka et al., 2022).

2.6. *Analysis of antioxidant metabolites in leaves of inoculated plants*

2.6.1. *Extracts preparation*

After six weeks post-treatment, plants (*T. violacea*) that were successfully colonised by *B. bassiana* and from which fungal colonies were re-isolated were randomly selected according to their fungal inoculation. Control plants were also included in the antioxidant capacity bioassays. They were oven-dried separately for a week at 35 °C in paper bags, then ground and the powder plant material were kept in airtight bags (Bulawa et al., 2022). A mass of 0.1 g each of powdered plant material was transferred into centrifuge tubes for phytochemical extraction. The samples were separately extracted with 25 mL of 60 % ethanol and placed inside the incubator for 24 h.

2.6.2. *Total alkaloids*

The spectroscopic method of Coe et al. (2010) was used to determine total alkaloids in the plant extracts. Briefly, 0.1 g of powdered *T. violacea* leaves were extracted with 25 mL of 60 % ethanol and 40 % of sterile distilled water for 24 h in total darkness, centrifuged (4000 x g for 10 min), and the supernatant was used for the assay. Subsequently, 2 mL of the extract supernatant and atropine standard solutions were mixed with 12 mL bromocresol green solution and 5 mL Concentration and pH of the buffer. Twelve mL of chloroform was added to the above-mentioned solution, and the solution was mixed using a vortex mixer. The spectrometric absorbance at 417 nm and a standard curve of atropine ranges between 1 and 20 μmol/L were used to determine the concentration of mg atropine equivalent per g dry weight (mg AE/g DW) in the sample.

2.6.3. *Total polyphenol*

The Folin-Ciocalteu method was used to determine the total polyphenol content of the crude extracts of leaves (Singleton et al., 1999). Twenty-five microlitres of the crude extract sample were mixed with 125 μL of 10 % Folin-Ciocalteu reagent (Merck, South Africa). One hundred microlitres (7.5% w/v) of aqueous sodium carbonate (Na_2CO_3) was then added to each well after 5 min. It was followed by the absorbance reading of the solution in the microplates. The results are expressed as mg gallic acid equivalent per g dry weight (mg GAE/g DW).

2.6.4. *Total flavonol*

The determination of flavonol content was done using the protocol described by (Daniels et al., 2011). Quercetin standard concentrations of 0, 5, 10, 20, 40, and 80 mg/L in 95 % ethanol were used. A 12.5 μL volume of the crude sample extracts was mixed with 12.5 μL of 0.1 % hydrochloric acid (HCl) in 95 % ethanol in the sample wells and then incubated for 30 min at room temperature. The results are expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

2.6.5. *Ferric reducing antioxidant power (FRAP)*

For the Ferric Reducing Antioxidant Power (FRAP) assay, a method described by (Benzie and Strain 1999) was followed. This assay is based on the reduction of the ferric-tripyridyl triazine complex to its ferrous in the presence of antioxidants. Reagents were prepared as required by mixing 2.5 mL of a 10 mmol/L TPTZ (2,4,6- tripyridyl-s-triazine) solution in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.3 mol/L acetate buffer, and maintained at pH 3.6 prepared freshly and warmed at 37 °C. Aliquots of 40 μL of the sample supernatant were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent. After incubation at 37 °C for 10 min, the spectrophotometric method was applied to read the absorbance of the reaction mixture at 593 nm. The standard solution was 1 mmol/L of $FeSO_4$, and the result was expressed as the concentration of antioxidants having a ferric-reducing ability equivalent to 1 mmol/L $FeSO_4$.

2.6.6. *Trolox equivalent antioxidant capacity (TEAC)*

The antioxidant activity of *T. violacea* was measured following the TEAC method described by (Tshayingwe et al., 2023). The TEAC values were measured on the antioxidants' ability to scavenge the blue-green coloured $ABTS^{\bullet+}$radical cation relative to the $ABTS^{\bullet+}$radical cation scavenging ability.

2.6.7. *DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)*

The DPPH radical's scavenging ability was determined according to the procedure described by (Jimoh et al., 2019) with minor modifications. Each sample with a fixed volume of 0.1 mL was mixed with 0.9 mL DPPH solution (0.15 mM in methanol) and stored in the dark at room temperature for 60 min. Then, its absorbance was measured at 517 nm using a microplate reader (Synergy H1, BioTek, CA, USA). The DPPH radical scavenging activity was expressed as the percentage of inhibition of the DPPH according to the expression: $[(A_0\text{-}A_t)/A_0] \times 100$ %, where A_0 is the initial absorbance and A_t is the absorbance at 60 min.

2.7. Gas chromatography-mass spectroscopy analysis (Head space)

2.7.1. GC–MS sample preparation

Fresh leaves and roots of fungus-treated *T. violacea* were frozen at −80 °C overnight, and liquid nitrogen (N_2) was added. Only plants from the fungus treatments that were successfully colonised were used for this analysis and three replicates were used per treatment. The samples were then crushed, 1 g was weighed into a solid phase microextraction (SPME) vial and 2 mL of 12 % alcohol solution (v/v) at pH 3.5 were added into the vial, followed by 3 mL of 20 % NaCl solution. The samples were vortexed, and the headspace of the samples was analysed using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Aliferis et al., 2010).

2.7.2. Chromatographic identification of constituents

Separation of volatile compounds was performed with a gas chromatograph (6890 N, Agilent Technologies Network) coupled to an inert XL Electron Ionization/Chemical Ionization (EI/CI) Mass Selective Detector (model 5975B, Agilent Technologies Inc., Palo Alto, CA). The GC–MS system was coupled to a CTC Analytics PAL autosampler, and the separation of volatiles present in the samples was achieved on a polar ZB-WAX (30 m, 0.25 mm ID, 0.25 μm film thickness) Zebron 7HGG007–11 capillary column. Helium gas was used as the carrier at a flow rate of 1 mL/min. The injector temperature was maintained at 250 °C, and the split ratio was set at 5:1. The oven temperature was programmed as follows: 35 °C for 6 min, at a rate of 3 °C/min to 70 °C for 5 mins, then at 4 °C/min to 120 C for 1 min and finally increased to 240 ° C at a rate of 20 °C/min and held for 2.89 min. The mass selective detector was operated in a full scan mode and the source and quad temperatures were maintained at 230 °C and 150 °C, respectively. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated under electron impact mode at an ionisation energy of 70 eV, scanning from 35 to 500 *m/z*. Relative ratios were calculated using the expression (peak area/IS peak area) × IS concentration (IS = internal standard), and hence, are only approximate values. Only the organic volatile compounds with a match quality of at least 90 % were identified and reported (Siddiquee et al., 2012).

2.7.3. Green synthesis of gold nanoparticles from T. violacea

The tested *T. violacea* materials were air-dried for 30 days and ground to powder with a milling machine. Ten grams of ground materials were used for extraction. One hundred millilitres (100 mL) of boiled distilled water was mixed with 10 g of ground plant material to reduce the chances of fermentation due to contamination. After cooling down, the gravimetric filtration was carried out in a centrifuge tube by centrifugation. The solution was then purified further using a filter syringe placed in the freezer and lyophilized. During the lyophilization process, the first step involves condensation-freezing liquid product to solid at a minimum temperature of −40 °C, followed by the sublimation process whereby moisture is extracted from the frozen product to gas (vapour), while still maintaining the material's crystalline structures. Thereafter, the vacuum removed the moisture and non-condensable vapour from the frozen product chamber or tray. This lyophilization process was carried out for 24 h.

Sixteen milligrams of the lyophilized *T. violacea* plant leaves and roots were dissolved in 2 mL of distilled water. After which, 8.5 mL of 0.01wt% of $NaAuCl_4 \bullet 2H_2O$ was measured and 1.7 mL of the plant extract was added to a 96-well cell culture plate dropwise. The solution was heated at 70 °C in a standard oven for 1 h. Thereafter, the solution was allowed to cool down at room temperature and diluted in a 1:1 ratio twice, resulting in two samples diluted from the solution sample treatment. This method was similar to that of Okaiyeto et al. (2021) with slight modifications.

2.7.4. Insecticidal bioassay

Adult females of *Planococcus ficus* were obtained from the ARC 21 Infruitec-Nietvoorbij (Agricultural Research Council) Stellenbosch, South Africa courtesy of Dr K.A. Achiano. The mealybugs were reared on butternut squash in a darkroom at 25 °C and 60 % RH (Fig. 3). The toxicity bioassay used was as described by (Nchu et al., 2016) with slight modification. After the extraction, the dried extracts from the four treatments: treatment 1 (1×10^6 conidia/mL), treatment 2(1×10^7 conidia/mL), treatment 3 (1×10^8 conidia/mL) and control treatment were mixed with DMSO (dimethyl sulfoxide). To achieve the final concentration of 0.005% w/v, 2 mg of ethanol extract in 22 mL of DMSO was prepared for the anti-insect bioassay. Each of the four treatments had three replicates. The negative control had only DMSO. KEMPRIN 200 EC from ARYSTA LifeScience South Africa (Pty) Ltd. was used as the positive control at the recommended dose of 1.0 mL/10 L. Each Petri dish was labelled accordingly. Ten adult female mealybugs (*P. ficus*) were treated with 2 μL of extract mixture dispensed topically on the dorsal side of the mealybugs in each petri dish. The percentage mortality was checked after 24 h. Dimethyl sulfoxide (DMSO) may be toxic certain to insects due to concentration. However, in this current study, it was not found toxic.

2.7.5. Toxicity of extracts of aunps to mealybugs (Planococcus ficus)

An immersion bioassay was performed following a method described by (Zaheer et al., 2021). For each aqueous nanoparticle solution,10 mealybugs were immersed in 1 mL of each solution of the replica for 1 min. Mealybugs were then transferred to Petri plates and kept in an incubator room at 25 °C for 4 days.

2.7.6. Statistical analysis

All data obtained from the study were analysed using one-way ANOVA (TIBCO Statistica® 13.3.0 Dell Inc., USA). The post hoc Turkey HSD was applied for the separation of means. Pearson Chi-square test was used to compare the number of volatile compounds produced by plants in fungus and control. BiosynthesisThe biosynthesisicles from *T. violacea* was analysed with UV–visible spectroscopy (UV–Vis) spectroscopy (BMG LABTECH-SPECTRO star-Nano).

Fig. 3. Mealybugs rearing on butternuts.

3. Results

3.1. Re-isolation of fungus from T. violacea

After four weeks of inoculation, *B. bassiana* was successfully isolated from three fungalngal treatments. Mycelia outgrowth on *T. violacea* leaves was 58.82 % in treatment 1 (1×10^6 conidia/mL), 47.06 % from treatment 2 (1×10^7 conidia/mL), and 64.71 % from treatment 3 (1×10^8 conidia/mL). No fungal outgrowth occurred in control plants.

3.2. Growth parameters of inoculated T. violacea

Results of the study showed that plants inoculated with *B. bassiana* and control treatment showed no significant difference ($P > 0.05$) at four weeks post-treatment (Table 1). There was no significant difference in heights among different treatments (DF = 3,76; $F = 0.293$; $P = 0.830$). However, T2 (1×10^7 conidial/mL) showed the highest height compared to other treatments. The number of leaves showed no significant difference between treatments (DF = 3, 96; $F = 2.014$; $P = 0.119$). Nevertheless, T2 (1×10^7 conidia/mL) and the control had more leaves than the other treatments. For the aerial part fresh weight (g), no significant difference (DF = 3.60; $F = 0.815$; $P = 0.491$) was observed among treatments, even though T1 (1×10^6 conidia/mL) led to the highest weight and T2 (1×10^7 conidia/mL) had the lowest weight (Table 1). Moreover, no significant difference was shown in the root fresh weights (g) (DF = 3.60; $F = 0.133$; $P = 0.939$). Also, no significant difference was recorded in the dry root weights (g) (DF = 3.60; $F = 0.593$; $P = 0.622$).

3.3. Effects of varying conidial suspensions of B. bassiana on secondary metabolites in inoculated T. violacea

The results showed that there was no significant difference between treatments (Table 2) in the total flavonol (mg QE/L) (DF = 3.8; $F = 2.05$; $p = 0.19$) and total polyphenol (mg GAE/g) contents. Flavonols were not detected in the roots of treated plants, while alkaloids (mg/L) were not detected in both leaves and roots.

3.4. Antioxidants content

Results of the antioxidant content of leaf and root samples of *T. violacea* inoculated with different concentrations of conidia of *B. bassiana* at four weeks post-treatment showed significant differences amongst treatments for DPPH (DF = 3.8 $F = 35.6$; $P>0.01$) in the leaf samples, while there was no significant difference ($P>0.05$) in the roots among treatments. Furthermore, no significant difference between TEAC (DF = 3.8; $p > 0.05$) and FRAP (DF = 3.8; $P> 0.05$) in both leaves and roots among treatments (Table 3).

3.5. GC–MS analysis (Head space)

Several volatile compounds were detected in the GC–MS analysis carried out on leaves and root extracts of *T. violacea* (Table 4). Some of the detected compounds included well-known anti-insect volatiles such as Methionol, Dimethyldisulfide, N-hexanal, Undecane, Verbenene, Isoamyl-acetate, Alpha-Terpinene, Limonene, Eucalyptol, Trans-2-hexenal, P-cymene, 1-Octen-3-ol, Phenylethyl_alcohol, Tetradecanoic acid, ethyl_ester, Trans-Caryophyllene, Gamma-terpinene and 2-pentylfuran (Table 5). The number of compounds obtained in plants subjected to different concentrations of fungal inoculum and control did not vary significantly in both leaves ($P>0.05$) and roots ($P>0.05$). There was no clear trend in the quantity (relative area ratio) of the detected compounds for both leaves and roots of *T. violacea* between plants exposed to different concentrations of fungal inoculum and control treatment ($P>0.05$). However, it was observed that roots had a higher number of compounds than leaves (Table 5).

Table 2
Effects of varying conidial suspensions of *B. bassiana* on secondary metabolites in inoculated *T. violacea*.

	Flavonols (mg QE/L)		Polyphenols (mg GAE/g)		Alkaloids (mg/L)	
Treatments	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	$8.5 \pm 1.5_a$	ND	$9.7 \pm 0.7_a$	$7.4 \pm 0.3_a$	ND	ND
T1	$6.4 \pm 2.7_a$	ND	$9.5 \pm 0.6_a$	$8.5 \pm 0.7_a$	ND	ND
T2	$5.0 \pm 1.0_a$	ND	$10.0 \pm 0.8_a$	$8.2 \pm 0.9_a$	ND	ND
T3	$4.9 \pm 2.3_a$	ND	$10.0 \pm 0.6_a$	$7.7 \pm 0.7_a$	ND	ND

This means with the same lowercase letters in the same column, roots and leaves are not significantly different ($P> 0.05$) following the comparison using Tukey's pairwise. T1= 1×10^6 conidia/mL, T2= 1×10^7 conidia/mL, and T3= 1×10^8 conidia/mL, **ND** = not detected.

3.6. Biosynthesis of gold nanoparticles

The addition of *T. violacea* leaf extract solution (50 μL) from different treatments to gold metal (250 μL) resulted in the development of an array of colours after the reaction mixture was heated at 70 °C, revealing the formation of gold nanoparticles (Fig. 4). No significant change in the appearance of the product was observed over several hours of maintaining the solution, indicating that the synthesized AuNPs were stable (Fig. 5).

3.7. Toxicity bioassay

There was a significant difference ($P<0.01$) in insect mortality among the treatments on both leaves (DF = 5.12; $F = 2.99$; $P = 0.05$; $P = 0.01$) and root extracts(DF = 5.12; $F = 5.39$; $P = 0.001$); leaves. Positive control had the highest mortality followed by extract from treatment 3 and negative control had the least mortality on leaves. The first control was a solvent and the other one was a treatment control (Tables 6 and 7).

3.8. Toxicity of extracts of aunps

Generally, results showed that the gold nanoparticle synthesized by extracts of *T. violacea* induced mealybug mortality. However, gold

Table 1
Effects of varying conidial suspensions of *B. bassiana* inoculation on different growth parameters of *T. violacea* at four weeks post-treatment.

Treatments	Shoot				Root		
Conidia/mL	Height (cm)	No of Leaves	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
Control	$5.3 \pm 0.6_a$	$3.6 \pm 04_a$	$10.8 \pm 0.9_a$	$1.3 \pm 0.1_a$	$25.8 \pm 0.8_a$	$18.3 \pm 1.6_a$	$2.8 \pm 0.3_a$
T1	$5.6 \pm 0.7_a$	$2.9 \pm 0.2_a$	$11.2 \pm 1.3_a$	$1.3 \pm 0.1_a$	$26.8 \pm 0.6_a$	$17.9 \pm 1.4_a$	$3.1 \pm 0.3_a$
T2	$6.1 \pm 05_a$	$3.7 \pm 0.2_a$	$9.5 \pm 0.5_a$	$1.3 \pm 0.1_a$	$27.7 \pm 0.5_a$	$17.5 \pm 1.3_a$	$2.6 \pm 0.3_a$
T3	$5.8 \pm 0.8_a$	$3.1 \pm 0.2_a$	$10.0 \pm 0.4_a$	$1.0 \pm 0.1_a$	$27.8 \pm 0.6_a$	17.1 ± 1.3	$2.9 \pm 0.2_a$

Means followed by the same lowercase letters in a column (Table 1) are not significantly different ($p > 0.05$) following the comparison of treatments using Tukey's test. T1= 1×10^6 conidial/mL, T2= 1×10^7 conidial/mL, and T3= 1×10^8 conidial/mL.

Table 3
Antioxidant capacity of the inoculated leaf and root samples of *T. violacea*.

	DPPH (μmol TE/L)		FRAP (μmol AAE/g)		TEAC (μmol TE/g)	
Treatments	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	$151.6 \pm 2.8_{a}$	$84.0 \pm 1.6_{a}$	$42.3 \pm 5.1_{a}$	$48.2 \pm 4.9_{a}$	$233.6 \pm 14.7_{a}$	$194.5 \pm 13.6_{a}$
T1	$101.7 \pm 1.2_{b}$	$71.5 \pm 0.9_{b}$	$43.4 \pm 4.2_{a}$	$59.0 \pm 6.7_{a}$	$214.6 \pm 8.1_{a}$	$189.7 \pm 2.7_{a}$
T2	$120.2 \pm 5.7_{c}$	$82.6 \pm 1.9_{a}$	$42.8 \pm 3.4_{a}$	$54.1 \pm 8.0_{a}$	$227.1 \pm 10.9_{a}$	$201.7 \pm 20.3_{a}$
T3	$135.6 \pm 3.0_{ac}$	$87.3 \pm 2.5_{a}$	$43.4 \pm 3.6_{a}$	$55.6 \pm 4.9_{a}$	$227.3 \pm 6.0_{a}$	$212.2 \pm 5.3_{a}$

This means with the same lowercase letters in the same column, leaves or roots are not significantly different ($P > 0.05$) following the comparison using the Tukey test. T1= 1×10^6 conidial/mL, T2= 1×10^7 conidial/mL, and T3= 1×10^8 conidial/mL, DF= Degree of freedom, DPPH= 2,2-diphenyl-1-picrylhydrazyl, FRAP= Ferric Reducing Antioxidant Power, TEAC= Trolox Equivalent Antioxidant Capacity.

Table 4
Volatile organic compounds with a match quality of at least 90 % present in fungal treated and control roots and leaves of *T. violacea*.

Compounds detected in root samples	Control	T1 (1×10^6)	T2 (1×10^7)	T3 (1×10^8)
Methionol	+	+	+	+
Dimethyldisulfide	+	+	+	+
N-hexanal	+	+	+	+
Ndecane	+	+	+	+
Verbenene	+	+	+	+
Isoamyl acetate	–	–	+	–
Alpha-terpinene	+	+	+	+
Limonene	+	+	+	+
Eucalyptol	+	+	+	+
Trans-2-hexenal	+	+	+	+
2-pentylfuran	+	+	+	+
Ethyl hexanoate	+	+	+	+
P-cymene	+	+	+	+
Cis-3-Hexenyl formate	+	+	+	+
2,4-dithiapentane	+	+	+	+
Methyl heptanoate	+	+	+	+
2-Pentyn-1-ol	+	+	+	+
Ethyl_n-heptanoate	+	+	+	+
Dimethyl trisulfide	+	+	+	+
Verbenyl ethyl ether	+	+	+	+
2 octenal	+	+	+	+
Ethyl octanoate	+	+	+	+
Acetic acid	+	+	+	+
1-Octen-3-ol	+	+	+	+
2-sec-Butyl-3-methoxypyrazine	+	+	+	+
Ethyl nonanoate	+	+	+	+
L-linalool	+	+	+	+
1-octanol	+	+	+	+
Alpha-trans-beta-bergamotene	+	+	+	+
Methyl methylthiomethyl disulfide	+	+	+	+
Trans-pinocarveol	+	+	+	+
Cis-verbenol	+	+	+	+
1,4-dimethyltetrasulfide	+	+	+	+
1,2,4-trithiolane	+	+	+	+
6-camphenol	+	+	+	+
Methyl-N-Hydroxybenzene carboximidoate	+	+	+	+
1,1 bis(methylmercapto)methyl sul	+	+	+	+
Hexanoic acid	+	+	+	+
Ethyl laurate	+	+	+	+
Benzyl alcohol	+	+	+	+
Phenylethyl alcohol	+	+	+	+
Tetradecanoic acid ethyl ester	+	+	+	+
Methyl palmitate	+	+	+	+
Ethyl palmitate	+	+	+	+
Bis(2-sulfhydrylethyl)-disulfide	+	+	+	+
Ethyl (11E)–11-hexadecenoate	+	+	+	+
Benzyl benzoate	+	+	+	+
Total number of compounds	46	46	47	46
Compounds detected in leaf samples				
1-propanethiol	+	+	+	+
Methionol	+	+	+	+
Dimethyldisulfide	+	+	+	+
Limonene	+	+	+	+
Trans-2-hexenal	+	+	+	+
2-pentylfuran	+	+	+	+

(*continued*)

Table 4 (*Continued*)

Compounds detected in root samples	Control	T1 (1×10^6)	T2 (1×10^7)	T3 (1×10^8)
Gamma-terpinene	+	+	+	+
Ethyl hexanoate	+	+	+	+
P-cymene	+	+	+	+
Cis-3-Hexenyl_formate	+	+	+	+
2,4-dithiapentane	+	+	+	+
Cis-3-Hexenyl acetate	+	+	+	+
Ethyl n-heptanoate	+	+	+	+
Dimethyl_trisulfide	+	+	+	+
Verbenyl ethyl ether	+	+	+	+
Cis-3-hexenol	+	+	+	+
2_octenal	+	+	+	+
Ethyl octanoate	+	+	+	+
Acetic acid	+	+	+	+
1-Octen-3-ol	+	+	+	+
Cis-3-hexenyl 2-methylbutanoate	+	+	+	+
Trans,trans-2,4-Heptadienal	+	+	+	+
L-linalool	+	+	+	+
1-octanol	+	+	+	+
Trans-caryophyllene	+	+	+	+
4-terpineol	+	+	+	+
Beta-cyclocitral	+	+	+	+
Methyl methylthiomethyl disulfide	+	+	+	+
(Z)−3-hexenyl pentanoate	+	+	+	+
Benzyl formate	+	+	+	+
Linalyl propionate	+	+	+	+
1,4-dimethyltetrasulfide	+	+	+	+
Dimethyl trithiocarbonate	+	+	+	+
B-phenylethyl formate	+	+	+	+
Methyl-N-hydroxybenzenecarboximidoate	+	+	+	+
Nerol	+	+	+	+
1,1_bis(methylmercapto)methyl sul	+	+	+	+
Benzyl alcohol	+	+	+	+
Phenylethyl alcohol	+	+	+	+
Beta-ionone	+	+	+	+
(-)-Nerolidol	+	+	+	+
Tetradecanoic acid ethyl ester	+	+	+	+
Methyl palmitate	+	+	+	+
Ethyl palmitate	+	+	+	+
Ethyl (11E)−11-hexadecenoate	+	+	+	+
Dihydroactinidiolide	+	+	+	+
1,2,4,6-tetrathiepane	+	+	–	+
8,11-Octadecadienoic acid methyl ester	+	+	–	+
Ethyl linoleate	+	+	+	+
Linolenic acid methyl ester	+	+	+	+
Benzyl benzoate	+	+	+	+
Total number of compounds	51	51	49	51

(+) and (–) signs respectively indicate that the compounds were detected and not detected in the treatments.

nanoparticle solution from the plants that were inoculated with fungus conidia mL^{-1} induced higher mortality compared with the control treatment (DF = 5.12; F = 17.3 $P < 0.01$). It is worth noting because the aqueous component of the gold solution was not evaporated and separated from the solid AuNPs, the exact concentration of the gold nanoparticle was not determined in this study. The two controls, one was a solvent and one a treatment control.

4. Discussion

Insect pests are mainly controlled using chemical insecticides, which are environmentally unfriendly (Khan and Ahmad, 2019). Also, pests tend to develop resistance towards synthetic chemicals over time (Auteri et al., 2018). This has motivated researchers to investigate alternative procedures to control insects (Zeng et al., 2010; Al-Ansari et al. 2021). The use of entomopathogenic fungi has gained interest among many researchers (Islam et al., 2021). Endophytic fungi do not only protect plants against pests but also influence plant growth positively (Mantzoukas and Eliopoulos 2020). In this study, conidia of *B. bassiana* successfully colonised 47the −64 % of leaf and root tissues of *T. violacea*. This was an indication that the fungus was moderately endophytic, and the inoculum was transfered from roots to the leaves. It is worth mentioning that successful (75 %) colonisation of *B. bassiana* in *T. violacea* has been reported on leaves (Staffa et al., 2020). However, the success of colonisation of *B. bassiana* may be influenced by the inoculation method, and biotic and abiotic factors (Tefera and Vidal 2009).

Despite evidence of successful colonisation of *T. violacea*, entomopathogenic *B. bassiana* had no significant effect ($P > 0.05$) on growth among treatments nor showed any significant difference in the number of leaves. Similar results were also obtained by (Moloinyane and Nchu 2019) in a study when the same strain of *B. bassiana* (SM3) was inoculated in *Vitis vinifera* L. Tall and Meyling (2018) reported that some strains of entomopathogenic fungi induce growth in plants. On the other hand, Espinoza et al. (2019) reported the limited effect of *B. bassiania* on the growth of *Allium schoenoprasum* L. (chives). Jaber et al. 2019 reported inconsistencies in the plant growth parameters of plants inoculated with entomopathogenic fungi. However,

Table 5
Selected and well-known insecticidal volatiles detected in *T. violacea* and their relative area ratios using GCM.

Compounds	Plant parts	T1 (1×10^6)	T2 (1×10^7)	T3 (1×10^8)	Control	References
Methionol*	Roots	1.88±0.52a	2.00±0.21a	2.07±0.96a	0.40±0.03a	(Liu et al., 2005; Wong et al., 2018)
	Leaves	1.07±1.07a	3.61±1.39a	4.31±0.22a	3.96±1.0 a	
Dimethyldisulfide*	Roots	3.53±1.17 a	2.85±0.41a	3.03±1.23a	1.28±0.14a	(Dugravot et al., 2003)
	Leaves	9.39±1.00 a	6.75±1.04a	8.17±1.34a	6.22±0.48a	
N-Hexanal	Roots	22.87±6.37a	15.02±3.20a	13.50±2.24a	11.68±0.42a	(Prates et al., 1998)
	Leaves	–	–	–	–	
Undecane	Roots	0.10±0.07a	0.06 ± 0.02a	0.05 ± 0.02a	0.31 ± 0.02a	(Ebadollahi and Taghinezhad, 2020)
	Leaves	–	–	–	–	
Verbenene	Roots	0.26 ± 0.03a	0.28 ± 0.03a	0.41 ± 0.02a	0.32 ± 0.09a	(Herrera et al., 2015)
	Leaves	–	–	–	–	
Isoamyl acetate	Roots	0.01±0.01a	0.04 ± 0.03a	–	0.33 ± 0.33a	(Velayutham and Ramanibai, 2016)
	Leaves	–	–	–	–	
alpha-Terpinene	Roots	0.40±0.12a	0.37±0.30a	0.60±0.38a	0.34±0.15a	(Pavela et al., 2009)
	Leaves	–	–	–	–	
Limonene*	Roots	1.03±0.06a	1.25±0.34a	1.11±0.07a	1.26±0.90a	(Hebeish et al., 2008)
	Leaves	0.42±0.16a	0.53±0.17a	0.72±0.21a	0.48±0.10a	
Eucalyptol	Roots	0.61±0.54a	1.41±0.18a	0.58±0.25a	0.24±0.03a	(Gharib et al., 2020)
	Leaves	–	–	–	–	
Trans-2-Hexenal*	Roots	0.29±0.02a	0.30±0.06a	0.32±0.07a	0.17±0.02a	(Lu et al., 2017)
	Leaves	9.17±2.79a	7.30±2.23a	6.62±1.00a	7.68±1.76a	
p-cymene*	Roots	0.35±0.21a	0.250.08 a	0.42±0.09a	0.210.07a	(Kordali et al., 2008)
	Leaves	0.48±0.06a	0.32±0.23a	0.76±0.20a	0.98±0.15a	
1-Octen-3-ol	Roots	0.35±0.12a	0.33±0.11a	0.58±0.04a	0.20±0.07a	(Herrera et al., 2015)
	Leaves	–	–	–	–	
Phenylethyl_Alcohol*	Roots	0.92±0.06a	1.11±0.40a	1.55±0.43a	1.12±0.11a	(Prates et al., 1998)
	Leaves	–	–	–	–	
Tetradecanoic acid, ethyl_ester	Roots	0.20±0.05a	0.92±0.44a	0.50±0.13a	0.93±0.41a	(Okonkwo and Onyeji, 2018)
	Leaves	–	–	–	–	
trans-Caryophyllene	Roots	–	–	–	–	(Lu et al., 2017)
	Leaves	0.54±0.21a	0.11±0.07a	0.31±0.16 a	0.20±0.07a	
gamma-Terpinene	Roots	.	–	–	–	(Al-Ansari et al., 2021)
	Leaves	1.41±1.30a	1.70±0.63a	1.82±0.58 a	1.76±0.69a	
2-Pentylfuran	Roots	0.80±0.12a	0.47±0.22a	0.50±0.08 a	0.33±0.18a	(Cha et al., 2021)
	Leaves	0.22±0.03a	0.17±0.11a	0.27±0.08 a	0.20±0.08a	
Total compounds found	**Roots**	**15**	**15**	**14**	**15**	
	Leaves	**8**	**8**	**8**	**8**	

researchers such as Bamisile et al. (2018) and Espinoza et al. (2019) have indicated that *B. bassiana* positively influences the growth, length, development, and health of chive and cabbage.

Manipulation of abiotic and biotic factors to improve the production of secondary metabolites in plants has yielded encouraging results. Factors such as light, water and nutrients can influence the production of secondary metabolites in plants (Borges et al., 2017). In recent times, there have been growing interest in the use of biotic agents, such as fungi and bacteria, to optimize secondary metabolite production in plants (Thakur et al., 2019). Some microbes perform the same role as chemical fertilizers and pesticides (Timmusk et al., 2017). *Tulbaghia violacea* inoculated with *B. bassiana* conidia was assessed for potential influence on bioaccumulation of polyphenols, flavonols and alkaloids. There was no significant difference ($P>0.05$) among treatments. Polyphenol contents were not influenced by the exposure of *B. bassiana* to leaves of *T. violacea* while flavonols also showed no significant influence on leaves. Alkaloids were not detected in both roots and leaves. Recently, Rocchetti et al. (2022) and Efiong et al. (2020) respectively reported low detection and absence of alkaloids in many *Allium* species. Noticeably, flavonols were highly concentrated in the control treatment in leaves compared to the inoculated treatments. Similar findings were reported by Espinoza et al. (2019) while investigating the effect of *B. bassiana* on the secondary metabolite content of chives.

Spectrophotometric analyses showed that the leaves of *T. violacea* inoculated with *B. bassiana* and the control plants contained

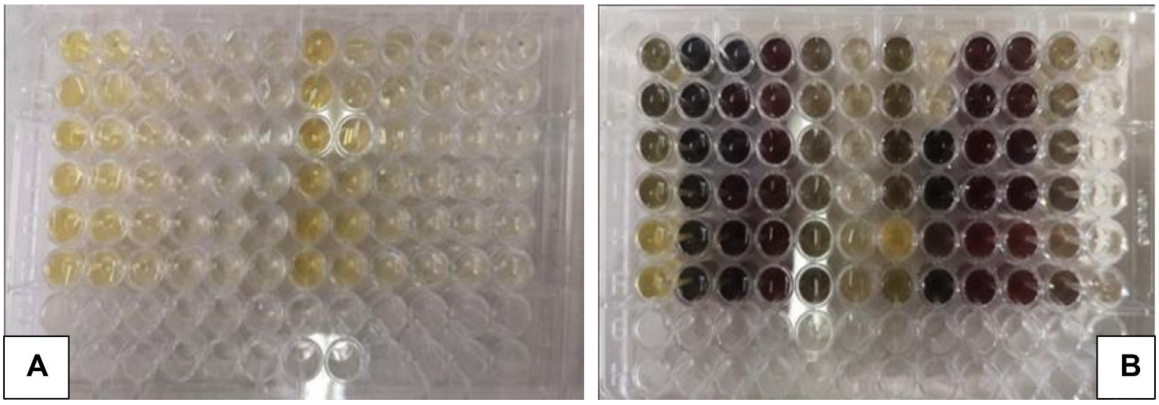

Fig. 4. A- showing plate before incubation in an oven and B showing plate after incubation in the oven at 70 °C.

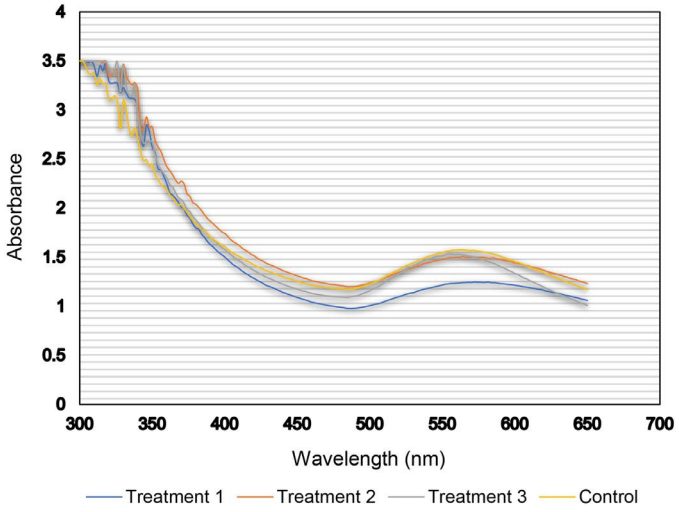

Fig. 5. UV-visible spectrums of synthesized AuNPs showing a peak at 570 nm. Different colours represent different treatments peaks and different wavelengths. Treatment 1 = 1 × 10^6 conidia/mL, Treatment 2 = 1 × 10^7 conidia/mL, and Treatment 3 = 1 × 10^8 conidia/mL.

Table 6
Effects of *B. bassiana* inoculation on *P. ficus* mortality of extracts of *T. violacea*.

Treatments	Insect mortality	
	Leaves 24h	Roots 24h
Control	7.00±0.58ab	8.67±0.88ab
T1	7.67±1.02ab	8.00±2.00ab
T2	7.33±1.45ab	10.00±3.33a
T3	8.00±2.00ab	7.67±1.20ab
Negative control	3.33±0.88b	3.33±0.88b
Positive control	10.00±3.33a	10.00±6.67a

Means with the same lowercase letters in the same column, roots and leaves are not significantly different (P> 0.05) following the comparison using the Tukey's pairwise. T1= 1 × 10^6 conidia mL^{-1}; T2= 1 × 10^7 conidia mL^{-1}; T3= 1 × 10^8 conidia mL^{-1}; Control= 0 conidia mL^{-1}; Negative control= DMSO; Positive control= 1.0 mL/10 L KEMPRIN 200 EC.

Table 7
Percentage of mortality mean ± SE of female *P. ficus* exposed to aqueous gold nanoparticles (AuNPs) solution of plants inoculated with *B. bassiana* at different treatments as well as a negative and positive control.

Treatments	Mortality
Control	1.00±0.33bc
Treatment 1	7.00±0.3ab
Treatment 2	5.00±0.33abc
Treatment 3	6.00±1.15ab
Negative control	0.00±0.00c
Positive control	10.00±0.00a

Means ± SE within columns followed by the salowercasease is not significantly different (P>0.05) following the comparison of the of Tukey HSD test. T1= 1 × 106 conidia mL−1; T2= 1 × 107 conidia mL−1; T3= 1 × 108 conidia mL−1; Control= 0 conidia mL−1; Negative control= DMSO; Positive control= 1.0 mL/10 L KEMPRIN 200 EC.

antioxidant capacity measured by FRAP, DPPH and TEAC assays. Results showed that there were significant differences (P < 0.05) between the different treatments in DPPH, TEAC and FRAP. However, there was no significant difference (P>0.05) in the roots among treatments. Interestingly, leaves from the control treatment had higher antioxidant capacity than leaves of the fungus-treated plants. Luximon-Ramma et al. (2002) reported that antioxidant capacity was strongly correlated with total phenols. Furthermore, the positive high correlation between antioxidant capacities and total phenolic content indicates that phenolic compounds are a major contributor to the antioxidant activity of these plants (Song et al., 2010; Jimoh et al., 2019). However, the antioxidant capacities of samples might be influenced by many factors such as test ssystemsand may not be fully described by a single method (Fu et al., 2011). Alcalde et al. (2019) opined that most natural antioxidants are multifunctional, and the behaviour of each compound varies in response to different methods. Therefore, in the current study, different antioxidant activity assessments could have yielded different results. Besides, the current results indicate that *B. bassiana* inoculation did not influence the antioxidant capacity of *T. violacea*.

Secondary plant products protect plants against various biotic and abiotic stresses (Akula and Ravishankar 2011; Jimoh et al., 2023). In this study it was observed that the leaves and roots extracts of *T. violacea* produced several well-known anti-insect compounds such as Methionol, Dimethyldisulfide, N-hexanal, Undecane, Verbenene, Isoamyl_acetate, Alpha-Terpinene, Limonene, Eucalyptol, Trans-2-hexenal, P-cymene, 1-Octen-3-ol, Phenylethyl_alcohol, Tetradecanoic acid, ethyl_ester, Trans-Caryophyllene, Gamma-terpinene 2-pentyl furanuran (Table 5). However, the number of compounds obtained in plants subjected to different concentrations of fungal inoculum and control did not vary significantly (P > 0.05) in both leaves and roots, which indicates that the fungal inoculum did not affect the number of compounds (Table 5). It is not surprising given that the secondary metabolite contents, both volatile and non-volatile constituents, did not vary significantly with fungal inoculation although the influence of endophytic fungi may be overwhelmed by the plant species and the portion of the plant colonized (Baron and Rigobelo 2022).

Green synthesis of gold nanoparticles by *T. violacea* inoculated with *B. bassiana* was successfully achieved in this study, and this was confirmed through a UV–Vis spectrophotometer. However, the nanoparticles were not characterized in this study. Future studies will characterize and test the exact concentration of synthesized gold nanoparticles. This was not the first time *T. violacea* was used for the green synthesis of nanoparticles. Similar findings were found in the study of Mbenga et al. (2022), which looked at nanoarchitectonics of ZnO nanoparticles mediated by the extract of *T. violacea* and their cytotoxicity. Similarly, the insect toxicity of aqueous solution of nanoparticles synthesised from extract of *T. violacea* was investigated for the different treatments. Although there was a significant difference in insect mortality, with aqueous AuNPs solutions of *B. bassiana* treated plants having higher mortality, these results need to be interpreted with caution because the exact concentration of the AuNPs in the solution was not determined. Benelli (2018) revealed that there is the nanotechnology and arthropod control science to manage pest population as reported by numerous researchers on the toxicity of nanoparticles towards various arthropod species (Pavoni et al., 2019; Pilaquinga et al., 2019). These results are supported by Staffa et al. (2020) who had positive findings on ticks' mortality when exposed to *T. violacea* inoculated with an endophytic arthropod-pathogenic fungus, and Nchu et al. (2016), who reported that dichloromethane extract of garlic, a close relative of *T. violacea* showed positive effects against ticks.

5. Conclusion

This study contributes to the current body of knowledge on the positive interaction between endophytic fungus and *T. violacea.* The

fungus successfully colonized the plants that were inoculated although with no significant influence on the growth and the antioxidant activity of *T. violacea*. Also, exposure to entomopathogenic fungus *B. bassiana* did not optimize the polyphenol and flavonol content of *T. violacea*. Remarkably, the extracts of *T. violacea* exposed to both fungus and control treatments were equally toxic to mealybug as they induced high mortality due to the fact that leaves and roots extracts of *T. violacea* produced several well-known anti-insect compounds. This study provides insights into the endophytic *B. bassiana*-*T. violacea*-mealybug relationship and recommends further studies on characteristization of nanoparticles induced by the fungal endophyte.

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Conflict of interest

Authors wish to declare no conflict of interest in the manuscript.

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