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Research article

Effects of *Beauveria bassiana* (Hypocreales) on plant growth and secondary metabolites of extracts of hydroponically cultivated chive (*Allium schoenoprasum* L. [Amaryllidaceae])

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ABSTRACT

The endophytic plant-fungi symbiotic relationship can be explored to improve cultivation of targeted medicinal plant species. The objective of this study was to assess the effects of the cultivation of chive (*Allium schoenoprasum*) in plant growth medium inoculated with the entomopathogenic fungus, *Beauveria bassiana* (Hypocreales). Twelve replicates of chive seedlings were exposed to *B. bassiana* inoculum formulated at concentrations of 0, 1×10^5, 1×10^4 and 1×10^3 conidia mL^{-1} in a completely randomized design. We assessed plant growth parameters, such as leaf number and plant height weekly and root length, leaf and root fresh and dry weights and secondary metabolites three weeks post-fungal inoculation. The fungus was re-isolated from some of the leaves and roots of the treated plants suggesting that the fungus successfully colonized the plant tissue. Generally, the results indicated that the fungal inoculation had minimal effect on most of the growth parameters assessed in relation to the control. Remarkably, plants exposed to the fungus recorded greater ($p < 0.05$) total alkaloid, ranging from 2.98 – 3.76 mg atropine equivalent (AE)/g dry weight (DW) compared to the control plants (1.96 mg AE/g DW) for the leaves. This study demonstrated that endophytic fungi could be used to improve the yield of active chemical constituents in cultivated medicinal plants.

1. Introduction

High demand has bolstered trade in medicinal plants and subsequently, creating the need for commercial cultivation of these plants. However, successful commercial cultivation of medicinal plants relies on achieving consistently high quality and quantity of medicinal materials at a reasonable cost of production. Consequently, efficient technologies and techniques are being developed. For example, greenhouse technologies circumvent the setbacks which are normally associated with open air conditions, such as variations in biotic and abiotic factors, and limited arable land (Canter et al., 2005).

A plethora of studies have examined the effects of varying levels of specific abiotic factors on the production of secondary metabolites (Pavarini et al., 2012). On the contrary, much fewer studies have investigated the effects of biotic factors on nutraceutical and medicinal plants (Gouvea et al., 2012). Some entomopathogenic fungi are endophytic; some are easily mass-produced *in vitro*; some are rhizospheric; they are quite ubiquitous (Vega et al., 2008, 2009). An endophytic fungus forms a mutually beneficial symbiotic relationship with a plant; it lives inside a plant's tissues without causing disease to the plant, meanwhile, boosting plant defenses and in return the plant acts as the host (Behie and Bidochka, 2014). Metabolites produced by some endophytic fungi have been reported to influence the reduction of insect infestations on their host plants (Jaber and Ownley, 2017). The increase in quantity and diversity of secondary metabolites in endophyte-containing plants are probably responsible for the reduction of insect herbivory on plants. The endophytic fungi-plant relationship can be explored for cultivation of targeted high value medicinal plant species with the view to optimizing medicinal properties by increasing quantity and quality of secondary metabolites in these plants. *Beauveria bassiana*, an entomopathogenic fungus which occurs naturally and ubiquitously in the soil (Keswani et al., 2013), is endophytic and an interesting candidate for the

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evaluation of endophytic fungal colonization on the medicinal properties of plants.

Plants belonging to the genus *Allium* including garlic, onion, and Chinese chive are known for their medicinal properties; they have proven pharmacological and nutraceutical activities. They contain organosulfur compounds such as allicin and phenolic compounds like gallic acid, quercetin, coumaric acid, and ferulic acid (Zeng et al., 2017; Kucekova et al., 2011). Phenolic compounds are bioactive substances that show antioxidant, anticancer, anti-inflammatory, and antimicrobial activity, and prevent chronic disease (Vlase et al., 2013). *Allium schoenoprasum*, which is commonly known as chive, is a perennial plant that is used as a popular seasoning and is cultivated globally (Zeng et al., 2017). Phytochemical analyses of *A. schoenoprasum* extracts revealed that its water extract contains flavonoid compounds, glycosides, and saponins while the methanol and ethyl acetate extracts contain alkaloids, flavonoids, glycosides, and saponins (Sihombing et al., 2018). Chive leaves have great medicinal values, such as positive effects on the circulatory system by reducing blood pressure and antimicrobial effects (Vlase et al., 2013). Chive leaf infusions, often rich in potassium, are used for treatment of kidney stone disease by dissolving calcium oxalate in kidneys (Tripathi et al., 2013). Kucekova et al. (2011) demonstrated that chive flower extract obtained by solid-liquid extraction have a great effect on human keratinocyte cell line (HaCaT) by decreasing cell proliferation, perhaps, due to their phenolic compounds. It is worth-mentioning that chives may contain many of the bioactive phenolic compounds such as gallic acid, coumaric acid, ferulic acid, and rutin that are commonly found in other *Allium* spp.

Hence, the objective of this study was to assess plant growth of chive (*A. schoenoprasum)* and secondary metabolites of chive leaves and roots following cultivation in a plant growth medium inoculated with conidia of an endophytic entomopathogenic fungus (*B. bassiana)* under greenhouse conditions. This is the first study on the experimental inoculation of chive with an endophytic fungus, and its subsequent effects on plant growth and production of secondary metabolites.

2. Material and methods

2.1. Fungus

An indigenous *B. bassiana* strain (SM3) that was previously isolated from a soil sample collected from the Cape Winelands in South Africa was used in this study. This strain was identified using molecular and morphological techniques as described in Moloinyane and Nchu (2019). The strain is being maintained at Cape Peninsula University of Technology in Bellville, South Africa. The fungus was cultivated on half-strength potato dextrose agar (PDA); 0.02 g/L of ampicillin (Sigma-Aldrich), and 0.04 g/L streptomycin (Sigma-Aldrich) in 9 cm and 14 cm diameters petri dishes. Fungal cultures were incubated at 25 °C in the dark for four weeks. Using a spatula, mature four-week-old *B. bassiana* conidia obtained from PDA plates were transferred to 2 L glass bottles containing sterile 0.01% Tween 80 (Polysorbate, Sigma-Aldrich, South Africa) and sterile water. Bottles were capped, mixed by shaking for 5 min and by using a magnetic stirrer (at 20 °C and 300 rpm for 30 min) to homogenous conidial suspensions. The conidia inoculum concentration was enumerated using a haemocytometer (Bright-Line, Sigma-Aldrich, South Africa) and observed with a light microscope at 400X magnification. In order to obtain the desired concentration (1×10^5 conidia mL^{-1}), the volume of sterile 0.01% Tween was increased or conidia were added to the glass bottle. This was followed by 10-fold serial dilutions to obtain lower concentrations of conidial inoculum suspensions; 1×10^4, 1×10^3 conidia mL^{-1}. A conidial germination test to determine conidial viability was carried out according to the method described by Inglis et al. (2012) and high spore germination of over 90% was obtained.

2.2. Plants

Chive (*A. schoenoprasum)* seedlings, were purchased from Stodels Nurseries (Pty) Ltd in Bellville, Western Cape Province, South Africa. Plants were maintained in the greenhouse at Cape Peninsula University of Technology in Bellville, South Africa at 23–25 °C, 60%–80% RH and 13/11 natural light/dark regime. Individual chive plants were separated from a clump and one plant was transplanted to each 10 cm diameter pot containing a substrate mixture of one-third of river sand, one-third of vermiculite, and one-third of perlite by volume. The substrate materials were sterilized using 1% sodium hypochlorite for 1h before rinsing with sterile distilled water. The plants were fed with water soluble, formulated hydroponic fertilizer, Nutrifeed (Starke Ayres Pty. Ltd., South Africa). The fertilizer was dissolved in sterile distilled water at a concentration of 10 g/5 L and 100 mL of the mixture was added to each plant once a week. Each plant was watered with 100 mL reverse osmosis water once a week.

2.3. Inoculation of plants

Three conidial inoculum suspensions (1×10^5, 1×10^4, 1×10^3 conidia mL^{-1}) were prepared as described above. All chive plants had a uniform age and twelve chive plants with a similar leaf length were randomly allocated to each of the treatments ($T1 = 1 \times 10^5$, $T2 = 1 \times 10^4$, $T3 = 1 \times 10^3$ conidia mL^{-1}). Ninety milliliters of each of the conidial concentrations was added manually and separately to the root system of each plant of the same treatment using a hand-held plastic dispenser at one week following the commencement of the experiment. Twelve plants were used as the control and treated with 90 mL of sterile 0.01% Tween 80 water.

2.4. Plant growth measurements

Leaf length (cm) from the soil surface to the top of the highest leaf and leaf number of each plant were measured weekly for three weeks. Leaf length increment (cm) was calculated as the difference between leaf length at weeks one and three and percentage growth was calculated as follows: 100 x the leaf length increment divided by week one leaf length. Similarly, leaf number was counted and leaf number increment and % increase were calculated. At the end of the experiment, three weeks post fungal inoculation, root length and fresh weights of plants were recorded. Root length (cm) was measured from the hypocotyl-root junction to the root tip with a ruler. Dry weights of the plants were determined by placing plants in paper bags in a drying oven at 35 °C for 7 days.

2.5. Fungal colonization

To determine fungal colonization of leaf tissue, three sections of leaf (5 × 5 mm) as well as root (5 mm length) sections were collected from each plant soon after harvesting. The excised material was surface sterilized by first dipping in 70% ethanol for 3 s and then rinsing in sterile distilled water for 1 min. Thereafter, the sterile leaf and root sections were placed on solid half-strength PDA, incubated in the dark at 25 °C, and were checked for outgrowth of *B. bassiana* in root and leaf tissues under stereo microscope after one and two weeks. Efficiency of surface sterilization was evaluated by placing drops of the previously used 70% ethanol and distilled water for surface sterilization on plates containing solid half-strength PDA, and then incubated and checked for fungal outgrowth.

2.6. Analysis of secondary metabolites in leaf and root extracts

Chive plants were harvested at three weeks post treatment and the leaves and roots were separated, placed in separate paper bags and dried in a drying oven at 35 °C for 7 days and the dry leaves and roots of each plant were ground separately using a ceramic mortar and pestle for 2 min and 1 min, respectively.

The spectroscopic method described by Fadhil and Reza (2007) was used to determine total alkaloids in the plant extracts. Briefly, 100 mg of the chive leaf and root materials were extracted separately with 10 mL of aqueous ethanol (mixture of 60% ethanol and 40% water) for 2 h, centrifuged (4000 x g for 10 min) and the supernatant was used in the assay. Two milliliters of the extract supernatant and atropine standard solutions were mixed with 5 mL sodium phosphate buffer and 12 mL bromocresol green solution. Thereafter, 12 mL of chloroform was added to the solution and the solution was mixed vigorously using a vortex mixer. The absorbance at 417 nm was determined and the concentration of mg atropine equivalent per g dry weight (mg AE/g DW) in the sample using a standard curve of atropine was calculated.

The total polyphenol content of the aqueous ethanol extracts of dried leaf and root dried materials of the chives were determined by the Folin-Ciocalteu method (Singleton et al., 1999; Swain and Hills, 1959). The method of Swain and Hills (1959) was adapted for the plate reader. Using a 96-well microplate, 25 μL of the sample was mixed with 125 μL Folin-Ciocalteu reagent (Merck, South Africa) and diluted 1:10 with distilled water. After 5 min, 100 μL (7.5%) aqueous Na_2CO_3 (Sigma-Aldrich, South Africa) was added to the well. The plates were incubated for 2 h at room temperature before the absorbance was read at 765 nm using a Multiskan plate reader (Thermo Electron Corporation, USA). The standard curve was prepared using 0, 20, 50, 100, 250 and 500 mg/L gallic acid in 10% ethanol and the results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

The flavonol content of the aqueous ethanol extracts of dried leaf and root materials of the chives were determined using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa) as standard. In the sample wells, 12.5 μL of the crude aqueous extracts were mixed with 12.5 μL 0.1% HCl (Merck, South Africa) in 95% ethanol, and 225 μL 2% HCl and incubated for 30 min at room temperature. The absorbance was read at 360 nm, at a temperature of 25 °C (Mazza et al., 1999). The results were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

2.7. Statistical analysis

All data were analyzed with Paleontological Statistics (PAST) (Hammer et al., 2001). Plant growth and secondary metabolite data were compared using one-way ANOVA at $p = 0.05$ level of significance. The significant difference between the means was determined with the Tukey-pairwise test ($P = 0.05$). Chive plants were arranged in a completely randomized design. Each treatment contained 12 replicate chive plants. The results are presented as mean ± SE.

3. Results

3.1. Re-isolation of fungus

B. bassiana was re-isolated from chive leaf and root samples from all fungal treatments, but not the controls. Fungal outgrowth on chive leaves was found in the fungus inoculated treatments after two weeks, but not from control treated plants (T1 = 33%, T2 = 22%, T3 = 33%, Control = 0% of leaf samples). The root samples of chive plants showed less fungal outgrowth in all treatments than the leaf samples (T1 = 11%, T2 = 11%, T3 = 11%, Control = 0% of root samples). Again, *B. bassiana* was also not detected in the root samples from control treated plants. Sterility of the re-isolation process was confirmed due to no outgrowth on petri dishes containing 70% ethanol and distilled water.

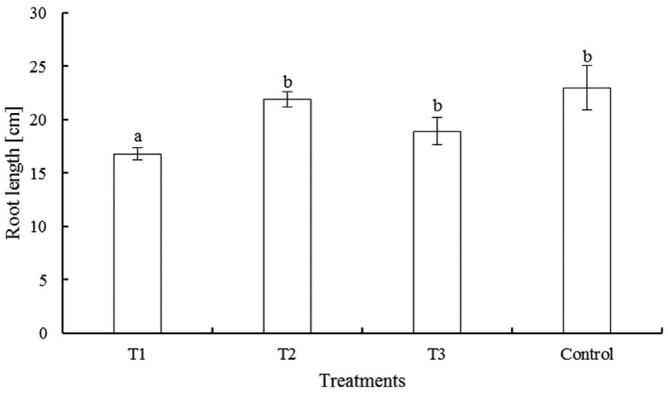

Figure 1. Root length (cm) of chive plants. treated with different concentrations of *Beauveria bassiana* three weeks after inoculation in the greenhouse at CPUT, Bellville, South Africa. N = 12. Bars with the same lowercase letters in the same column are not significantly different following Tukey test at the $p < 0.05$ level of significance and error bars represent standard error. T1 = 1×10^5, T2 = 1×10^4, T3 = 1×10^3 *B. bassiana* conidia mL^{-1}.

3.2. Chive growth

Generally, plant growth increased over time in both test and control. However, there was no significant difference ($df = 3, 44$; $p > 0.05$) in the leaf length between the *B. bassiana* treated and the control plants for all weeks (Table 1). Interestingly, the only exception was the root length; root lengths were statistically different among treatments ($df = 3, 44$; $p < 0.05$) with the highest concentration of conidial suspension yielding the shortest root length (Figure 1). While there were no significant differences ($df = 3, 44$; $p > 0.05$) among treatments for the number of leaves or leaf number increment, chives exposed to the highest concentration of conidial suspensions showed a slightly greater percentage increase in leaf number compared to the control and the other two fungus treatments from first to third week post-treatment (Table 2). Although root and leaf fresh and dry weights were comparatively lower in the fungus treated plants compared to their control counterparts, the differences were not significant ($df = 3, 44$; $p > 0.05$) (Table 3).

3.3. Quantification of secondary metabolites

Results from the chemical analysis showed that the secondary metabolite concentrations were statistically greater ($p < 0.05$) in the leaves than

Table 1. Leaf length (cm), total plant growth (cm; the difference between week one and week three), and percentage growth (%; the percentage change from week one to week three) of chive treated with different concentrations of *Beauveria bassiana* at one, two and three weeks after inoculation in the greenhouse at CPUT, Bellville, South Africa. N = 12.

	Leaf length (cm)				
Treatment[a]	Week one (baseline)	Week two	Week three	Total plant growth (cm)	Percentage growth (%)
T1	19.10 ± 0.70	25.20 ± 1.00	30.50 ± 1.10	11.40 ± 1.20	59.70
T2	19.60 ± 1.10	25.50 ± 0.80	32.50 ± 1.40	12.80± 1.40	65.80
T3	20.30 ± 1.10	26.80 ± 0.80	33.60 ± 0.80	13.30 ± 1.30	65.50
Control	21.20 ± 1.10	26.90 ± 0.90	33.50 ± 1.60	12.30 ± 1.50	61.10

[a] T1 = 1×10^5, T2 = 1×10^4, T3 = 1×10^3 *B bassiana* conidia mL^{-1}. There was no significant difference ($df = 3, 44$; $p > 0.05$) in the leaf length between the treated and the control plants at weeks 1, 2 and 3 post treatment.

Table 2. Number of leaves, increase of leaf number (the difference in the number of leaves between week one and week three), and percentage increase (%; the percentage change in the number of leaves from week one to week three) of leaves of chive treated with different concentrations of *Beauveria bassiana* at one, two and three weeks after inoculation in the greenhouse at CPUT, Bellville, South Africa. N = 12.

	Leaf number (cm)				
Treatment[a]	Week one (baseline)	Week two	Week three	Increase of leaf number	Percentage increase (%)
T1	9.50 ± 0.60	11.70 ± 0.5	13.40 ± 1.00	3.90	41.10
T2	8.60 ± 0.70	10.70 ± 0.80	11.70 ± 1.10	3.10	36.00
T3	8.50 ± 0.50	9.80 ± 0.80	11.70 ± 0.90	3.20	37.60
Control	10.80 ± 1.00	13.00 ± 1.30	14.70 ± 1.40	3.90	36.10

[a] T1 = 1×10^5, T2 = 1×10^4, T3 = 1×10^3 *B bassiana* conidia mL^{-1}. There was no significant difference (df = 3, 44; $p > 0.05$) in the leaf number between the treated and the control plants at weeks 1, 2 and 3 post treatment.

Table 3. Fresh weight (g) and dry weight (g) of roots and leaves of chive plants treated with different concentration of *Beauveria bassiana* entomopathogenic endophytic fungi three weeks after inoculation in the greenhouse at CPUT, Bellville, South Africa. N = 12.

	Fresh weight (g)		Dry weight (g)	
Treatment[a]	Roots	Leaves	Roots	Leaves
T1	4.10 ± 0.60	5.90 ± 0.90	0.48 ± 0.09	1.16 ± 0.33
T2	3.70 ± 0.90	5.20 ± 1.30	0.45 ± 0.14	0.99 ± 0.41
T3	3.80 ± 0.70	5.20 ± 0.90	0.40 ± 0.09	1.03 ± 0.35
Control	4.90 ± 0.80	6.40 ± 1.00	0.62 ± 0.15	1.18 ± 0.47

[a] T1 = 1×10^5, T2 = 1×10^4, T3 = 1×10^3 *B. bassiana* conidia mL^{-1}. There was no significant difference (df = 3, 44; $p > 0.05$) in the dry and fresh weights between the treated and the control plants.

in the roots, irrespective of whether plants were exposed to fungus or not (Table 4). However, while noticeably greater polyphenol and flavonol contents in the leaves were yielded by plants exposed to fungal inoculum at a concentration of 10^4 *B. bassiana* conidia mL^{-1}(T2) compared to the other plants, the difference was not significant ($p > 0.05$). Remarkably, the alkaloid content in the leaves of fungus exposed plants were significantly (df = 3, 8; F = 3.06; $p < 0.05$) greater than that of the control plants with T2 being about twice that of the control plant. Otherwise, there was no difference in the polyphenol and flavonol concentrations among treatments (df = 3, 8; $F_{polyphenol} = 0.44$; $F_{flavonols} = 1.33$; $p > 0.05$).

4. Discussion

The *B. bassiana* isolate used in the present study was able to endophytically colonize chive plants. The re-isolation of the fungus from chive leaf samples showed that the fungus was systemic, i.e., inoculum was transferred from the growth medium to the leaves. This is the first record of successful experimental inoculation and colonization of *B. bassiana* in chives. Previous studies have reported the colonization of *B. bassiana* in other plant species with different inoculation methods (Akutse et al., 2013; Quesada-Moraga et al., 2009).

Inoculation with *B. bassiana* did not improve the growth of chives over the control treatment (Tables 1, 2, and 3). In fact, the control plants had slightly higher biomass and root growth compared to the treated plants. These results differ from those reported in previous studies, which showed that *B. bassiana* promotes plant growth of cassava (*Manihot esculenta*), faba bean (*Vicia faba*) and cotton (*Gossypium hirsutum*) (Lopez and Sword, 2015; Greenfield et al., 2016; Jaber and Enkerli, 2016a and b). However, Lewis et al. (2001) reported no significant difference in the growth of maize (*Zea mays*) plants exposed to seed treatments with *B. bassiana* and the corresponding control treatment. Jaber and Enkerli (2016b) reported inconsistent endophyte-induced plant growth promotion across sampling dates following foliar inoculation of faba bean plants with *B. bassiana* (Naturalis®), *B. brongniartii* (BIPESCO2 and 2843) and *M. brunneum* (BIPESCO5). The root lengths varied significantly among treatments, and the shortest length was observed in plants

Table 4. Content of polyphenols (mg GAE/g DW), flavonols (mg QE/g DW), alkaloids (mg AE/g DW) in leaf and root samples of chives inoculated with *Beauveria bassiana* entomopathogenic endophytic fungi in the greenhouse at Bellville, South Africa. Data are the mean of 3 replicate chive plants. GAE = gallic acid equivalent, QE = quercitin equivalent, AE = atropine equivalent, DW = Dry weight.

Treatment[a]	Polyphenols (mg GAE/g DW)	Flavonols (mg QE/g DW)	Alkaloids (mg AE/g DW)
Leaves			
T1	7.41 ± 0.68aA	3.25 ± 0.40aA	3.14 ± 0.37aA
T2	8.16 ± 1.40aA	4.30 ± 0.41aA	3.76 ± 0.62aA
T3	6.78 ± 0.84aA	2.90 ± 0.66aA	2.98 ± 0.04aA
Control	7.00 ± 0.43aA	3.41 ± 0.55aA	1.96 ± 0.02bA
Roots			
T1	3.55 ± 0.65aB	0.70 ± 0.23aB	1.13 ± 0.04aB
T2	3.70 ± 0.66aB	0.65 ± 0.06aB	1.03 ± 0.05aB
T3	3.81 ± 0.78aB	1.08 ± 0.16aB	1.06 ± 0.03aB
Control	4.03 ± 0.46aB	0.96 ± 0.25aB	1.09 ± 0.05aB

[a] T1 = 1×10^5, T2 = 1×10^4, T3 = 1×10^3 *B. bassiana* conidia mL^{-1}. Means with the same lowercase letters in the same column, for roots or leaves, are not significantly different when leaf or root data for the different treatments are separately compared using Tukey test at the $p < 0.05$ level of significance. Means with the same uppercase letters in the same column are not significantly different when leaves and roots for corresponding treatments were compared using Tukey test at the $p < 0.05$ level of significance.

exposed to the highest concentration of fungal treatment, which may suggest that fungus might have had a negative effect on root length. In a more recent study, *B. bassiana* inoculation had a positive influence on plant growth parameters including root length of common beans (*Phaeseolus vulgaris*) (Afandhi et al., 2019). Nevertheless, the colonization of plant tissues by fungal endophytes can be influenced by many factors, such as inoculation method, species and fungal strain (Muvea et al., 2014; Afandhi et al., 2019). Fungal endophytes might affect the nutrient cycle and uptake of nutrients from the soil by plants (Saikkonem et al., 2015).

In this study, chemical analysis revealed that the leaves and roots of both *B. bassiana*-exposed and unexposed chive contained polyphenols, alkaloids and flavonols, and also that these secondary metabolites were significantly more concentrated in the leaves than in the roots. Chives and other *Allium* species, such as onion and garlic contain polyphenols, alkaloids, flavonoids, glycosides and organosulfur (Gulfraz et al., 2014; Soto et al., 2016; Sihombing et al., 2018). Furthermore, higher alkaloid content was detected in the leaves of plants inoculated with *B. bassiana* than in the control treated plants (Table 4). In a previous study, which involved the same fungal strain (strain: SM3) used in this study, drenching potted grapevine plants with its conidial suspension induced higher production of anti-insect volatile compounds including Naphthalene in the fungus-exposed plants compared to the control (Moloinyane and Nchu, 2019). The better yield of alkaloids in fungus-treated plants could be due to the synthesis of secondary metabolites by fungus in the plant tissues (Alvin et al., 2014; Lugtenberg et al., 2016). Also, endophytes can potentially induce host plants to accumulate secondary metabolites (Lugtenberg et al., 2016). Zhang et al. (2012) reviewed a wide range of bioactive alkaloids that are produced by endophytic fungi. Lozano-Tovar et al. (2013, 2017) reported that *B. bassiana* produce secondary metabolites that can induce antifungal activity. *B. bassiana* produces several biological active metabolites of the class of alkaloids such as tennelin, bassianin, pyridovericin, and pyridomacrolidin (Patocka, 2016). In the current study, since the specific alkaloid compounds were not detected, it is not possible to establish with certainty whether the higher total alkaloid content detected in the fungus-treated plants in this study was due to the direct production of alkaloids by *B. bassiana* or the fungus physiologically influenced the plant cells to produce more alkaloids. It is worth mentioning that fungal endophytes can produce mycotoxins in their host that are potentially harmful to livestock and humans (Azevedo et al., 2000).

In conclusion, this study demonstrated that endophytic entomopathogenic fungi could be used to improve the yield of alkaloids in medicinal plants. In order to further understand the influence of fungal endophytes on plant production of bioactive compounds, future studies involving detailed phytochemical elucidation of the bioactive constituents of fungus-treated plants are warranted.

Declarations

Author contribution statement

Friederike Espinoza: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Stefan Vidal, Francis Lewu: Analyzed and interpreted the data; Wrote the paper.

Fanie Rautenbach: Performed the experiments; Analyzed and interpreted the data.

Felix Nchu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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