Journal of Advanced Zoology

ISSN: 0253-7214

Volume 44 Special Issue-2 Year 2023 Page 2622:2636

The Potential of Beauveria against Root Mealybugs Formiccoccus sp. (Homoptera: Pseudococidae) Black Pepper in Dak Lak Province of Vietnam

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Article History

Received: 08 Aug 2023

Revised: 29 Sept 2023

Accepted: 12 Oct 2023

Abstract

Root mealybugs Formicoccus sp.(Hemiptera: Pseudococcidae) currently emerge as an economically important soil-borne insect pest in the production of black pepper (Piper nigrum L.) in Dak Lak province of Vietnam. Entomopathogenic fungi can be used in agricultural production as feasible safe biocontrol agents and plant growth promoters. This study aimed to isolate, select and identify the local Beauveria sp. strains from the black pepper fields, as well as evaluate the effects of the selected strains on the growth of black pepper. The results showed that the frequency of positive Beauveria sp. soil samples collected from organic black pepper fields was significantly higher than that from conventional black pepper fields. Twenty-one Beauveria sp. strains in black pepper organic fields and four Beauveria sp. strains in black pepper conventional fields were successfully isolated, among them, the Beauveria bassiana BB1 was examined and evaluated for its biocontrol potential against Formicoccus sp. and its ability to promote the growth of black pepper seedlings. In the laboratory experiments, the mortality of both adults and first instar nymph root mealybugs Formicoccus sp. caused by B. bassiana BB1 culminated 100% at 21 days after treatments. In the nethouse, at 6 months after treatment, the efficacy of B. bassiana BB1 against black pepper root mealybugs reached 99.18%. The levels of photosynthetic pigments in the leaves of the black pepper seedlings increased about 2 times compared to the control. Moreover, B. bassiana BB1 strain also remarkably promoted the growth of the black pepper seedlings under the nethouse conditions.

Keywords: Black pepper, Beauveria bassiana, root mealybugs, strain, nethouse, isolate,

1. Introduction

Black pepper (Piper nigrum L.) is a commercial spice crop that has significantly contributed to the economy of Vietnam's Central Highlands [1-3]. From 2014 to 2018, the price of peppercorns steadily increased, leading to a dramatic increase in the area under cultivation of black pepper in Vietnam [4]. However, due to severe infestation of pests, black pepper production in Vietnam is facing numerous challenges[1]. In recent years, the root mealybugs have emerged as one of the most significant insect pests impeding black pepper production in Vietnam[2] and India[5-6]. The black pepper root mealybug lives beneath the soil and feeds on the roots and underground stems of the black pepper vine[6]. This insect pest species attacks crops directly by sucking sap, causing symptoms such as yellowing, wilting, leaf defoliation, stunted growth, falling fruit, and crop death[5-6]. Seriously, because root mealybug is a soil-dwelling insect pest, it causes indirect black pepper vine damage by increasing the infectivity of soil-borne plant pathogens[7].

Generally, farmers cultivating black pepper in Vietnam's Dak Lak province often use chemical insecticides to control root mealybugs because it is simple to apply to manage root mealybugs. However, this method appears inefficient because root mealybugs typically associate with fungi to form thick mycelium layers that prevent insecticides from coming into contact with their bodies[8]. Furthermore, chemical insecticides have a negative impact on product quality, nontarget organisms, the ecological environment, and human health[9-12]. As the results attained, there has been a growing interest in researching alternative biopesticides to chemical pesticides in order to develop sustainable agriculture around the world[13]. Root mealybugs are living organisms in agricultural ecosystems that are also controlled by other animal species namely natural enemies. In which, entomopathogenic fungi are mentioned as a potential biological control agent for root mealybugs[6].

Beauveria is a well-known genus of entomopathogenic fungi that control a variety of agricultural insect pests. It includes several important species such as B. bassiana, B. brongniartii, B. amorpha, and B. caledonica[14-15]. Many commercial biopesticides based on B. bassiana and B. brongniartii have been approved and are being used for the biological control of various insect pests[14,16]. Beauveria can be found in most agricultural systems' soil, but its presence is influenced by a variety of factors, including cultivated practices associated with conventional or organic farming[17-18]. A large number of Beauveria isolates from various insects and mites have been reported around the world[19]. Beauveria isolates have also been reported as the crop growth promoters on grape vine[20](Vitis vinifera), chives (Allium schoenoprasum)[21], maize (Zea mays)[22-23], red chilli (Capsicum annuum) [24], and tomato (Solanum lycopersicum)[25]. However, there are differences in pathogenicity among Beauveria isolates against different hots insect species, as well as the ability of each plant species to promote the growth relevant to crop species[26-30]. Therefore, the objectives of this study aimed to (1) determine the presence of Beauveria sp. in soil between black pepper fields in the Dak Lak province of Vietnam; (2) isolate, select, and identify local Beauveria sp. strains from the black pepper field; and (3) then evaluate the potential of locally selected Beauveria sp. strains against black pepper root mealybugs (Formicococcus sp.) and their effects on the growth of black pepper seedlings.

2. Materials and Methods

2.1. Sample collection

Two hundred seventy soil samples were collected from black pepper fields in Dak Lak province's Buon Ma Thuot City, Cu Kuin District, and Krong Nang District. The samples were collected from nine organic fields and nine conventional fields at each location. Soil samples were collected at 5 points in each field using a diagonal system. After removing surface litter, the soil was collected with a trowel (at 20 cm of depth and 20 cm from the main root of the black pepper vines). The trowel was sterilized with 85% ethanol after each sample was collected. Soil samples were placed in 2.0-liter clean polyethylene bags, the necessary information was recorded, and the bags were stored in the lab's refrigerator at 3^{o}C.

2.2 Isolation and Identification

Each twenty-gram sample's soil was diluted with 180 ml distilled water containing 0.1% Tween 80 and vigorously shaken for 30-40 seconds in a 250 ml Erlenmeyer flask, yielding a 10^1 dilution. Transferred 1 ml of 10^1 dilution factor suspension to a 15 ml falcon tube with 9 ml distilled water resulting in 10^2 dilution factor. On separate 9-cm Petri dishes containing SDA 50 media, 0.1 ml of 102 dilutions was spread[15]. Dishes were incubated at 25^{o}C for 24 h, and colonies of individual fungal species were subcultured several times to obtain a pure strain.

Twenty grams of each sample were diluted with 180 ml distilled water containing 0.1% Tween 80 and vigorously shaken for 30-40 seconds in a 250 ml Erlenmeyer flask, yielding a 10^1 dilution. After that transferring 1 ml suspension 10^1 dilution factor to a 15 ml falcon tube with 9 ml distilled water yielded 10^2 dilution factor. The 0.1 ml of 10^2 dilutions was spread onto separate 9-cm Petri dishes containing SDA 50 media[15]. Dishes were placed in incubators at 25^{o}C for 24 h, and colonies of individual fungal species were subcultured sometimes to gain a pure strain.

A single spore was inoculated at the center of a 9-cm Petri dish containing PDA media and placed in an incubator maintained at 25°C and relative humidity of 65-70% for 14 days for morphological characterization. To compare, several colony characteristics (color, shape, edge, elevation, and texture), as well as some morphological characterizations of hyphae, conidiophores, and conidia (size, arrangement) were determined[31-32].

The fungal ITS gene was amplified for molecular identification using universal primers ITS1 and ITS4. The total reaction volume of 25 ml included gDNA purified using an in-house optimized protocol, 0.5 pmol of each primer, 200 M deoxynucleotides triphosphates (dNTPs), 0.5U thermostable DNA polymerase, supplied PCR buffer, and water. The PCR was carried out as follows: One cycle (98°C for 2 m) for initial denaturation; 25 cycles (98°C for 15 seconds; 60°C for 30 seconds; 72°C for 30 seconds) for annealing and extension; and one cycle (72°C for 10 m) for final extension. The PCR products were purified using a standard PCR clean-up procedure. Using the BigDye® Terminator v3.1 Cycle Sequencing Kit, the purified PCR products were subjected to bidirectional sequencing with universal primers M13F (-20) and M13R-pUC (-26). (Applied Biosystems). To identify the taxonomic positions of isolated strains, the nucleotide sequences of the 16S rRNA genes were compared to the published sequences in the DDBJ/Genbank/EMBL databases using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The Mega software version 6.0 was used to generate the phylogenetic tree with multiple data alignments.

2.3 Preparation of Conidial Suspensions

Conidia were harvested from each B. bassiana isolate after 14 days of growth on PGA medium at 25^{o}C, 12:12. (L:D). Conidia were scraped from the surface of the PGA medium and suspended in falcon tubes in sterile distilled water containing 0.05% Tween 80 to create a homogeneous conidial suspension. The spore concentration was measured using a Neubauer Improved Hemocytometer and adjusted to (10^9 cfu.ml-1) concentrations for the in vitro and nethouse trials.

2.4 Collection and rearing of Formicoccus sp.

In Dak Lak province, adults Formicoccus sp. were collected from the root of black pepper. Formicoccus sp. was grown on pumpkin to be used as a source for assays in the laboratory. The pumpkin was washed with tap water and disinfected with natri hypochlorite before use. To increase the chances of Formicoccus sp. sticking and establishing a colony on the pumpkin, each pumpkin was wrapped in a foam mesh bag[33-34]. A mealybug-infected pumpkin was placed in a plastic container and transferred to insect-rearing cages.

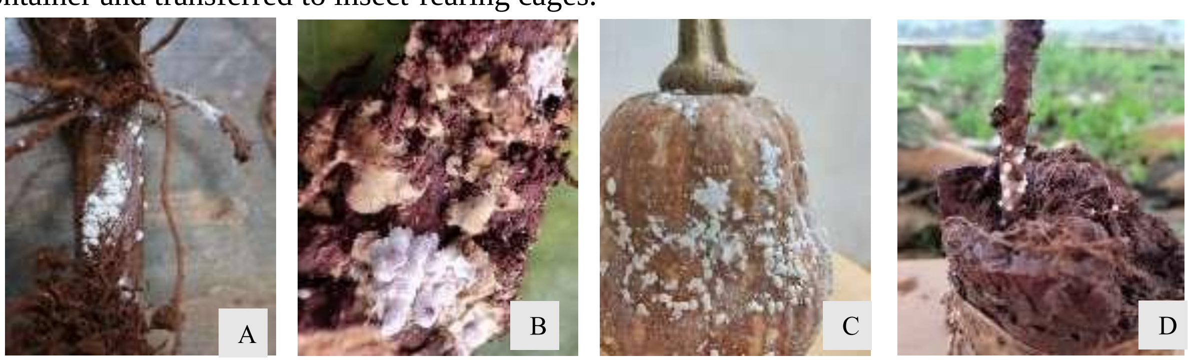

Figure 1. Black pepper root mealy bug: infested the roots and basal stem region underground (A), forming “crust over” on roots and basal stem region underground (B), reared on pumpkin (C), infested the basal stem region underground of seedlings

2.5 Pathogenicity of Beauveria sp. strains against mealybugs Formicococcus sp. in the laboratory

In quintuplicate, treatments containing isolated Beauveria sp. strains and one control treatment (CK) containing sterile water containing 0.05% Tween-80 were prepared. In each replicate, thirty female adults and thirty-first instar nymph mealybugs were dipped in 30s for each treatment. They are placed in a plastic container in the laboratory (25-30^{o}C room temperature, 70-80% relative humidity) after being transferred to the pumpkin wrapped in a foam mesh bag. The number of dead mealybugs was counted at 14 days after treatment.

2.6 Effect of B. bassiana on controlling mealybugs and the growth of black pepper in nethouse conditions

The Randomized Complete Block Design (RCBD) was set up with three blocks and five treatments randomized within each block for one replicate. Three experimental groups were treated with three B. bassiana isolates, control 1 (untreated with B. bassiana suspension) and control 2 (untreated with B. bassiana suspension and no infection with Formicoccus sp.). As a result, the experiment had fifteen plots, each with thirty pepper seedlings. One black pepper seedling (Vinh Linh local variety) was grown in a polythene bag with 0.5 kg of sterilized soil, sand, and organic fertilizer in a 2:1:1 ratio respectively[35]. When seedlings with five leaves were treated with 100 ml of B. bassiana suspension (10^9 cfu ml^{-1}). Ten Formicoccus sp. adults were released into each root of a black pepper seedling after 14 days. To keep moisture, tap water was used for irrigation once a day. This experiment lasted 6 months in total. The black pepper seedlings were removed from the soil to count the mealybugs, percentage of control (%)[36] and to determine some black pepper seedlings' growth parameters as formula below:

$$\text{Percent control } (\%) = (1 - \frac{\text{Population treated plot after treament}}{\text{Population in control plot after treatment}}) * 100$$

2.7 Data analysis

The Statistical Package for the Social Sciences 20 (SPSS 20) software is used for all analyses. The presence of Beauveria sp. in organic and conventional fields was investigated using the Chi-squared test. Other values were analyzed using simple variance analysis (ANOVA) to compare average values ($p \leq 0.05$ was considered significant).

3. Results and Discussion

3.1 Presence of Beaveria sp.

In all three locations, the presence of Beauveria sp. in soil samples from black pepper organic fields was statistically significantly higher than in conventional fields (Figure 1). The frequency of positive samples in organic fields in Buon Ma Thuot city was 83.7%, more than five times that of conventional fields (X^2 = 34.00, df=1, P 0.001). In the Cu Kuin district, the percentage of soil samples containing Beauveria sp. was 74.5% in organic fields and only

25.5% in conventional fields (X^2 = 23.00, df=1, P 0.001). Beauveria sp. was also statistically more common in organic fields rather than in conventional fields in Krong Nang district, with 76.2% and 23.8%, respectively (X^2 = 22.00; df = 1; P 0.001).

Many studies have been conducted to compare the effect of cultivated practice on the occurrence of entomopathogenic fungi. This is the first report of the presence of Beauveria sp. in soil from black pepper fields in Vietnam's Central Highlands. This finding also supports the conclusion that organic soils provide an ideal environment for the development of entomopathogenic fungi[13,37-39].

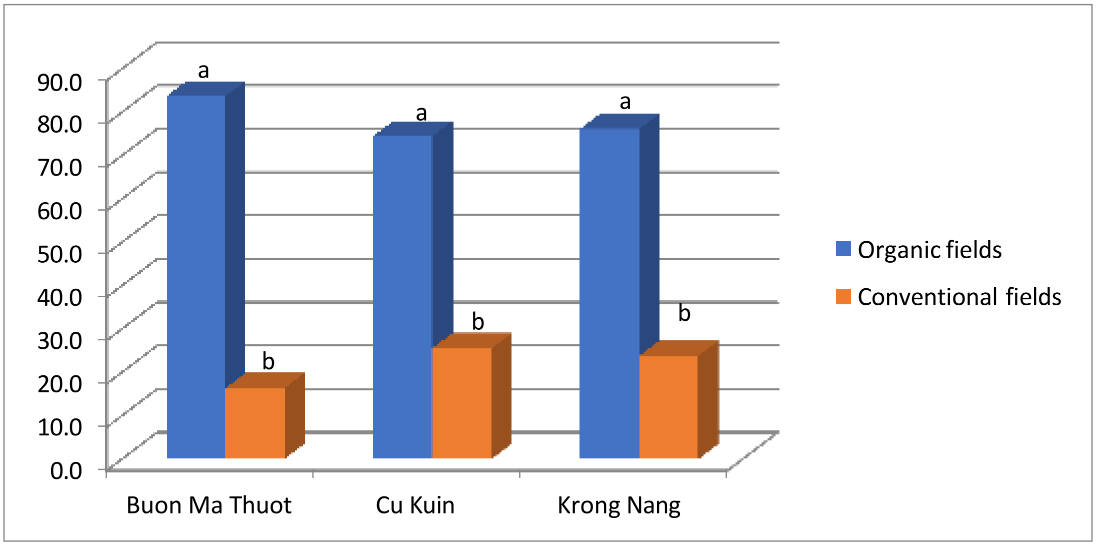

Figure 1. The frequency of Beauveria sp. isolation in soils from black pepper fields
The different superscript letter(s) above the column implied significantly different ($p \leq 0.05$) based on Duncan's multiple range test (alpha = 0.05)

3.2 Evaluation and identification of the high pathogenicity of Beauveria sp. Strains
3.3

Twenty-one Beauveria sp. strains were isolated from organic black pepper fields, but only four Beauveria sp. strains were isolated from conventional black pepper fields (Figure 2 and Figure 3). Table 1 shows the results of an in vitro test to assess the pathogenicity of twenty-five isolated local Beauveria sp. strains against root mealybugs (Formicoccus sp.). The pathogenicity of isolated local Beauveria sp. strains killed both adults and mealybugs first instar nymphs with statistical significance ($p \leq 0.05$). When compared to the control group, all Beauveria sp. strains caused statistically significant higher mortality in both adults and first instar nymphs (Tween 80 0.01%). In which, three strains (BB1, BC9, and BK17) demonstrated potent pathogenicity, with root mealybugs mortality reaching over 70%. The BB1 strain resulted in the highest mortality rate for both stages (100%), followed by BC9 (73.32% for female adults, 86.00% for first instar nymph) and BK17 (73.32% for female adults, 84.0% for first instar nymph), respectively.

Figure 2. Morphology of Beauveria bassiana

(A) colonies BB1 strain on PGA; (B)colonies BC9 strain on PGA; (C)colonies BK17 strain on PGA; (D) Conidia and phialides; (E) Conidia, Leng of one cell = 1µm.

Figure 3. Morphology of Formicoccus sp. Female adults uninfected (E); cadaver of Formicoccus sp. female adults infected with Beauveria bassiana BB1 (F); cadaver of Formicoccus sp. Female adults infected with Beauveria bassiana BC9 (G), cadaver of Formicoccus sp. female adults infected with Beauveria bassiana BK17 (H). Scale bars = 1cm.

Table 1. Mortality of Formicococcus sp. treated with Beauveria sp. strains in vitro test (21 days after treatment)

No	Isolates	Practice	**Mortality (%)** ± SD	
			Female adult	**First instar nymph**
1	BB1	Organic	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}
2	BB2	Conventional	18.02 ± 1.81 ^{i}	30.00 ± 2.33 ^{h}
3	BB3	Organic	53.32 ± 2.37 ^{cd}	57.34 ± 2.80 ^{cd}
4	BB4	Organic	50.66 ± 2.76 ^{cd}	57.36 ± 1.48 ^{cd}
5	BB5	Organic	40.66 ± 2.76 ^{ef}	48.02 ± 1.81 ^{ef}
6	BB6	Organic	52.68 ± 3.67 ^{cd}	56.00 ± 2.81 ^{cd}
7	BB7	Organic	54.68 ± 3.00 ^{c}	58.66 ± 3.80 ^{c}
8	BB8	Organic	52.66 ± 2.78 ^{cd}	60.00 ±2.33 ^{c}
9	BC9	Organic	73.32 ± 2.37 ^{b}	86.00 ± 4.35 ^{b}
10	BC10	Organic	49.3 ± 1.48 ^{d}	53.98 ± 1.52 ^{d}
11	BC11	Organic	54.0 ± 3.67 ^{c}	56.66 ± 3.35 ^{cd}
12	BC12	Organic	38.66 ± 5.05 ^{fg}	49.34 ± 1.47 ^{e}

13	BC13	Organic	43.34 ± 3.35 ^{e}	48.00 ± 2.99 ^{ef}
14	BC14	Organic	42.00 ± 3.35 ^{ef}	46.02 ± 1.52 ^{efg}
15	BC15	Conventional	35.32 ± 6.05 ^{gh}	44.02 ± 3.67 ^{fg}
16	BC16	Organic	51.32 ± 2.95 ^{cd}	56.00 ± 2.81 ^{cd}
17	BK17	Organic	73.32 ± 2.37 ^{b}	84.00 ± 4.35 ^{b}
18	BK18	Organic	19.34 ± 2.76 ^{i}	32.00 ± 2.99 ^{h}
19	BK19	Organic	33.3 ± 2.37 ^{h}	44.00 ± 2.81 ^{fg}
20	BK20	Organic	40.66 ± 2.76 ^{ef}	43.32 ± 2.37 ^{g}
21	BK21	Organic	54.00 ± 2.81 ^{c}	58.00 ± 5.04 ^{cd}
22	BK22	Conventional	21.98 ± 1.81 ^{i}	28.00 ± 2.99 ^{h}
23	BK23	Conventional	20.00 ± 2.33 ^{i}	28.68 ± 1.81 ^{h}
24	BK24	Organic	33.32 ± 2.37 ^{h}	48.00 ± 3.79 ^{ef}
25	BK25	Organic	34.66 ± 3.81 ^{gh}	49.98 ± 4.08 ^{e}
26	Control (Tween 80 0.01%)		5.34 ± 1.86 ^{j}	7.34 ± 2.80 ^{i}
F Value			213.7	202.5
Pr > F			<0.001	<0.001
CV (%)			6.88	5.83

The mortality of root mealybugs (Formicococcus sp.) in the same column within the same superscript letter(s) are not significantly different, based on Duncan's multiple range test (alpha = 0.05)

The 16S rRNA gene was sequenced to classify three Beauveria sp. strains that caused high mortality in vitro tests (Figure 4). The results revealed that all three potent strains (BB1, BC9, and BK17) were B. bassiana (Table 2). Our findings on the pathogenicity of local Beauveria sp. bolstered the conclusion that different entomopathogenic fungi strains have varying pathogenicity to adults and larvae of various insect pests[27]. The pathogenicity of the BB1 strain for black pepper root mealybugs is comparable to that of B. bassiana PPRC-56 (100%) but higher than that of B. bassiana FF (92%)[26]. Other locally isolated Beauveria sp. strains, on the other hand, were less pathogenic.

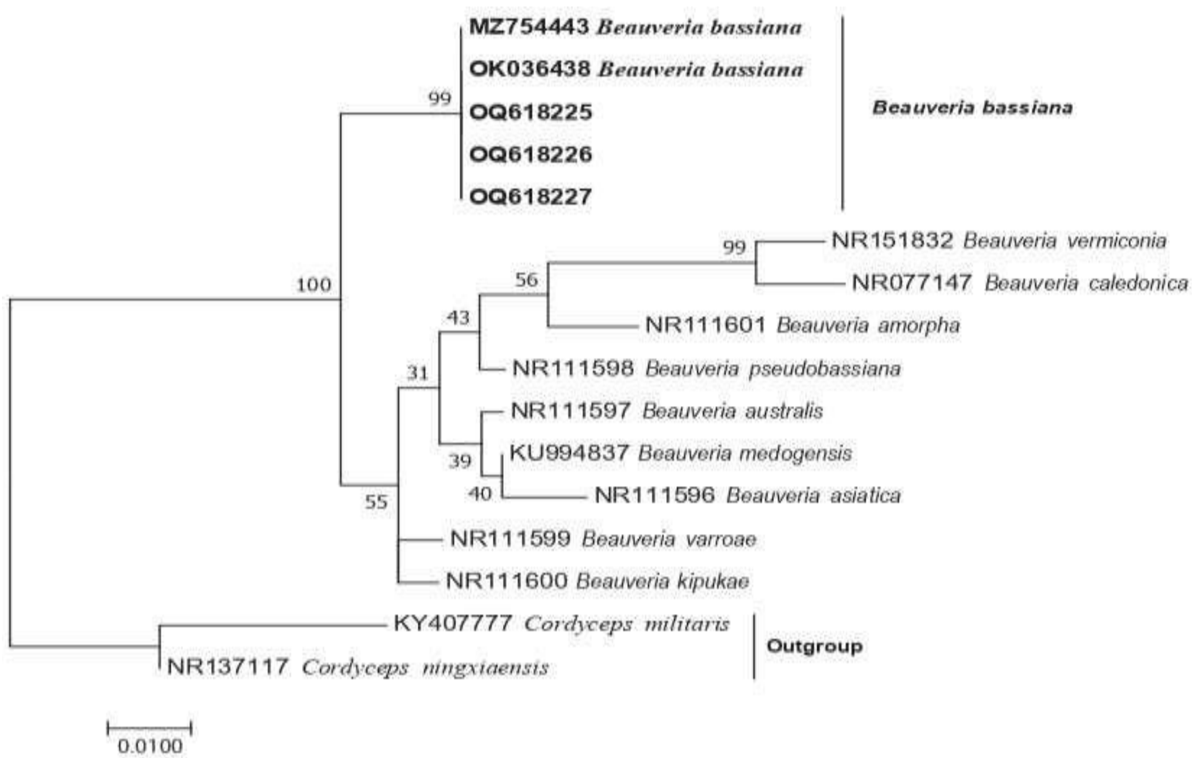

Figure 4. Phylogenetic analysis of Beauveria bassiana strains based on the ITS1-5.8S-ITS2 rRNA gene sequences. The phylogenetic tree was made as per Tamura 3-parameter model[41]. Estimation of the number of nucleotide substitutions when there were strong transition-transversion and G + C-content biases. Molecular Biology and Evolution 9:678-687.) and created using Mega software version 7.0[42]. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.) after multiple alignments of the data by Clustal W. The outgroup Cordyceps militaris and Cordyceps ningxiaensis and reference sequences were downloaded from Genbank.

Table 2. The accession IDs of 16s rRNA genes of the selected endophytic bacterial strains.

No	Fungal name	Accession Number
1	Beauveria bassiana BB1	OQ618225
2	Beauveria bassiana BC9	OQ618226
3	Beauveria bassiana BK17	OQ618227

3.4 Evaluation of potent of Beauveria bassiana strains in controlling Formicoccus sp. under Nethouse Conditions

The potency of three selected B. bassiana strains against Formicoccus sp. under nethouse conditions was assessed by the number of mealybug per plant and percent control (Table 3.). The findings revealed a significant difference in the potential biocontrol of selected B. bassiana strains. In which, BB1 strain had the highest biological potential against black pepper root mealybugs. After six months, the number of mealybugs in treatment with BB1 was only 0.18 individuals, which was 97 times lower than in control 1 (19.46 individuals). The selected B. bassiana strains exerted percent control against black pepper root mealybugs reaching from 87.04% to 99.18% after six months. The percent control of BB1 (99.18%) was statistically significantly higher than that of BC9 and BK17 (88.49% and 87.04%, respectively).

Table 3. The efficacy of Beauveria bassiana strains in controlling the number of black pepper root mealybugs under nethouse conditions (6 months after treatment)

No	Treatments	Mean number of mealybugs	Percent control (%)
1	BB1	0.18 ± 0.23^{a}	99.18 ± 1.10^{a}
2	BC9	2.22 ± 0.55^{ab}	88.49 ± 2.98^{b}
3	BK17	2.50 ± 0.22^{b}	87.04 ± 1.64^{b}
4	Control 1	19.46 ± 2.62^{c}	-
5	Control 2	0.00^{a}	-
	F Value	149.81	23.46
	Pr > F	<0.001	0.017
	CV (%)	23.92	2.59

Value in the same column within the same superscript letter(s) is not significantly different, based on Duncan's multiple range test (alpha = 0.05)

Table 4. The effect of local Beauveria bassiana strains on the levels of photosynthetic pigments in the leaves of the black pepper seedlings grown under nethouse conditions (6 months after treatment)

No	Treatments	Chlorophyll a ($mg \cdot g^{-1}$ FW)	Chlorophyll b ($mg \cdot g^{-1}$ FW)	Carotenoid ($mg \cdot g^{-1}$ FW)
1	BB1	0.58 ± 0.038^{a}	0.44 ± 0.051^{a}	0.40 ± 0.045^{a}
2	BC9	0.48 ± 0.032^{b}	0.34 ± 0.025^{bc}	0.31 ± 0.012^{bc}
3	BK17	0.47 ± 0.027^{b}	0.35 ± 0.014^{b}	0.29 ± 0.009^{c}
4	Control 1	0.28 ± 0.035^{c}	0.26 ± 0.004^{c}	0.19 ± 0.010^{d}
5	Control 2	0.52 ± 0.005^{ab}	0.40 ± 0.065^{ab}	0.36 ± 0.048^{ab}
	F value	41.762	18.296	19.294
	Pr > F	<0.001	<0.001	<0.001
	CV (%)	6.78	12.52	10.23

Value in the same column within the same superscript letter(s) is not significantly different, based on Duncan's multiple range test (alpha = 0.05), using SPSS version 20. FW: Fresh Weight.

The effect of local B.bassiana strains on the chlorophyll and carotenoid content in the leaves of the black pepper seedlings grown under nethouse conditions is shown in Table 4. The results showed that BB1 strain exerts a significantly higher influence on all three content of photosynthetic pigments than those of BC9, BK17 strains and control 1, but not significantly higher than that of control 2. The chlorophyll a, chlorophyll b and carotenoid content of black pepper leaves in the treatment with BB1 respectively reached (0.58, 0.44 and 0.40 $mg \cdot g^{-1}$ FW), whereas these figures in control 1 were only (0.28, 0.26 and 0.19 $mg \cdot g^{-1}$ FW), in treatment with BC9 strain were (0.48, 0.34 and 0.31 $mg \cdot g^{-1}$ FW), in treatment with BK17 strain were (0.47, 0.35 and 0.29 $mg \cdot g^{-1}$ FW).

The effects of selected B. bassiana strains on the growth-promoting activity of black pepper plants are shown in Table 5. The result indicated that BB1 had a positive effect on the growth, but negative effect on the percentage of yellowing symptoms. Length and fresh biomass of the root, plant height, leaf number per plant, and leaf area were significantly higher in treatment with BB1 than that in control 1 (no treatment with Beauveria sp. but infecting with Formicoccus sp.). The length of the root, fresh biomass of the root, plant height and the leaf number per plant in control 1 was 18.77 cm, 11.83 g, 119.70 cm, 17.07 leaves, which were about 3 folders higher than that of control 1 (6.27 cm, 3.71 grams, 30.27 centimeters and 5.94 leaves) respectively. Leaf yellowing is one of the symptoms caused by root mealybugs infecting the roots of black pepper. The percentage of yellowing symptoms in group 1 was only 8.84%, whereas there were 79.24% in control 1.

Our results showed that treatment with B.bassiana BB1 has a positive correlation on the growth of black pepper seedlings. This result could be due to a number of reasons: Firstly, B.bassiana BB1 killed and almost completely reduced the number of Formicoccus sp which led to the black pepper seedling was not damaged which increased the growth parameters. Whereas, treatments with a high number of root mealybugs result in black pepper seedlings being sucked sap which caused a decline in photosynthetic pigments, increase in the percentage of yellow leaves, and a decrease in growth indicators. These were equivalent to previous studies on symptoms caused by root mealybugs[5-6]. Secondly, the cadaver of root mealybugs being infected B.bassiana BB1 was able to be a source of nutrients for the growth of black pepper seedling[43-44]. Furthermore, B.bassiana BB1 could produce chemical compounds as plant-growth-improving agents[45].

Table 5. The effect of local Beauveria bassiana strains on some plant-promoting parameters of the black pepper seedlings grown under nethouse conditions (6 months after treatment)

T	LR (cm)	FBR (g)	PH (cm)	LN/Pt	LA (cm^2)	PYL (%)
BB1	18.77 ± 0.66 ^{a}	11.83±0.53^a	119.70±0.70^a	17.07±0.55^a	67.01±2.44^a	8.84±0.86^c
BC9	15.13 ± 1.12 ^{b}	6.40±0.55^c	82.96±2.65^b	14.91±1.30^b	51.94±0.90^c	24.65±1.43^b
BK17	14.70±1.53^b	6.13±0.52^c	80.77±2.08^b	13.84±0.34^b	50.71±1.30^c	27.31±1.69^b
Control 1	6.27±0.40^c	3.71±1.38^d	30.27±3.67^c	5.94±0.10^c	42.56±4.55^d	79.24±2.85^a
Control 2	17.03±0.12^a	10.36±0.08^b	114.74±3.55^a	16.78±0.70^a	62.02±1.02^b	12.54±0.86^c
F value	149.81	97.32	480.78	96.69	47.42	270.947
Pr > F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	6.70	7.58	3.291	5.83	4.44	9.22

T: Treatment; LR: Length of root; FVR: Fresh biomass of root; PH: Plant height; LN/P: Leaf number per plant; LA: Leaf area; PYL: % of yellow leaves. Value in the same column within the same superscript letter(s) is not significantly different, based on Duncan's multiple range test (alpha = 0.05), using SPSS version 20.

4. Conclusions

In conclusion, we have determined the positive effect of organic practices on the presence of Beauveria sp. in soil-cultivated black pepper in Dak Lak province of Vietnam. Notably, the local B. bassiana BB1 strain was successfully selected and identified as the most effective strain. B. bassiana BB1 strain demonstrated high efficacy in controlling root mealybugs. Moreover, this strain plays a role as a growth promoter for black pepper under nethouse conditions. The application of B. bassiana BB1 has resulted in a significant reduction in the population of root mealybugs, minimized yellowing symptoms as well as enhanced some plant- promoting parameters of the black pepper seedlings. Therefore, our finding suggests that B.bassiana BB1 may be used as both a biopesticide and biofertilizer for sustainable production of black pepper for sustainable agriculture production in this country.

Acknowledgements

We would like to express our sincere thanks to Dr. Dao Thi Hang (Vietnam Plant Protection Institute) for supporting this study in the identification of the black pepper root mealybugs Formicoccus sp. We also would like to thank some farmers in Dak Lak province for allowing us to collect soil and root mealybugs in their black pepper fields.

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