HUE UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY
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NGUYEN XUAN HIEU

STUDY ON THE BIOLOGICAL ANTAGONISM AGAINST *ASPERGILLUS* sp. CAUSING COLLAR ROT DISEASE ON GROUNDNUT (*ARACHIS HYPOGAEA*) IN QUANG TRI PROVINCE AND HUE CITY

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LIST OF PUBLISHED WORKS

[1]. Nguyen Xuan Hieu, Nguyen Thi Minh Nga, Nguyen Duc Huy, Nguyen Quang Co, Cao Thi Thuyet, Pham Thi Thuy Hoai, Nguyen Thi Thu Thuy (**2023**). **Identification and characterization of *Aspergillus niger* causing collar rot of groundnut (*Arachis hypogaea*)**. *Biodiversitas*, *24*(5). 2556-2562.

https://doi.org/10.13057/biodiv/d240507

[2]. Nguyen Thi Minh Nga, **Nguyen Xuan Hieu**, Nguyen Tien Long, Nguyen Duc Huy and Nguyen Thi Thu Thuy (2024). **Isolation and characterization of antagonistic bacterial strains for biocontrol of collar rot (*Aspergillus niger*) in groundnut (*Arachis hypogaea* L.).** *Chiang Mai J Sci 2024* 51(2): e2024022.

https://doi.org/10.12982/CMJS.2024.022

[3]. Nguyen Xuan Hieu, Nguyen Duc Huy, Nguyen Tien Long, Cao Thi Thuyet, Pham Thi Thuy Hoai and Nguyen Thi Thu Thuy (**2024**). **Controlling soil-borne fungus *Aspergillus niger* in groundnut by optimizing the function of isolated *Bacillus* bacteria**. *Malaysian Applied Biology*, 53(2), 134-144.

https://doi.org/10.55230/mabjournal.v53i2.3012

[4]. Nguyen Xuan Hieu, Nguyen Đuc Huy, Ton Đuc Hong, Nguyen Tien Long, Thai Thi Huyen, Nguyen Thi Thu Thuy (**2024**). **Evaluation of *Trichoderma asperellum* HL6 against *Aspergillus niger* Van Tieghem causing collar rot in groundnut.** Hue University Journal of Science: *Agriculture and Rural Development*, 133(3C), 41-55.

https://doi.org/10.26459/hueunijard.v133i3C.7496

INTRODUCTION

1. RATIONALE AND SIGNIFICANCE OF THE STUDY

Peanut (*Arachis hypogaea* L.) is an important upland crop in central Vietnam, contributing to sandy soil improvement and farmers' livelihoods. However, its productivity is severely constrained by collar rot caused by *Aspergillus niger*, leading to serious yield losses and potential food safety risks due to ochratoxin A production. Current disease control relies mainly on chemical fungicides, which cause environmental pollution and resistance. Therefore, the study entitled "**Biological antagonism against *Aspergillus* sp. causing collar rot of peanut in Quảng Trị and Huế City**" aims to develop safe and effective biological control solutions to support sustainable peanut production.

2. RESEARCH OBJECTIVES

To screen and select indigenous microbial strains producing extracellular enzymes (chitinase and β-1,3-glucanase) with strong antagonistic activity against *Aspergillus* sp. causing peanut collar rot; to evaluate their antagonistic efficacy under in vitro and in vivo conditions; and to elucidate the underlying mechanisms as a scientific basis for proposing safe and effective biological control strategies.

3. RESEARCH CONTENTS

Content 1: Isolation and identification of *Aspergillus* sp. causing peanut collar rot (black mold).

Content 2: Screening and selection of indigenous microbial strains antagonistic to pathogenic *Aspergillus* sp.

Content 3: Determination of the activity of extracellular enzymes antagonistic to *Aspergillus* sp.

Content 4: Evaluation of the inhibitory effects of selected microbial strains against pathogenic *Aspergillus* sp. under in vitro and in vivo conditions.

4. RESEARCH SUBJECTS

- *Aspergillus* sp. strains causing peanut collar rot in Quảng Trị and Huế.

- Indigenous antagonistic microbial strains with strong production of extracellular enzymes (chitinase and β-1,3-glucanase) isolated from peanut-cultivated soils in Quảng Trị and Huế.

- The local peanut cultivar L14.

5. SCOPE OF THE STUDY

5.1. Study period: From December 2021 to June 2025.

5.2. Study locations: Disease and soil sampling and field experiments were conducted in peanut-growing areas of Quảng Trị and Huế; laboratory and net-house experiments at Hue University; SEM analysis at the University of Science and Education – The University of Danang.

6. NOVEL CONTRIBUTIONS OF THE DISSERTATION

The study identified *Aspergillus niger* as the causal agent of peanut collar rot in Quảng Trị and Huế and selected three indigenous *Bacillus* strains (*B. siamensis* 101, *B. siamensis* 112, and *B. velezensis* 137) with strong antagonistic activity. The β-1,3-glucanase–mediated antagonistic mechanism was clarified, culture conditions were optimized for high enzyme activity, and the efficacy of the mixed formulation BAZ04 was preliminarily evaluated under in vitro and in vivo conditions.

7. SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE DISSERTATION

7.1. Scientific significance

The dissertation identified the causal agent of peanut collar rot, selected indigenous *Bacillus* strains with strong antagonistic activity, and elucidated the antagonistic mechanism mediated by β-1,3-glucanase.

7.2. Practical significance

The results demonstrate the potential application of the BAZ04 formulation as a safe, effective, and sustainable biological control solution for peanut production.

Chapter 1
OVERVIEW OF THE RESEARCH PROBLEM

Peanut production in central Vietnam is severely constrained by collar rot caused by the soil-borne fungus *Aspergillus niger*. As chemical control poses environmental and resistance risks, biological control using antagonistic microorganisms such as *Bacillus* and *Trichoderma*, particularly through β-1,3-glucanase activity, represents a sustainable alternative. However, studies under local ecological conditions in Quảng Trị and Huế remain limited, highlighting the need to select indigenous antagonistic microorganisms and clarify the role of β-1,3-glucanase.

Chapter 2
RESEARCH METHODS

Isolation and screening of microorganisms: Pathogenic *Aspergillus* sp. were isolated and identified, and indigenous antagonistic microorganisms were screened by dual-culture and agar diffusion assays, with molecular identification based on ITS (fungi) and 16S rRNA (bacteria) gene sequences.

Enzyme characterization and antagonistic mechanisms: β-1,3-glucanase activity and optimal conditions were determined, and antagonistic mechanisms were evaluated through minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays, fungal biomass reduction, and scanning electron microscopy (SEM).

Efficacy and biosafety evaluation: Antifungal efficacy was evaluated under in vitro, in vivo, and net-house conditions, while biosafety was assessed based on hemolytic activity, antibiotic susceptibility, effects on soil organisms, and indole-3-acetic acid (IAA) production.

Chapter 3
RESEARCH CONTENTS AND RESULTS

3.1. Isolation and identification of the pathogenic fungus

Nine representative pathogenic fungal strains from diseased areas were selected for detailed morphological analysis (Figure 3.2).

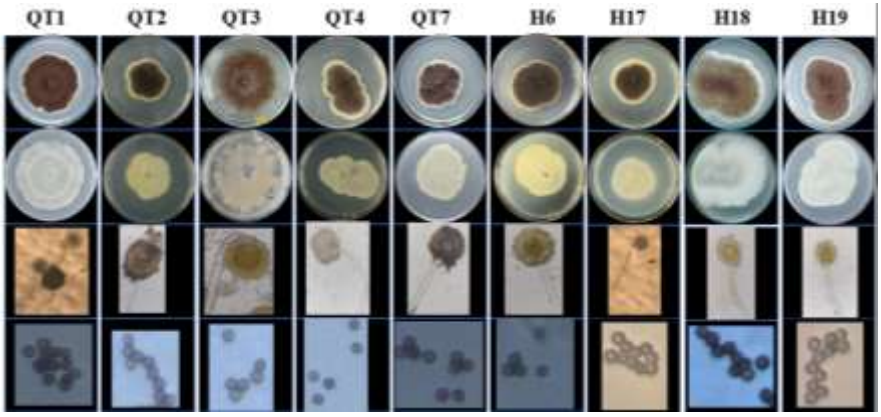

Figure 3.2. *Morphological characteristics of fungal isolates causing peanut collar rot (black mold) cultured on PDA medium*

Pathogenicity test based on Koch's postulates: All nine fungal strains were pathogenic, causing symptoms within 3–6 days, with plant mortality ranging from 85.7% to 100%; strains QT1 and H17 showed the highest and statistically significant mortality.

Molecular identification: were identified by morphological characteristics and ITS1–ITS4 sequence analysis, showing 100% similarity to *Aspergillus niger*, thereby confirming the pathogenic strains as A. niger (Figure 3.5).

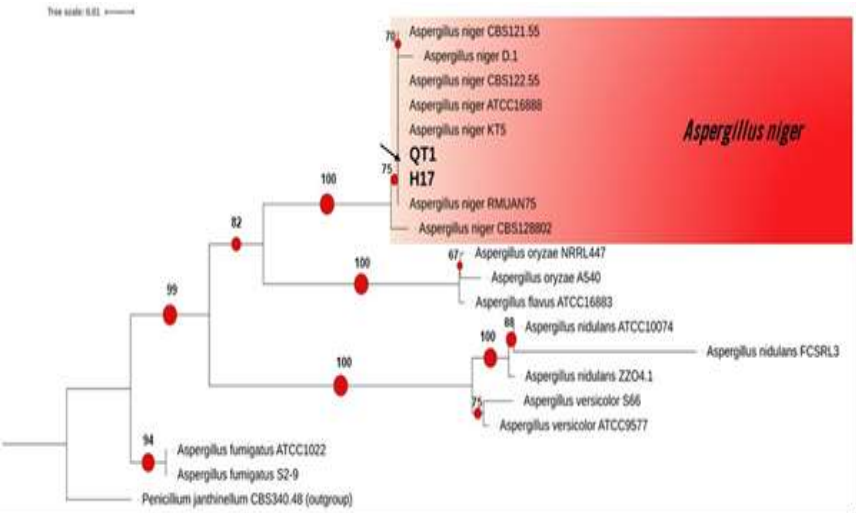

Figure 3.5. *Phylogenetic tree of A. niger strains QT1 and H17*

3.2. Isolation and screening of antagonistic microorganisms against *A. niger*

3.2.1. Isolation and primary screening of antagonistic strains

From 12 soil samples collected from peanut fields in Quảng Trị and Huế, 144 microbial strains antagonistic to *A. niger* QT1 and H17 were isolated. Among these, 16 potential strains were selected for in vitro antagonistic evaluation (Table 3.5; Figure 3.6).

Table 3.5. *Inhibition rates of microbial strains antagonistic to pathogenic fungi QT1 and H17 after 6 days of incubation*

No.	Isolated strain	Inhibition rate (%)	
		QT1	H17
1	H1	45,20 ± 0,46 ^{gh}	45,21 ± 0,35 ^{d}
2	**K1**	**61,60 ± 0,05** ^{b}	**45,50 ± 0,35** ^{d}
3	K2	49,86 ± 0,13 ^{ef}	41,15 ± 0,18 ^{e}

No.	Isolated strain	Inhibition rate (%)	
		QT1	H17
4	**B8**	**55,38 ± 0,18** ^{d}	**45,61 ± 0,08** ^{d}
5	45K	41,05 ± 0,19 ^{i}	46,44 ± 0,20 ^{dc}
6	46K	45,10 ± 0,03 ^{h}	45,54 ± 0,08 ^{d}
7	**101**	**58,60 ± 0,18** ^{c}	**54,56** ± 0,12 ^{b}
8	**112**	**52,10 ± 0,04** ^{e}	**52,28** ± 0,07 ^{b}
9	118	46,90 ± 0,06 ^{gh}	42,63 ± 0,12 ^{e}
10	**137**	**57,14 ± 0,13** ^{cd}	**57,63** ± 0,15 ^{a}
11	VL1	37,04 ± 3,40 ^{k}	35,82 ± 3,36 ^{f}
12	Đak3	46,67 ± 2,23 ^{gh}	40,83 ± 2,28 ^{e}
13	**HL6**	**77,41 ± 1,28** ^{a}	**48,30 ± 1,22** ^{c}
14	PhĐ3	47,78 ± 2,94 ^{fg}	27,64 ± 2,61 ^{g}
15	TPH4	50,37 ± 2,31 ^{e}	26,91 ± 2,18 ^{g}
16	PhV1	61,48 ± 0,64 ^{b}	23,28 ± 2,31 ^{h}

Different letters within the same column indicate statistically significant differences among means at p < 0.05 (Duncan's test). M ± SD: mean ± standard deviation.

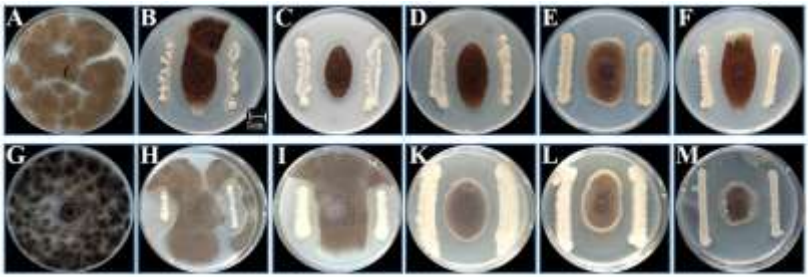

Figure 3.6. *Antagonistic activity of bacterial strains against the pathogenic fungus on PDA after 6 days (QT1: A–F; H17: G–M; strains B8, K1, 101, 112, and 137).*

3.2.2. Selection of antagonistic microorganisms with strong extracellular enzyme activity

Further screening based on hydrolysis zone diameters identified 11 strongly antagonistic strains with notable extracellular enzyme activity (Table 3.6; Figure 3.9).

Table 3.6. *Extracellular enzyme production capacity of strongly antagonistic microbial strains (mm)*

No.	Microbial strain	Hydrolysis zone diameter (mm)		
		1,3- β -glucanase	**Chitinase**	**Cellulase**
1	H1	$18,50 \pm 0,20^{d}$	$20,47 \pm 0,15^{c}$	$17,70 \pm 0,62^{d}$
2	K1	$24,97 \pm 0,15^{ab}$	$25,83 \pm 0,15^{a}$	$25,47 \pm 0,06^{a}$
3	K2	$22,77 \pm 0,25^{bc}$	$23,30 \pm 0,20^{b}$	$24,03 \pm 0,15^{ab}$
4	B8	$26,17 \pm 0,42^{a}$	$26,10 \pm 0,10^{a}$	$25,77 \pm 0,25^{a}$
5	45K	$20,07 \pm 0,31^{cd}$	$19,37 \pm 0,15^{cd}$	$18,73 \pm 0,21^{cd}$
6	46K	$22,23 \pm 0,38^{bc}$	$22,93 \pm 0,21^{bc}$	$22,97 \pm 0,15^{b}$
7	101	$23,20 \pm 0,36^{b}$	$22,53 \pm 0,06^{bc}$	$21,23 \pm 0,31^{bc}$

No.	Microbial strain	Hydrolysis zone diameter (mm)		
		1,3- β -glucanase	Chitinase	Cellulase
8	112	23,93 ± 0,31 [b]	22,87 ± 0,23 [bc]	21,90 ± 0,79 [bc]
9	118	21,03 ± 0,15 [c]	18,17 ± 0,15 [d]	20,10 ± 0,10 [c]
10	137	21,03 ± 0,15 [c]	20,47 ± 0,06 [c]	20,67 ± 0,31 [c]
11	HL6	18,30 ± 0,18 [d]	18,45 ± 0,15 [d]	17,68 ± 0,20 [d]

Different letters within the same column indicate statistically significant differences among means at $p < 0.05$ (Duncan's test). M ± SD: mean ± standard deviation.

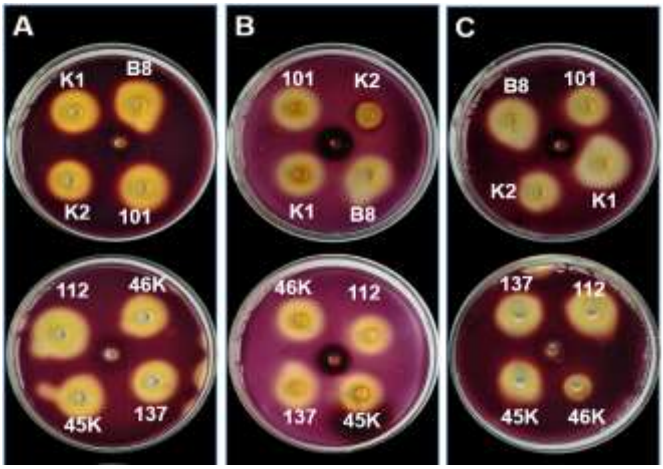

Figure 3.9. *Enzymatic activities of β-1,3-glucanase (A), chitinase (B), and cellulase (C) of selected isolated microbial strains*

3.2.3. Identification of antagonistic Microorganisms

Six strongly antagonistic isolates with high extracellular enzyme activity were identified by morphological and molecular analyses, including five *Bacillus* strains (*B. siamensis* B8, *B. siamensis* 101, *B. siamensis* 112, *B. velezensis* K1, and *B. velezensis* 137) and one fungal isolate identified as *Trichoderma asperellum* (Figures 3.11 and 3.13).

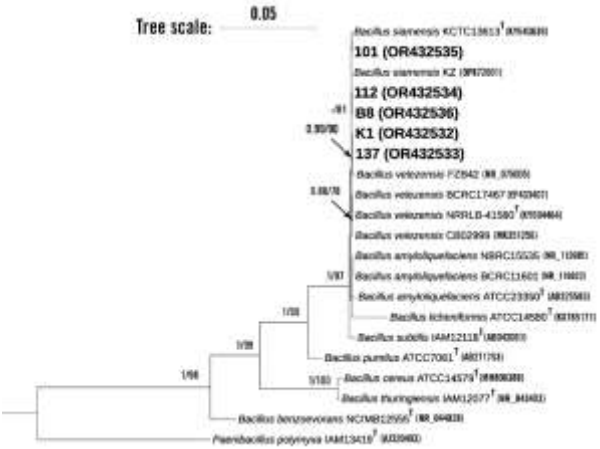

Figure 3.11. *Phylogenetic tree based on nucleotide sequences of the 16S rRNA gene of isolates B8, K1, 101, 112, and 137 and reference strains, with Paenibacillus polymyxa used as the outgroup.*

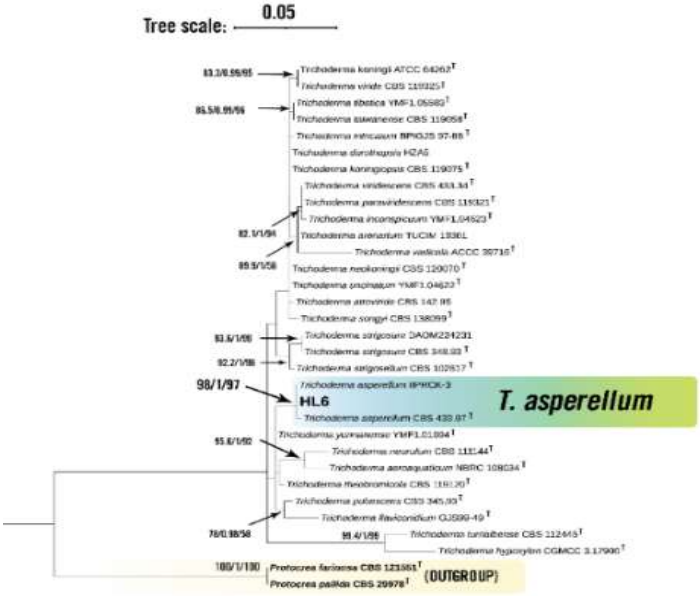

Figure 3.13. *Phylogenetic tree based on ITS sequences of the fungal isolate HL6.*

3.2.4 Screening antagonistic microorganisms safe for peanut germination

Peanut seeds (L14) treated with 6 selected microbial strains showed high germination rates (98.33–99.67%), comparable to the control with no statistically significant difference.

3.2.5. Screening for antagonism against *A. niger* in net-house conditions

Across both pathogenic strains (QT1 and H17), *Bacillus* 101 and 112 achieved the highest efficacy (86.67%), followed by Bacillus 137 (83.33%). *Trichoderma* HL6, *Bacillus* B8, and K1 showed lower efficiencies, all below 80%.

3.2.6. Compatibility assessment of antagonistic bacterial strains

Figure 3.14. *Antagonistic interaction among the three selected Bacillus strains on LBA medium after 48 h of incubation.*

3.2.7. Role and antagonistic mechanism of beta-1,3-glucanase against *A. niger* in vitro

3.2.7.1. Evaluating *A. niger* inhibition on solid media

Table 3.12. *Antagonistic activity against A. niger of cell-free culture filtrates from individual and mixed Bacillus strains assessed by the agar diffusion method*

Bacterial strain	Diameter of growth inhibition of pathogenic fungal strains (mm)	
	***A.niger* QT1**	***A.niger* H17**
B. siamensis 101	26,6± 0,54 [c]	21,1± 0,44 [c]
B. siamensis 112	25,4±0,46 [c]	20,3±0,46 [c]
B. velezensis 137	28,3±0,61 [b]	23,5±0,53 [b]
Mixture of three *Bacillus* strains	**30,8±0,58 [a]**	**26,8±0,66 [a]**
Control	0,0±0,00 [d]	0,0±0,00 [d]

3.2.7.2. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Individual beta-1,3-glucanase from *B. siamensis* 101, 112, and *B. velezensis* 137 showed MIC = MFC = 0.4 U/mL against *A. niger* QT1 and H17. When combined at a 1:1:1 ratio, fungicidal efficacy significantly increased, with MIC/MFC reaching 0.1/0.2 U/mL (QT1) and 0.2/0.4 U/mL (H17), maintaining an MFC/MIC ratio of 2 (Table 3.13).

Table 3.13. *Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of the tested strains determined using a 96-well microplate assay*

Enzyme source	Pathogen control *A. niger* QT1			Pathogen control *A. niger H17*		
	MIC	**MFC**	**MFC/MIC**	**MIC**	**MFC**	**MFC/MIC**
B. siamensis 101	0,4	0,4	1	0,4	0,4	1
B. siamensis 112	0,4	0,4	1	0,4	0,4	1
B. velezensis 137	0,4	0,4	1	0,4	0,4	1
Hỗn hợp 3 vi khuẩn	0,1	0,2	2	0,2	0,4	2

MFC was defined as the concentration at which no fungal growth was observed on PDA plates after 48 h following subculturing from the corresponding microplate wells.

3.2.7.3. Evaluating A. niger Inhibition in Liquid Media

The inhibition of pathogenic fungal biomass by individual and combined bacterial strains is shown in Table 3.13. and 3.14.

Table 3.14. *Effect of beta-1,3-glucanase on the (dry) biomass of A. niger QT1*

Beta-1,3-glucanase source	**Nồng độ enzyme *β-1,3-glucanase* (U/mL)**				
	0	0,2	0,4	0,8	1,6
B. siamensis 101	3,92±0,12 ^{e}	3,63±0,11 ^{dC}	2,32± 0,22 ^{cC}	1,81±0,11 ^{bC}	1,35±0,07 ^{aC}
B. siamensis 112	3,92±0,12 ^{e}	3,60±0,10 ^{dC}	2,28±0,18 ^{cC}	1,73±0,08 ^{bC}	1,26±0,14 ^{aC}
B. velezensis 137	3,92±0,12 ^{e}	3,57±0,18 ^{dB}	2,11±0,04 ^{cB}	1,38±0,06 ^{bB}	1,06±0,04 ^{aB}
Enzyme blend from three sources	**3,92± 0,12 ^{e}**	**3,53±0,12 ^{dA}**	**1,67±0,15 ^{cA}**	**1,05±0,04 ^{bA}**	**0,64±0,07 ^{aA}**
ANOVA analysis (P-value)					
Enzyme source (Factor 1)				p < 0,001	
Enzyme concentration (Factor 2)				p < 0,001	
Interaction (Enzyme source × Enzyme concentration)				p < 0,001	

Different letters (a, b, c...) indicate statistically significant differences between enzyme concentrations at p < 0.05 (Duncan's test). Different capital letters (A, B, C...) indicate statistically significant differences between enzyme sources at p < 0.05 (Duncan's test). M±SD: Mean ±Standard deviation.

Table 3.15. *Effect of beta-1,3-glucanase on the (dry) biomass of A. niger H17*

Beta-1,3-glucanase source	**Nồng độ enzyme *β-1,3-glucanase* (U/mL)**				
	0	0,2	0,4	0,8	1,6
B. siamensis 101	3,19±0,20 ^{eC}	3,06±0,16 ^{dC}	2,25±0,13 ^{cC}	1,63±0,11 ^{bC}	1,39±0,08 ^{aC}
B. siamensis 112	3,19±0,20 ^{eBC}	3,01±0,20 ^{dBC}	2,13±0,22 ^{cBC}	1,52±0,12 ^{bBC}	1,29±0,10 ^{aBC}
B. velezensis 137	3,19± 0,20 ^{eB}	2,99±0,13 ^{dB}	1,94±0,51 ^{cB}	1,32±0,10 ^{bB}	1,06±0,16 ^{aB}
Enzyme blend from three sources	**3,19±0,20 ^{eA}**	**2,97±0,23 ^{dA}**	**1,54±0,19 ^{cA}**	**1,03±0,02 ^{bA}**	**0,75±0,10 ^{aA}**
ANOVA analysis (P-value)					
Enzyme source (Factor 1)				p < 0,001	
Enzyme concentration (Factor 2)				p < 0,001	
Interaction (Enzyme source × Enzyme concentration)				p = 0,085	

Different letters (a, b, c...) indicate statistically significant differences between enzyme concentrations at p < 0.05 (Duncan's test). Different capital letters (A, B, C...) indicate statistically significant differences between enzyme sources at p < 0.05 (Duncan's test). M±SD: Mean ±Standard deviation.

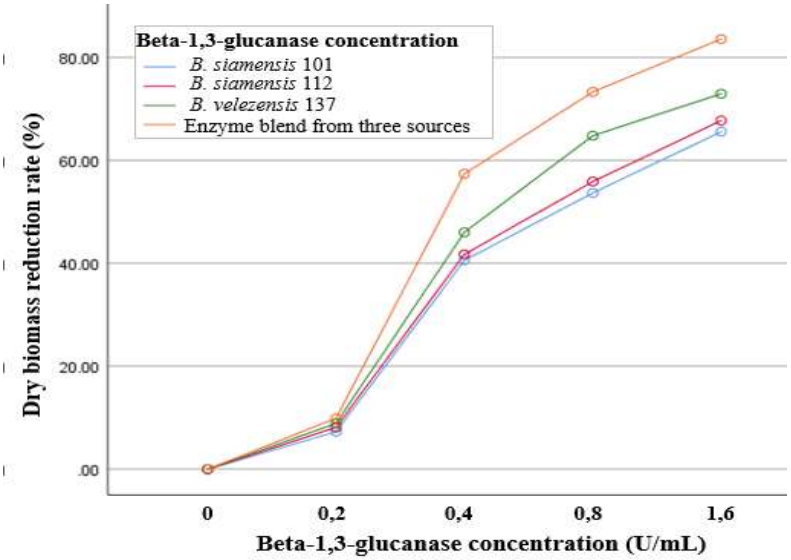

Figure 3.18. *Percentage reduction of dry biomass in pathogenic A. niger QT1 under the effect of beta-1,3-glucanase*

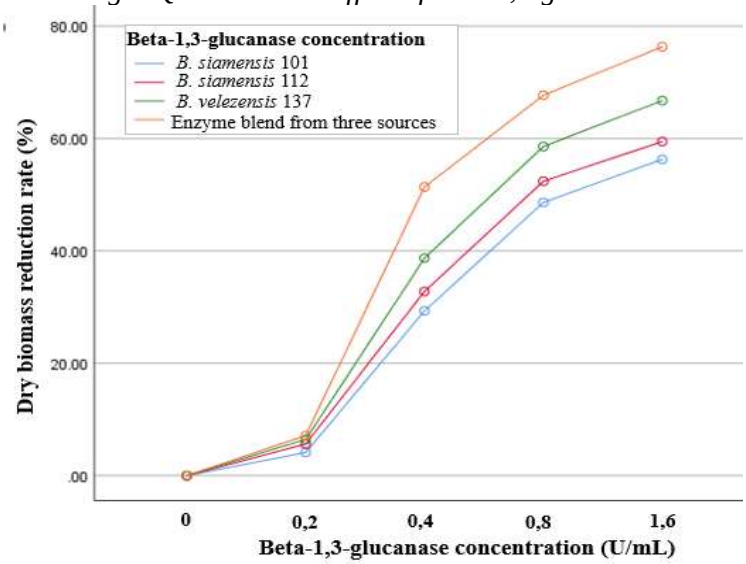

Figure 3.19. *Percentage reduction of dry biomass in pathogenic A. niger H17 under the effect of beta-1,3-glucanase*

3.2.7.4. Correlation between beta-1,3-glucanase activity and antagonistic efficacy against *A. niger*

Table 3.16. *Antifungal activity of purified β-1,3-glucanase enzyme mixtures from bacterial strains at different concentrations against A. niger, assessed by the agar diffusion method*

Concentration (U/mL)	Diameter of growth inhibition of pathogenic fungal strains (mm)	
	***A.niger* QT1**	***A.niger* H17**
0 (Control)	0,0± 0,00	0,0± 0,00
0,2	ND	ND
0,4	23,5±0,49 [c]	20,7±0,43 [c]
0,8	26,4±0,17 [b]	25,2±0,17 [b]
1,6	33,3±0,19 [a]	30,3±0,31 [a]

Note: ND (Not determined): not determined.

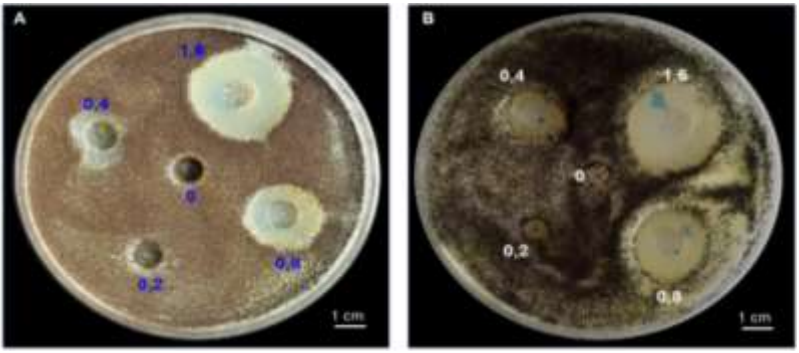

Figure 3.20. *Inhibitory effects of the mixed β-1,3-glucanase enzymes from isolated Bacillus strains against A. niger QT1 (A) and A. niger H17 (B)*

3.2.7.5. Impact of beta-1,3-glucanase on A. niger

Cell WallsSEM analysis showed that while control samples maintained normal spore and hyphal morphology, beta-1,3-glucanase treatment caused hyphal deformation and cell wall rupture. Additionally, the enzyme completely inhibited spore germination on PDA medium.

3.2.8. Biosafety and Plant Growth Promotion (PGP) of Selected Bacillus Strains

Hemolytic activity: *B. siamensis* 101, 112, and *B. velezensis* 137 showed no hemolytic activity on blood agar.

Antibiotic sensitivity: All three strains were sensitive to six antibiotics: Tetracycline, Chloramphenicol, Trimethoprim, Levofloxacin, Gentamicin, and Streptomycin.

Impact on soil fauna: Single and combined *Bacillus* strains (10^7 – 10^9 CFU/mL) showed no acute toxicity toward *Eisenia fetida*. Mortality and biomass loss were within ISO 11268-1:1993 standards and not statistically different from the control ($p > 0.05$).

IAA production: All three strains synthesized IAA, peaking at 72 hours (0.025–0.027 mg/mL), indicating strong plant growth promotion potential (Figure 3.24).

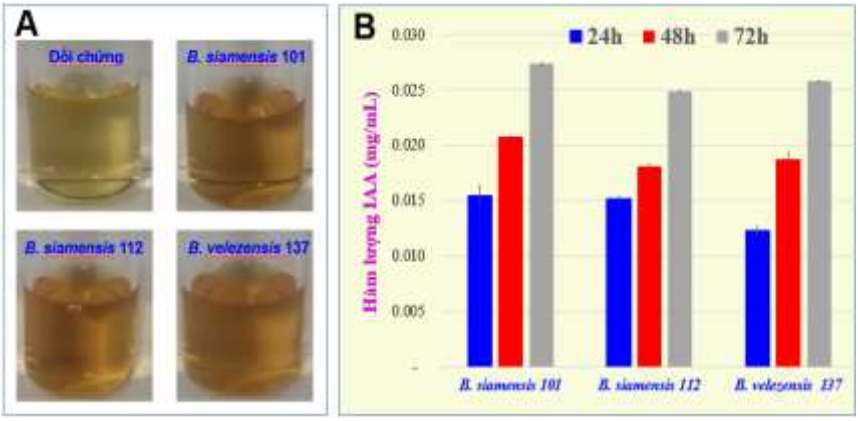

Figure 3.24. *Qualitative analysis of Indole-3-acetic acid (IAA) production capacity by selected Bacillus strains (A) and quantification of IAA content (B)*

3.3. Activity and influencing factors of beta-1,3-glucanase from antagonistic strains

3.3.1. beta-1,3-glucanase accumulation curve

Optimal enzyme harvest time is 20–24 hours for all three *Bacillus* strains*: B. siamensis* 101, 112, and *B. velezensis* 137 (Figs. 3.25–3.27).

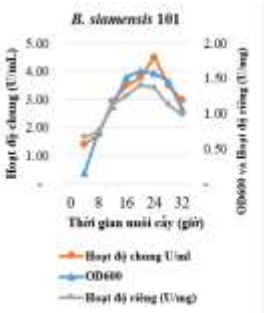

Figure 3.25. *Cumulative curve of beta-1,3-glucanase production by B. siamensis 101*

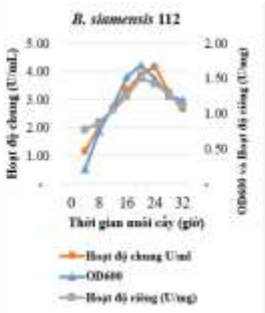

Figure 3.26. *Cumulative curve of beta-1,3-glucanase production by B. siamensis 112*

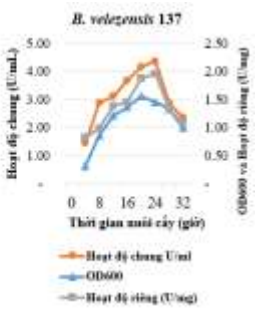

Figure 3.27. Cumulative curve of beta-1,3-glucanase production by B. velezensis 137

3.3.2. Factors influencing beta-1,3-glucanase

Activity effect of temperature: beta -1,3-glucanase activity for the three *Bacillus* strains peaked between 50–60°C. Specifically, *B. siamensis* 101 optimized at 50°C, while *B. siamensis* 112 and *B. velezensis* 137 optimized at 60°C (Figure 3.28).

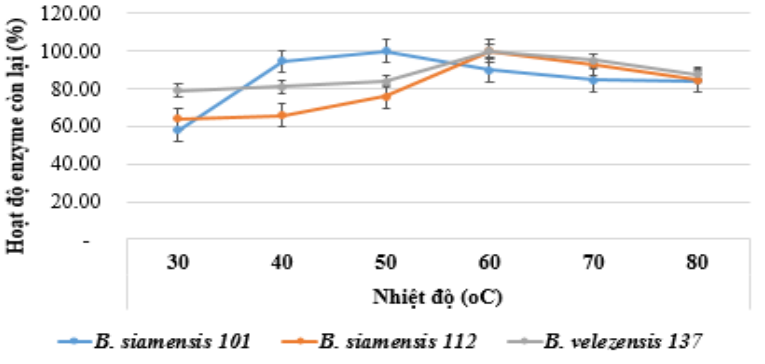

Figure 3.28. *Effect of temperature on enzyme activity of the bacterial strains*

Effect of pH: The β-1,3-glucanase of all three *Bacillus* strains exhibited optimal activity at pH 5,0 with a sharp decrease in activity under highly acidic or alkaline conditions.

Effect of metal ions and chemical agents: Mn^{2+} and Co^{2+} markedly enhanced β-1,3-glucanase activity of the three strains (*B. siamensis* 101, *B. siamensis* 112, and *B. velezensis* 137), increasing activity to 132–202% relative to the control. In contrast, EDTA, urea, and Tris significantly reduced enzyme activity to 70–91%.

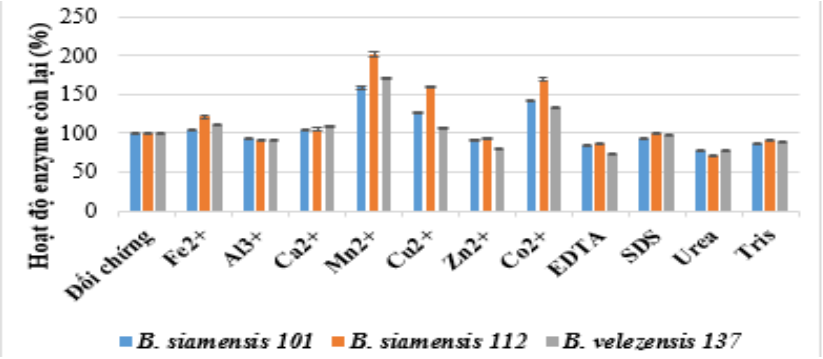

Figure 3.20. *Effects of metal ions and chemical agents on β-1,3-glucanase activity of the bacterial strain*

3.3.3. Factors affecting beta-1,3-glucanase biosynthesis

Effect of carbon sources: Corn starch and curdlan used as carbon sources resulted in β-1,3-glucanase production within 24 h comparable to curdlan and markedly higher than that obtained with barley and wheat.

Effect of culture media: LB medium was identified as the most suitable medium for primary seed culture of *Bacillus* strains.

Effect of nitrogen sources: Yeast extract was determined to be the optimal nitrogen source for enzyme production.

Effect of inoculum size: An inoculum size of 7% produced the best results for bacterial growth and β-1,3-glucanase accumulation (Table 3.23).

Table 3.23. *Effect of inoculum size on growth and β-1,3-glucanase accumulation of Bacillus strains*

Inoculum size (%)	*B. siamensis* 101		*B. siamensis* 112		*B. velezensis* 137	
	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)
1	0,93±0,44^c	1,51±0,21^b	1,70±0,20^c	2,40±0,41^b	1,88±0,51^c	1,81±0,34^b
5	3,89±0,23^b	**7,20±0,61^a**	**3,92±0,21^a**	**8,40±0,31^a**	3,90±0,11^b	**7,33±0,54^a**
7	**4,98±0,11^a**	**7,71±0,43^a**	**4,32±0,20^a**	**8,50±0,22^a**	**4,39±0,41^a**	**8,80±0,41^a**
10	**3,92±0,12^a**	**7,42±0,41^a**	3,72±0,24^b	**8,11±0,63^a**	3,80±0,40^b	**8,32±0,32^a**

Different letters in the same column indicate statistically significant differences at p < 0.05 (Duncan's test). M± SD: Mean ± Standard deviation. Culture conditions: 1% (w/v) corn flour, 1% (w/v) yeast extract, 30°C, pH 7.0, 150 rpm, 24 hours.

Effect of incubation time: An incubation period of 24 h resulted in the highest enzyme activity and biomass for all three Bacillus strains (Table 3.24)

Table 3.24. *Effect of incubation time on growth and β-1,3-glucanase accumulation of Bacillus strains during primary seed culture*

Time (hours)	*B. siamensis* 101		*B. siamensis* 112		*B. velezensis* 137	
	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)	Enzyme activity (U/mL)	Biomass (x10^9 CFU/mL)
0	0,19±0,10^c	**0,00002±0,3^a**	2,60±0,13^b	**0,00002±0,51^a**	0,58±0,33 ^{c}	**0,000015±0,50^a**

Time (hours)	*B. siamensis* 101		*B. siamensis* 112		*B. velezensis* 137	
	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)
24	**5,15±0,40[a]**	**7,52±0,51[a]**	**4,40±0,21[a]**	**8,94 ±0,90[a]**	**4,41±0,51[a]**	**8,44±0,53[a]**
36	3,66±0,52[b]	**6,34±0,53[a]**	3,92±0,24[b]	**7,72 ±0,91[a]**	**3,90±0,14[a]**	**7,34±0,81[a]**
48	1,63±0,53[c]	**3,62±1,14[a]**	1,96±0,41[c]	**4,22 ±0,54[a]**	2,08±0,93[b]	**3,82 ±1,14[a]**

Different letters in the same column indicate statistically significant differences at $p < 0.05$ (Duncan's test). M± SD: Mean ± Standard deviation. Culture conditions: 1% (w/v) corn flour, 1% (w/v) yeast extract, 30°C, pH 7.0, 150 rpm.

Effect of cultivation temperature: A cultivation temperature of 30 °C resulted in the highest enzyme activity and biomass for all three Bacillus strains (Table 3.25).

Table 3.25. *Effect of cultivation temperature on growth and β-1,3-glucanase accumulation of Bacillus strains during primary seed culture*

t°C	*B. siamensis* 101		*B. siamensis* 112		*B. velezensis* 137	
	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)
25	3,01 ±0,62[b]	0,33±0,12[b]	**3,06±0,63[a]**	0,40±0,34[b]	3,17[b]±0,73	0,50±0,21[b]
30	**5,24 ± 0,44[a]**	**7,42±0,23[a]**	**4,49± 0,11[a]**	**9,11±0,52[a]**	**4,52±0,54[a]**	**8,24±0,41[a]**
35	3,9±0,42[b]	**7,02±0,60[a]**	**3,66±0,32[a]**	**8,82±0,43[a]**	**3,52±0,52[a]**	**8,52±0,54[a]**
37	2,91±0,60[b]	**7,04±0,51[a]**	**2,79±0,44[a]**	**8,14±0,31[a]**	2,59±0,40[b]	**7,91±0,72[a]**

Effect of culture pH: A pH of 7,0 resulted in the highest enzyme activity and biomass for all three *Bacillus* strains (Table 3.26).

Table 3.26. *Effect of culture pH on growth and β-1,3-glucanase activity of Bacillus strains during primary seed culture*

pH	***B. siamensis* 101**		***B. siamensis* 112**		***B. velezensis* 137**	
	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)	Enzyme activity (U/mL)	Biomass ($x10^9$ CFU/mL)
5,0	3,01±0,91^{c}	0,16±0,3^{c}	2,80±0,50^{b}	0,52±0,4^{b}	2,85±0,41^{c}	0,23±0,62^{c}
6,0	3,51±0,32^{c}	0,31±0,62^{c}	3,62±0,44^{b}	**0,71±0,41^{a}**	3,64±0,10^{b}	0,50±0,31^{c}
6,5	4,21±0,50^{b}	**7,12±0,74^{a}**	3,67±0,32^{b}	**8,40±0,82^{a}**	3,74±0,34^{b}	8,11±0,50^{ab}
7,0	**5,43±0,34^{a}**	**7,63±0,82^{a}**	**4,53±0,10^{a}**	**8,84±0,53^{a}**	**4,52 ± 0,51^{a}**	**8,51±0,62^{a}**
7,5	4,17±0,11^{b}	4,51±0,50^{b}	3,50±0,42^{b}	**6,40±0,33^{a}**	3,04±0,62^{b}	7,01±0,22^{ab}
8,0	2,86±0,21^{c}	2,30±0,44^{b}	3,21±0,50^{b}	3,82±0,60^{b}	2,24±0,63^{c}	5,23±0,44^{b}

Different letters in the same column indicate statistically significant differences at $p < 0.05$ (Duncan's test). M ± SD: Mean ± Standard deviation. Culture conditions: 1% (w/v) corn flour, 1% (w/v) yeast extract, 7% inoculum size, 30°C, 150 rpm, 24 hours.

Effect of agitation speed: An agitation speed of 150 rpm resulted in the highest enzyme activity and biomass for all three Bacillus strains.

Culture conditions for β-1,3-glucanase production: Liquid LB medium; supplementary nitrogen source: yeast extract; inoculum size: 7%; incubation time: 24 h; cultivation temperature: 30–35 °C; culture pH: 7.0; agitation speed: 150 rpm.

3.4. *In vivo* Antagonistic Efficacy of *Bacillus* Strains Against *A. niger*

3.4.1. Inhibition of *A. niger* in Net-house Conditions Post-infection, the control group showed a rapid increase in mortality (60% after 7 days; 100% after 14–21 days). In contrast, peanut plants treated with the 3-strain bacterial consortium (coded **BAZ04**) showed no symptoms, maintaining a 0% mortality rate throughout the experiment (Fig 3.31)

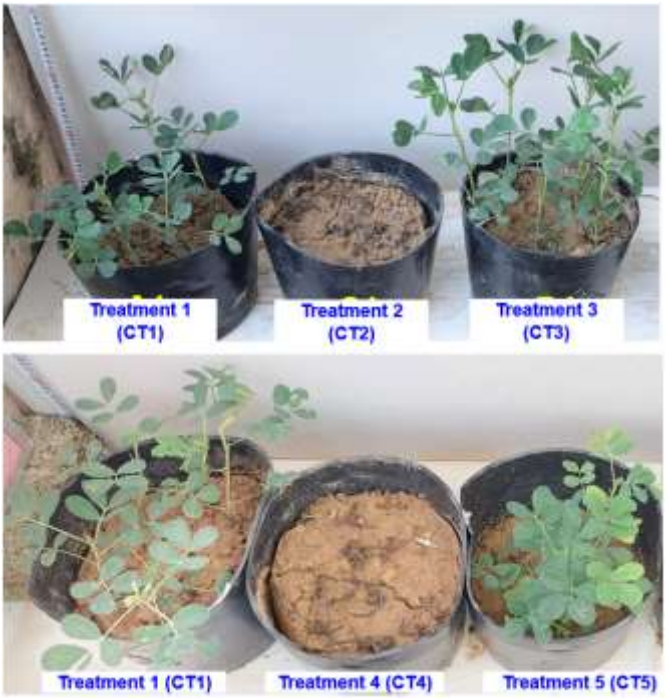

Figure 3.31. *Biological control of groundnut collar rot using Bacillus strains under net-house conditions following artificial inoculation with Aspergillus niger. Treatments included:* ***CT1****, non-inoculated plants treated with water only (negative control);* ***CT2****, plants inoculated with A. niger QT1 and treated with water only (positive control);* ***CT3****, plants inoculated with A. niger QT1 and treated with the BAZ04 formulation;* ***CT4****, plants inoculated with A. niger H17 and treated with water only (positive control); and* ***CT5****, plants inoculated with A. niger H17 and treated with the BAZ04 formulation.*

3.4.2. Field efficacy of the *Bacillus* consortium (BAZ04)

Effect of the BAZ04 formulation on peanut growth and development: The BAZ04 formulation increased main stem height and total branch number of the peanut cultivar L14 at different growth stages in both Huế and Quảng Trị.

Effect of the BAZ04 formulation on collar rot (black mold) disease: The BAZ04 formulation markedly reduced the incidence and severity of peanut collar rot on the cultivar L14 at experimental sites in both Huế and Quảng Trị (Table 3.32).

Table 3.32. *Percentage of plant mortality due to peanut collar rot (black mold) under different experimental treatments (%)*

Treatment	Phu Vang, Hue	Huong Tra, Hue	Hai Lang, Quang Tri	Trieu Phong, Quang Tri
BAZ04 (CT1)	**3,57 ±0,33 [a]**	**1,16 ± 0,06 [a]**	**1,38 ± 0,11 [a]**	**2,11 ± 0,20 [a]**
Trichoderma (CT2)	**4,08 ± 0,31[a]**	**1,75 ± 0,11 [a]**	**1,78 ± 0,14 [a]**	**2,21 ± 0,28 [a]**
Control (CT3)	10,71±1,05[b]	6,57 ± 0,62 [b]	4,13 ± 0,35 [b]	8,16 ± 0,69 [b]
LSD $_{0.05}$	*2,61*	*3,05*	*1,07*	*2,13*

Different letters within the same column indicate statistically significant differences among means at $p < 0.05$.

Effect of the BAZ04 formulation on peanut yield and yield components: The BAZ04 formulation improved yield components and increased the actual yield of the peanut cultivar L14 under field production conditions in the experimental areas of Huế and Quảng Trị (Figure 3.32).

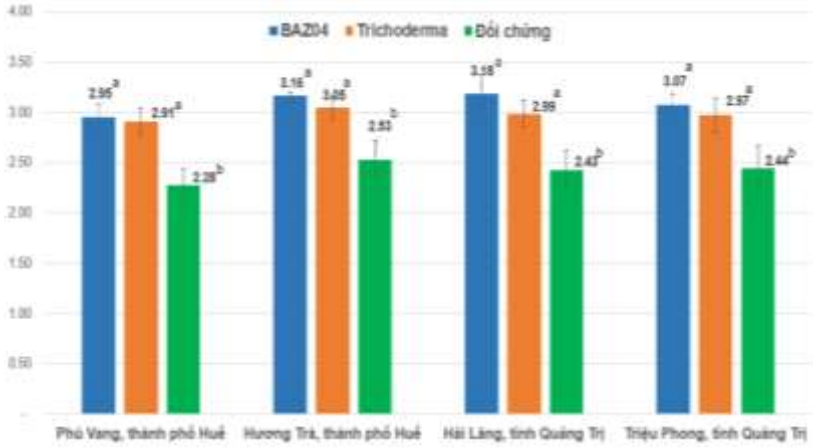

Figure 3.32. *Peanut yield at testing sites in Huong Tra, Phu Vang, Trieu Phong, and Nam Hai Lang. Different letters within the same location indicate statistically significant differences at $p < 0.05$ (Duncan's test). M ± SD: Mean ± Standard deviation.*

CONCLUSIONS AND ECOMMENDATIONS

Conclusions

Based on the experimental results obtained, the thesis draws the following conclusions:

1. A total of 60 fungal isolates were isolated from peanut plants exhibiting black mold wilt symptoms in Quang Tri and Hue City. Confirmation via Koch's postulates and pathogenicity evaluation of 09 selected strains showed plant mortality rates of 85.7–100%. Based on the combination of morphological traits and ITS-rDNA sequence analysis, two representative strains with the highest virulence causing 100% plant mortality (QT1 and H17) were preliminarily identified as *Aspergillus niger*, designated as *A. niger* QT1 and *A. niger* H17.

2. Three bacterial strains (*Bacillus siamensis* 101, *Bacillus siamensis* 112, and *Bacillus velezensis* 137) and one fungal strain (*Trichoderma asperellum* HL6) indigenous to the region were selected for their strong antagonism against *A. niger*. The three selected *Bacillus* strains exhibited high extracellular enzymatic activities (chitinase, beta-1,3-glucanase, and cellulase), produced IAA, and demonstrated biosafety compliance: non-hemolytic, non-toxic to earthworms (at 10^9 CFU/mL), sensitive to six tested antibiotics (tetracycline, chloramphenicol, trimethoprim, levofloxacin, gentamicin, and streptomycin), and had no adverse effect on peanut seed germination.

3. In single-factor experiments, the beta-1,3-glucanase enzyme produced by the three bacterial strains (*B. siamensis* 101, *B. siamensis* 112, and *B. velezensis* 137) achieved maximum activity at pH 5.0 and 50–60 °C, was enhanced by Mn^{2+} and Co^{2+}, and was strongly inhibited by EDTA and urea. Optimal culture conditions for beta-1,3-glucanase harvesting from the three *Bacillus* strains were: Liquid LB broth (supplemented with 1% cornmeal and 1% yeast extract), pH 7.0, 30–35 °C, 150 rpm, 7% inoculum size, and a incubation time of 24 hours. Formulating the enzymes at a 1:1:1 ratio reduced both MIC (0.1–0.2 U/mL) and MFC (0.2–0.4 U/mL), inducing cell wall deformation and rupture in *A. niger* as observed via SEM.

4. A culture broth mixture containing the three *Bacillus* strains (*B. siamensis* 101, *B. siamensis* 112, and *B. velezensis* 137) combined at a 1:1:1 volume ratio at a concentration of 10^8 CFU/mL inhibited *A. niger* more effectively than individual strains under both *in vitro* and greenhouse conditions. The consortium maintained a low incidence of collar rot-induced mortality (1.16–3.56%), comparable to commercial *Trichoderma*, while increasing peanut yield by 20.5–22.7% relative to the control.

Recommendations

Conduct field trials using the culture broth consortium of the three *Bacillus* strains (*B. siamensis* 101, *B. siamensis* 112, and *B. velezensis* 137) to optimize the production process, aiming toward product commercialization and expanding its application to other crops in Central Vietnam./.