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Research

Controlling Soil-Borne Fungus *Aspergillus niger* in Groundnut By Optimizing The Function of Isolated *Bacillus* Bacteria

Nguyen Xuan Hieu[1], Nguyen Duc Huy[1], Nguyen Tien Long[1], Cao Thi Thuyet[2], Pham Thi Thuy Hoai[3,4], Nguyen Thi Thu Thuy[2]*

1. Institute of Biotechnology, Hue University, Thua Thien Hue 49000, Vietnam
2. University of Agriculture and Forestry, Hue University, Thua Thien Hue 49000, Vietnam
3. MienTrung Institute of Scientific Research, Vietnam National Museum of Nature, Vietnam
4. Academy of Science and Technology (VAST), Thua Thien Hue 49000, Vietnam
*Corresponding author: nguyenthithuthuy@huaf.edu.vn, nttthuy.huaf@hueuni.edu.vn

ABSTRACT

Collar rot is a devastating disease caused by the soil-borne pathogen *Aspergillus niger* that greatly affects groundnut production worldwide. The long-term persistence of the fungus in the soil can reduce the effectiveness of synthetic fungicides. Recently, significant attention has been raised to the use of the biological control method such as the application of antagonistic microorganisms, which potentially decline the number of spores and eradicated *A. niger* from the soil. In the present study, three *Bacillus* strains (*Bacillus siamensis* 101, *B. siamensis* 112 and *B. velezensis* 137) isolated from the rhizosphere soil of groundnut cultivation farms were found to inhibit the growth of *A. niger* mycelia by 53.6% to 60.8% *in vitro*. In pot experiments, the supplementation of this mixture of three bacterial strains (namely BAZ04) strongly reduced the collar rot symptoms of groundnut with a biocontrol efficacy of 100% compared to nil (no treatment). Field trials demonstrated the efficiency of BAZ04 in controlling collar rot disease, which increased the yield by 20.5–22.7% compared to the untreated plots. These results suggest that BAZ04 is a potential biocontrol agent for collar rot disease.

Key words: *Aspergillus niger*, *Bacillus*, biocontrol, collar rot disease, groundnut

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) or peanuts is a species belonging to the family Fabaceae and ranks second after soybeans as the essential oil crop grown worldwide. Due to its wide adaptability to different climates and soils, groundnuts are widely grown in subtropical and tropical areas with the largest groundnut cultivation in Asia, Africa, North America, and South America. The top countries growing groundnuts include India, China, America, Senegal, Myanmar, Brazil, and Argentina (Arya *et al*., 2016; Le *et al*., 2018; Lora *et al*., 2019; Nathawwat *et al*., 2021). Currently, the areas cultivated by groundnut are expanding in some countries such as Malawi, Sudan, Thailand, and Vietnam. In Vietnam, groundnuts have been widely cultivated and become one of the top groundnut producers in the world, (Fabra *et al*. 2010). In 2020, the total area under cultivation was 177.040 thousand ha, with an average yield of 2.51 tons/ha and a total production of 438.860 tons (Nguyen *et al*., 2023). However, groundnut crops are strongly vulnerable to diseases from plant pathogenic fungi, particularly those originating in the soil such as *Aspergillus spp*., *Sclerotium rolfsii*, *Rhizoctonia solani* (Janila *et al*., 2013; Le *et al*., 2018; Lora *et al*., 2019; Nguyen *et al*., 2023). These fungal diseases cause several damages including plant death, yield loss, decreasing nutrition value, and secreting toxins in seeds that lead to dangerous diseases for humans and livestock (Qaiser *et al*., 2018; Navale *et al*., 2021; Wei *et al*., 2023).

Collar rot is a disease caused by the population of *A. niger* which may lead to significant yield loss globally (Janila *et al*., 2013; Mohammed *et al*., 2014; Le *et al*., 2018; Lora *et al*., 2019; Nguyen *et la*., 2023). For example, annual global yield losses due to this disease account for approximately 13% - 52% (Kumari & Singh, 2017). In Vietnam, the mortality rate of plants by the fungus ranges from 9% to 30% (Nguyen *et al*., 2023). This disease occurs in two phases: pre-emergence and post-emergence. In the pre-emergence phase, the symptom of collar rot may include rotted seeds, thus reducing the emergence rate. In the post-emergence phase, the disease attacks the stem tissues near the ground surface, resulting in rot, wilting, and death of the plant (Harsukh *et al*., 2011).

Collar rot disease in groundnuts has been commonly controlled through cultural practices and fungicide application (Omugo *et al*., 2018; Gikas *et al*., 2022). The use of fungicides often results in chemical residues both in soils and nuts, which risks to human health after consumption (Okigbo, 2016). Moreover, the application of fungicides over time can result in reduced biodiversity, environmental pollution, and increased fungal resistance (Rao & Nnaji, 2017). Due to these concerns, synthetic chemical fungicides have been alerted to minimize their application and/or replaced with biological methods (Li *et al*., 2016).

The use of antagonistic bacteria for biocontrol has become increasingly popular and widespread (Putri *et al*., 2020; Kim *et al*., 2021). Among these, *Bacillus* spp. is a well-known bacteria that can be a biocontrol agent (Kuzina *et al*., 2019). Several reports suggest that Bacillus species can promote plant growth and inhibit the growth of fungal pathogens in plants. In particular, Kim *et al*. (2021) found that *Bacillus* subtilis had a high level of antagonism against five phytopathogenic fungi, including *Sclerotinia minor*, *Fusarium oxysporum, Colletotrichum gloeosporioides, Alternaria solani, Sclerotinia sclerotiorum,* and *Rhizoctonia solani.* Additionally, a study by Xu *et al*. (2016) demonstrated the effectiveness of *Bacillus* in controlling *Magnapothe grisea, R. solani,* and *C. gloeosporioides.* Moreover, a *Bacillus* sp. strain S20D12 produces the lipopeptide surfactin, therefore inhibiting the growth of *Sclerotium* fungus that causes groundnut stem rot (Le *et al*., 2018). However, little information is known about the effectiveness of *Bacillus* strains in controlling groundnut collar rot disease in Central Vietnam.

This study aimed to assess the effectiveness of three *Bacillus* strains previously isolated from peanut growing areas in Central Vietnam in preventing groundnut collar rot disease under *in vitro*, glasshouse, and field conditions.

MATERIALS AND METHODS

Materials

The pathogenic strain: *A. niger* QT1 was supplied by the Laboratory of Enzyme and Protein Technology at the Institute of Biotechnology, Hue University. It was isolated from groundnut plants infected with collar rot disease on variety L14 and identified through morphological characteristics, Koch's postulates, and molecular biology analysis (Nguyen *et al*., 2023).

Antagonist bacteria: Three bacterial strains, *Bacillus siamensis* 101, *Bacillus velezensis* 137, and *Bacillus siamensis* 112 were isolated and identified by our team (Nguyen *et al*., 2024). These strains were stored in glycerol stock at -80 °C until use.

The groundnut variety used in the glasshouse and field experiments, L14, was provided by the Institute of Biotechnology, Hue University.

Methods

Antagonist assay of *Bacillus* strains

Two qualitative methods (Antagonistic assay & diffusion method) were used to test the antagonistic ability of *Bacillus* strains against *A. niger* QT1. Prepare fungus: *A. niger* QT1 was grown on PDA medium in petri dishes and incubated at 28 °C ± 2 for seven days. Sterilized distilled water was added to the plates and fungal cells were scraped with a sterile inoculating loop. The cell suspensions were then filtered through sterile papers, and fungal spores were quantified using a Neubauer chamber under a light microscope. The fungal cell concentration was adjusted to 1×10^6 spores/mL for further study (Nguyen *et al*., 2024). Prepare antagonistic bacteria: Three bacterial strains (*B. siamensis* 101, *B. velezensis* 137 & *B. siamensis* 112) activated in LB (Luria-Bertani) medium and incubated at 30 °C for 24 hr at 180 rpm. The concentration of cell suspension was adjusted according to the 0.5 McFarland scale until reaching approximately 1×10^8 cfu/mL with sterile distilled water (Nguyen *et al*., 2024).

Antagonistic assay was performed using the dual culture technique on the potato dextrose agar PDA, as described by Anith *et al*. (2021). A well with a 5 mm diameter was created in PDA plates using a corkborer. Following this, 50 microliters of bacterial suspension were added to the well. An agar piece

of 3-day-old mycelia of QT1 strain was dropped at the opposite side of the well containing bacterial suspension. An identical set was prepared as the control except for *Bacillus* suspension culture. All plates were incubated at 30 °C until the fungal mycelia in the control plate reached the corners. The growth of *A. niger* QT1 mycelia was measured and compared with the control of average *A. niger* QT1 growth of the control without treatment. The assay was replicated three times. The Growth Inhibition Rate (GIR) was measured as described by Li *et al*. (2016).

$$\% \text{ GIR} = \frac{C-T}{C} \times 100$$

Where,

C: Size of fungal mycelial growth in the control (mm)

T: Size of fungal mycelial growth in treatment (mm)

In the second method, The antifungal activity of *Bacillus* strains against *A. niger* QT1 was tested using the agar diffusion method. Briefly, one milliliter of *A. niger* QT1 spores suspension (1×10^6 spores/mL) was melt-inoculated onto PDA (Potato Dextrose Agar) medium plates. Then, a corkborer was used to make wells with a 5 mm diameter in PDA medium. Fifty microliters of bacterial suspension were added to each well (Kuzina *et al*., 2019). A well added with sterile distilled water was assessed as the control. The results were evaluated after 48 hr of incubation. The antagonistic potential of each bacterium was determined by the diameter of growth inhibited zone on the agar plate. The experiment was done in duplicate and carried out three times to ensure the reproducibility of results.

Glasshouse experiments

The effectiveness of a mixture of three bacterial strains (*B. siamensis* 101, *B. velezensis* 137, and *B. siamensis* 112) in controlling groundnut collar rot disease was tested in the glasshouse at the Institute of Biotechnology, Hue University. To prepare for the infection study: Three bacterial strains (*B. siamensis* 101, *B. velezensis* 137 & *B. siamensis* 112) were grown separately until reaching approximately $1x10^8$ cfu/mL. Then 30 mL of cell suspension containing a mixture of three Bacillus strains was prepared in a 1:1:1 ratio for each replication.

The experiment was conducted in a randomized complete design with three replicates and five plants per replication. The treatments were as follows:

N1: Plants were uninfected *A. niger* QT1, groundnut seeds, and plants were treated with sterile water (uninfected plants);
N2= Plants were infected with *A. niger*, groundnut seeds, and plants treated with sterile water (negative control);
N3 = Plants were infected *A. niger*, groundnut seeds, and plants were treated with a mixture of three *Bacillus* strains;
N4= Plants were infected with *A. niger*, groundnut seeds, and plants were treated with a commercial fungicide, Ridomil Gold 68WG (Metalaxyl M + Mancozeb) (Syngenta Company, Vietnam) (positive control).

In this experiment, methods of infection with *A. niger* fungus and spraying antagonistic bacteria on groundnut plants were carried out as described by Nguyen *et al*. (2024). Groundnut seeds were soaked in 10 mL of mixed bacterial (1×10^8 cfu/mL) for 1 hr, then 5 seeds were sown in each pot. For N1 and N2 treatments, the groundnut seeds were treated with sterile water. Groundnut seeds of treatment N4 were treated with Ridomil Gold 68WG. Then, groundnut seeds were cultivated in a plastic pot measuring 35 × 30 × 30 cm containing 5 kg of sterilized soil. Five seeds were planted in each pot. All pots were placed in a glasshouse condition. Fourteen days after sowing (4 leaf stage), each pot poured 20 mL of mixed bacterial suspension at $1x10^8$ cfu/mL into the soil around the roots. After one day, each pot was inoculated with 20 mL of *A. niger* QT1 spore suspension $1x10^6$ spores/mL into the soil around the roots. The same solution without fungal inoculum was used to set the N1 treatment. For N4 treatment, seeds and plants were treated with Ridomil Gold 68WG as per the manufacturer's instructions.

Mortality rates (MR) were observed and recorded at 5, 7, 14, and 21 days post-infection (dpi) for each treatment. Biocontrol efficacy is calculated according to Yadav *et al*., 2023.

$$\text{Biocontrol effication (\%)} = \frac{\text{MR of control - MR of treatment}}{\text{MR of control}} \times 100$$

Field trials

The mixture of three antagonistic bacteria (*B. siamensis* 101, *B. venelensis* 137, and *B. siamensis*) was hereafter referred to as BAZ04. A field trial was conducted to test the effectiveness of BAZ04 against *A. niger* in L14 variety groundnut. The trials were carried out in two sandy soils, central provinces of Vietnam including Quang Tri (16°51′N 107°06′E) and Thua Thien Hue (16°26′N 107°42′E), where collar rot disease in groundnut was severely affected (Nguyen *et al*., 2023).

The experiment was conducted following a randomized complete block design (RCBD) with two treatments: CT1 (control, the untreated farmer field) and CT2 (treatment with biocontrol product BAZ04). The experiment consisted of three blocks with three replications (a, b, c), and each plot had a size of 30 m^2 (15 m × 2 m). The plants were spaced 10 cm within each row and 30 cm between each row. Before sowing groundnut seeds, BAZ04 product is applied to the beds with a final density of Bacillus of 10^6 cfu/cm^2 of soil.

The treated and untreated groundnut fields were applied with chemical fertilizer (per hectare) of 40kg of nitrogen (N), 60 kg of phosphorus (P_2O_5), 80 kg of potassium (K_2O), and 500 kg of calcium ($Ca(OH)_2$). Before sowing, 100% P2O5, 50% K2O, 50% N and 50% Ca(OH)2 are applied. When the seedlings have 3 - 4 leaves, 50% N and 50% K2O are applied. The remainder of Ca(OH)2 fertilizer is applied at flowering.

The collar rot (*A. niger*), stem rot (*Sclerotium rolfsii*), and root rot (*Rhizoctonia solani*) diseases were monitored weekly by evaluating a square of 10 m² (2 m × 5 m) area in the center of each plot. The plant mortality rate (MR) was counted. The groundnut yield obtained from the experimental plots was used to calculate the yield per hectare.

$$\text{MR (\%)} = \frac{\text{Total dead plants}}{\text{Total number of plants observed}} \times 100$$

Statistical analysis.

The data presented in this study are shown as mean ± SD (standard deviations). The data collected from both glasshouse and field experiments were statistically analyzed by the ANOVA method (SPSS software, version 22). The significant differences (p<0.05) among the treatments were determined by Duncan's test or t-test.

RESULTS

Evaluation of antagonist activity of *Bacillus* strains *in vitro* condition

The results of the antagonistic assay indicated that *Bacillus* strains were effective in inhibiting the mycelium growth of *A. niger* QT1. *B. siamenses* 101 inhibited *A. niger* growth diameter by 56.5%, while the inhibition rate by the treatment of *B. siamenses* 112 and *B. velezensis* 137 were 53.6% and 58.1%, respectively. Meanwhile, the mixture of three bacteria (*B. siamenses* 101, *B. siamenses* 112, and *B. velezensis* 137) increased the inhibition up to 60.8% (Table 1, Figure 1).

Table 1. The antifungal activity of *Bacillus* strains against *A. niger* QT1 by dual culture method

S. No	Isolate	Growth Inhibition Rate (%)
1	*B. siamenses* 101	56.5^c
2	*B. siamenses* 112	53.6^d
3	*B. velezensis* 137	58.1^b
4	Mixture of three bacteria	60.8^a

Means in the identical column with varying letters exhibit significant differences (*P≤0.05*)

In the test measuring the fungal inhibition zone of *Bacillus* strains, the results showed that different *Bacillus* strains reduced the growth rate of *A. niger* QT1 at different degrees. *B. siamenses* 101, *B. siamenses* 112, and *B. velenzesis* 137 had inhibition zones of 26.6, 25.4, and 28.3 mm respectively. The mixture of three bacterial strains yielded the highest inhibition zone diameter of 30.5 mm (Table 2, Figure 2). Overall, the three-strain combination expressed the most effective against *A. niger* QT1, based on both mycelium growth and inhibition zones *in vitro* tests.

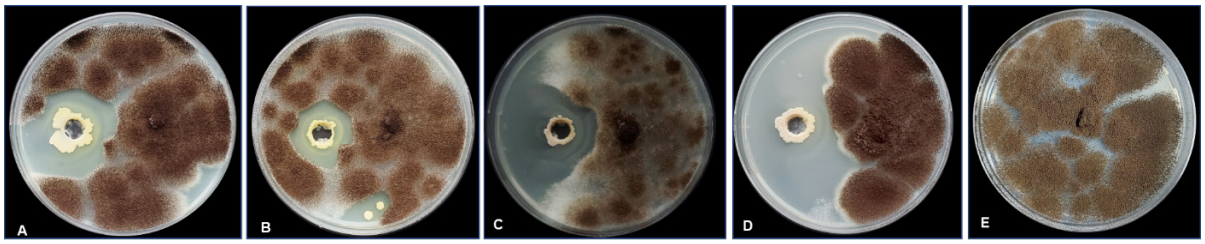

Fig. 1. Antagonist assay of *Bacillus* strains against *A. niger* QT1 by dual culture method on PDA. (A) *Bacillus* sp. 101, (B) *Bacillus* sp. 112, (C) *B. velezensis* 137, (D) Mixture of three bacteria, (E) Control

Table 2. The antifungal activity of *Bacillus* strains against *A. niger* QT1 by agar diffusion method

S. No	Isolate	Diameter of inhibition zones (mm)
1	*B. siamenses* 101	26.6^{c}± 0.54
2	*B. siamenses* 112	25.4^{c}±0.46
3	*B. velezensis* 137	28.3^{b}±0.61
4	A mixture of three bacteria	30.8^{a}±0.58
5	Control	0.0^{d}

Means ± SD in the identical column with varying letters exhibit significant differences (*P≤0.05*)

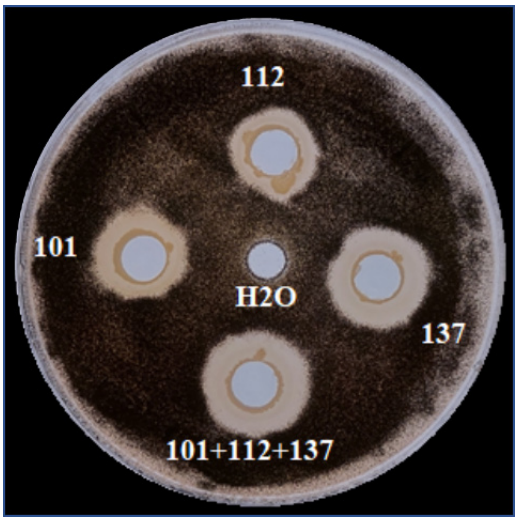

Fig. 2. Antagonist assay of *Bacillus* strains against *A. niger* QT1 by agar diffusion method (101. *B. siamenses* 101; 112. *B. siamenses* 112; 137. *B. velezensis* 137; 101+112+137. Mixture of three bacteria; H2O: Sterile water)

Evaluation of antagonistic activities of *Bacillus* strains under glasshouse conditions

The results showed that the biocontrol agents were successful in suppressing the disease (Table 3). At 5 days after inoculation, the antagonistic bacteria treatment was found to be highly effective in inhibiting the mortality rate of groundnut (MR=0) while the control was severely affected by the disease (MR=26.7%). The chemical fungicide treatment showed a decrease in the mortality rate of plants compared with the control. At 7 days, the mortality rate of plants increased significantly in both the control (MR=60%) and the chemical fungicide treatment (MR=26.67%). However, there were no disease symptoms observed in plants following the application of antagonistic bacteria (Figure 3). At 14 and 21 days, the control had the highest mortality rate (100%), while the lowest mortality rate (0%) was observed in plants inoculated with the mixture of three bacterial strains. In the uninfected group, groundnut plants grew well and there were no symptoms of collar rot disease observed throughout the experiment. From the results above, it was also observed that the mixture of three bacterial strains was the most promising for controlling *A. niger* QT1, with a biocontrol efficacy of 100%.

Table 3. Biocontrol efficacies of *Bacillus* strains against groundnut collar rot under glasshouse condition

Treatment	Mortality rate (%) of groundnut at day post inoculation				Biocontrol Efficacy 21DAI (%)
	5 dpi	7 dpi	14 dpi	21 dpi	
Uninfected plants	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	100.00
Negative control	26.67^{a}	60.00^{a}	100.00^{a}	100.00^{a}	-
Positive control	6.67^{b}	26.67^{b}	46.67^{b}	46.67^{b}	53.33
A mixture of three bacteria	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	100.00

Means in the identical column with varying letters exhibit significant differences ($P<0.05$)

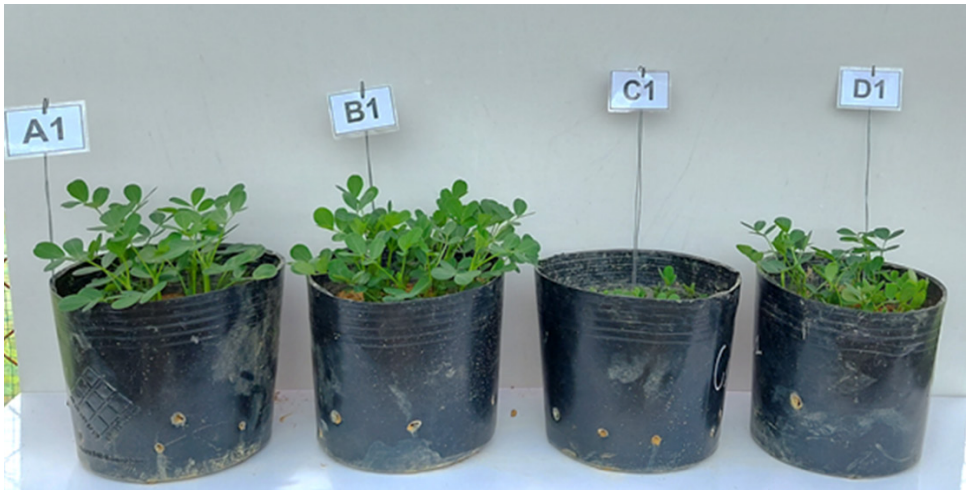

Fig. 3. Biological control of collar rot of groundnut by *Bacillus* strains under net house condition at 7 DAI (A1. Uninfected plants; B1. Mixture of three bacteria treatment; C1. Negative control; D1. Chemical treatment)

Evaluation of biological control of *Bacillus* strains under field condition

The plant mortality rate caused by collar rot (*A. niger*), stem rot (*Sclerotium rolfsii*), and root rot (*Rhizoctonia solani*) diseases differed in the nil and BAZ04 treatment at both experimental sites. In the Quang Trị site, the application of BAZ04 resulted in a decrease of 51.2 to 69.2% in plant mortality rate as compared to the control fields. Similarly, in the Thua Thien Hue site, BAZ04 had a much lower rate of plant mortality compared with nil (Table 4, Figure 4).

The yield of groundnut in the Quang Tri site was 2.44 tons/ha for nil but 3.07 tons/ha for the BAZ04 treatment. there was a significant increase in the yield of groundnut under the supplementation of BAZ04 up to 20.5% compared to the control.

Similarly, in Thua Thien Hue, the yield of groundnut in fields treated with BAZ04 was 2.95 tons/ha compared to 2.28 tons/ha in control fields. BAZ04 was found to significantly increase the yield of groundnut up to 22.7%, which is notably different compared to the untreated field (Figure 5).

Table 4. Biological control of collar rot in groundnut by *Bacillus* strains under farmer field conditions

Disease	Quang Tri				Thua Thien Hue			
	MR (%) control	MR (%) BAZ04	% difference between control and BAZ04	T-test (p)	MR (%) control	MR (%) BAZ04	% difference between control and BAZ04	T-test (p)
Collar rot (*A. niger*)	11.40	3.50	69.2	0.0231	6.5	1.1	83.1	0.0025
Stem rot (*Sclerotium rolfsii*)	8.60	4.20	51.2	0,0462	4.2	1.0	76.2	0.0361
Root rot (*Rhizoctonia solani*)	5.20	2.10	59.6	0.0414	4.0	1.2	70.0	0.0392

DISCUSSION

The present study demonstrated the effectiveness of using a mixture of three isolated Bacillus strains to control the mycelium growth of *A. niger*, suggesting its potential replacement for chemical fungicides against collar rot disease in groundnut. Data from 2 field trials showed that using the biological product BAZ04 (A mixture of three strains of *B. siamenses* 101, *B. siamenses* 112 & *B. velezensis* 137) resulted

in decreased plant mortality rates. Additionally, BAZ04 was found to be beneficial for crop growth and improved the yield of groundnut. Therefore, the mixture of *B. siamenses* 101, *B. siamenses* 112, and *B. velezensis* 137 holds the potential to be developed as agricultural inoculants to prevent phytopathogens and increase crop yield. Our results support previous findings by Xu *et al*. (2016) that antagonists were effective in managing plant diseases. *B. velezensis* by inhibiting the growth of many pathogenic fungi such as *Cylindrocladium quinqueseptatum*, *Helicobasidium purpureum*, and *Cryphonectria parasitica*. In other reports, *B. velezensis* acts as biocontrol agent to inhibit various pathogen fungi such as *Rhizoctonia solani*, *Aspergillus flavus*, and *Fusarium oxysporum* (Chowdhury *et al*., 2013; Cao *et al*., 2018; Chen *et al*., 2019). Additionally, Shereen *et al*. (2023) showed that *B. amyloliquefaciens* RaSh1 expresses excellent biocontrol ability to fungus *Alternaria alternata* and positively increases the growth rate of black pepper. Moreover, *Bacillus* strains can enhance several physiological processes of plants such as emergence, root length, and plant height extension, which contribute to increasing the yield of crops (Sayyed *et al*., 2010; Hashem *et al*., 2019; Dong *et al*., 2023). Dong *et al*. (2023) reported that *B. cereus* DW019 enhanced the productivity of cherry tomatoes and promoted the accumulation of phytochemicals.

Fig. 4. Biological control of collar rot in groundnut by *Bacillus* strains under farmer field condition (The circles indicate the symptoms of plant wilting & death)

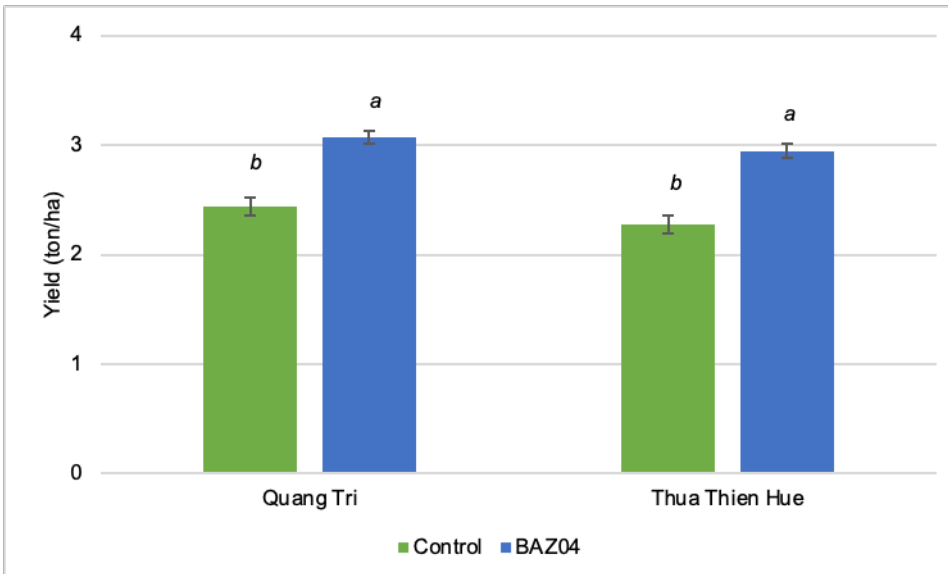

Fig. 5. Effect of Bacillus strains on the yield of groundnut cultivar L14 under field conditions in Quang Tri and Thua Thien Hue provinces, Vietnam. Bars are standard errors. Different letters indicate significant differences between the treatments within the site.

The reduction in plant mortality rates in the presence of the three *Bacillus* strains tested in this study may be related to the ability of these strains to inhibit mycelia growth of *A. niger* QT1. Each bacterial strain had inhibitory effects on *A. niger* (>50%, Table 1) in the PDA medium. The mixture of three bacteria (*B. siamenses* 101, *B. siamenses* 112, and *B. velezensis* 137) increased the growth inhibition by 60.8% compared with that measured in the control. This is consistent with our previous study (Nguyen *et al*. 2024) that these *Bacillus* strains can inhibit the growth of fungus *A. niger* by producing cell wall hydrolytic enzymes (endo-1,3-β-glucanase, chitinase & cellulase).

The application of BAZ04 to soils (a mixture of three *Bacillus* strains) appeared to be more effective and beneficial than the use of a commercial fungicide in controlling collar rot disease. The results from the glasshouse experiment found an immediate effect of Ridomil Gold 68WG (Metalaxyl M + Mancozeb) within 5 days after infection. However, its effectiveness reduced over time, with an increase in MR to 26% after 7 days and 46% after 21 days. The growing reliance on chemical fungicides to protect crops and maximize yields has negative impacts on human health, animal welfare, and the environment (Dadrasnia *et al*., 2020). Meanwhile, using rhizosphere microorganisms proves to be an efficient method for disease control, promoting growth, and ultimately enhancing crop productivity (Huruna *et al*., 2021; Shereen *et al*., 2023). Several studies reported that rhizosphere bacteria such as *Streptomyces* spp., *Pseudomonas* spp., and *Bacillus* spp. act as potential biocontrol agents for various pathogen fungi in replacement of chemical fungicides (Jangir *et al*., 2018; Andric *et al*., 2020; Stefany Castaldi *et al*., 2021; Shereen *et al*., 2023). Importantly, numerous *Bacillus* species have been used as effective biocontrol agents for the inhibition of plant pathogens (Jangir *et al*., 2018; Andric *et al*., 2020). *Bacillus* sp. is a versatile bacterium that employs various direct and indirect mechanisms to increase plant growth rate and control pathogen proliferation. These mechanisms include nitrogen fixation, mineralization and solubilization of nutrients such as phosphorus and potassium, production of hydrolytic enzymes, phytohormones, antimicrobial compounds, and abiotic stress tolerance through competition for nutrients, parasitism, and stimulation of host plant defenses (Zhang *et al*., 2020; Sharma *et al*., 2021; Yánez-Mendizábal *et al*., 2021; Mirskaya *et al*., 2022; Bathke *et al*., 2022; Ajuna *et al*., 2023).

Although the present study observed the effectiveness of using BAZ04 against collar rot disease in groundnut and to increase the yield from field sites, the influence of these bacterial strains on nodulation and nitrogen fixation of groundnut needs further investigation.

CONCLUSION

The results from *in vitro* conditions showed that the mixture of three antagonistic bacteria strains (*B. siamensis* 101, *B. siamensis* 112, *B. venelensis* 137) had a greater ability to inhibit *A. niger* compared to each bacterium alone. In the glasshouse and field conditions, it has been proven that applying the mixture of antagonistic bacteria to soils had the highest effectiveness in reducing plant mortality caused by *A. niger* on groundnut crops grown in two field trials in Quang Tri and Thua Thien Hue provinces, Vietnam. Importantly, our findings suggested that an appropriate application of three antagonistic bacteria strains together could increase the yields in groundnuts by 20.5 - 22.7 %.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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