INTRODUTION

1. General information

Endophytic bacteria are among beneficial microorganisms that have been increasingly interested. These bacteria reside inside plants and have no visibly harmful effects on host plants (Schulz, 2006, Wang *et al.*, 2009); on the contrary, they stimulate the growth of host plants directly or / and indirectly by a wide variety of mechanisms (Bent & Chanway, 1997, Ryan *et al.*, 2008).

Coffee is one of the strategic agricultural commodities, contributing more than US $ 3.5 billion to the state budget (Nguyen Thi Lai & Do Thi My Hien, 2019). However, Vietnamese coffee production in general is currently facing many challenges, including chemical fertilizer abuse (Truong Hong *et al.*, 2013). This has not only increased production costs but also reduced the resistance of coffee trees, leading to disease outbreaks, quality reduction. It can also lead to arable land degradation, water and environment pollution

The initial research results have revealed that some coffee endophytic bacteria strains have been able to fix biological nitrogen, solubilize phosphorus, synthesize plant growth promoters and antagonize to some coffee pathogens (Shiomi *et al.*, 2006; Mekete *et al.*, 2009; Nguyen Ngoc My, 2012; Truong Vinh Thoi, 2012; Ngo Van Anh *et al.*, 2017). However, these studies have been limited in collecting, isolating and analyzing some of their biological activities *in vitro* and on seedlings in greenhouses.

From the above reasons, we conducted the research "Study on the effects of some selected endophytic bacterial strains on growth and development of Robusta coffee (*Coffea canephora* Pierre var. *robusta*)".

2. Objectives and scope

a. **Objectives:** The aims were to evaluate the effects of some selected endophytic bacteria strains on growth, development of Robusta coffee in

a greenhouse and in field conditions. Based on these results, further studies were conducted to determine the effective dose and compatible combination of these strains on the growth and yield of the coffee in the field.

b. Scope

From the results of the Institute of Biotechnology and Environment, Tay Nguyen University, on the isolation and screening of coffee endophytic bacteria (Nguyen Ngoc My, 2012, Truong Vinh Thoi, 2012 and Ngo Van Anh *et al.*, 2017), this research selected 9 strains with high biological activities for evaluating their capacities in growth prommotion of coffee seedlings in greenhouse and coffee trees in field conditions. This research did not develop bacteria formulation but focused on evaluating their abilities in growth promotion of Robusta coffee using endophytic bacteria susspensions in greenhouse and field conditions in Buon Ma Thuot city.

3. Scientific and practical significances

- Scientific significances*:* The research results revealed roles of some selected coffee endophytic bacteria in coffee growth promotion. This dissertation is also a useful reference for further insight study on endophytic bacteria and development of biologial fomulations from coffee endophytic bacteria.

- Practical significances*:* The research results are scientifical bases for selecting coffee endophytic bacteria strains using in research and development of bio-fertilizers and bio-formulations applied in sustainable coffee production.

4. Innovative contributions

- New issue of the effects of endophytic bacteria strains on the Robusta coffee growth and development at various stages (seedlings, vegetative and productive stages) was studied.

- The research evaluated the effects of endophytic bacteria mixtures on the coffee parasitic nematode densities in field conditions.

- The research evaluated the effects of endophytic bacteria mixtures on leaf chlorophyll, carotenoid, N and P contents. This is obvious evidence for the effect of endophytic bacteria on growth, development, productivity and quality of Robusta coffee.

CHAPTER 1. LITERATURE REVIEW

1.1. Definition of endophytic bacteria

According to Bacon và White (2000), endophytic bacteria are those that colonize living plant tissues and reside inside plants without causing any apparent negative effects. Root is considered to be the most preferred site where bacteria penetrate plant tissues (Verma *et al.*, 2001). After penetrating into the host plants, endophytic bacteria will reside in endophytic niches. These endophytic niches will protect endophytic bacteria from negative effectes of environment, while helping them colonize and establish inside plant cells and tissues (Oliveira *et al.*, 2013).

1.2. Roles of endophytic bacteria

The role of bacteria has been recognized in a wide range of research. The impacts and applications of endophytic bacteria are summarized in the figure 1.1.

The mechanism of beneficial effects of endophytic bacteria on host plants is similar to that of plant growth promoting rhizobacteria (Kloepper *et al.*, 1991). This is because most endophytic bacteria are isolated from inside of healthy plants and able to live outside the plant tissue as rhizobacteria. (Di Fiori & Del Gallo, 1995, cited by Lodewyckx *et al.*, 2002).

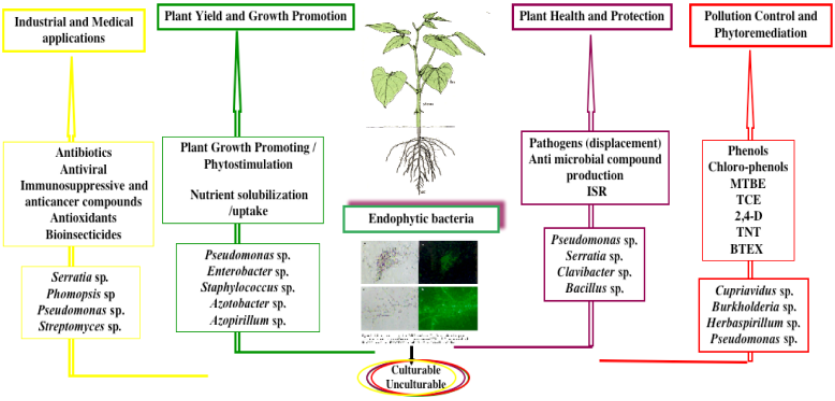

Fig. 1.1. Roles and applications of endophytic bacteria

(Ryan et al., 2008)

1.3. Applications of endophytes in agriculture

Results from many research have revealed that endophytic bacteria play an important role in rice, sugarcane and wheat production. Endophytic bacteria are able to fixe nitrogen, thereby reducing the amount of nitrogen fertilizer needed. For examples, inoculating *Rhizobium* into rice saved two-thirds of the nitrogen fertilizer needed, equivalent to 96 kg N / ha (Yanni *et al.*, 1997), inoculating *Burkholderia* MG43 into sugarcane saved more than 50% of N fertilizer needed (140 kg N / ha). Adding *H. seropedicae* to corn seeds grown in greenhouses, yield increased from 49 to 82% as compared to chemical nitrogen fertilizer (Baldani *et al.*, 2000).

Many endophytic phosphorous solubilizing bacteria strains have been employed in bio-fertilizers production, such as: *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium* and *Erwinia* (Goldstein, 1986).

Results from some field experiments showed that application of phosphorus solubllizing bacteria on plants increased the efficiency of P uptake, thus, stimulating plant growth (Muhammad *et al.*, 2013, Niazi *et al.*, 2015).

Application of multiple endophytic bacteria strains increased the plant growth better than single species addition. Applying the mixture

of *H. seropedicae, Azospirillum lipoferum, Gluconacetobacter* and *B. vietnamiensis* (10^8 CFU / ml) on 5-day-old rice increased yield by 14.4% whereas single strain application increased yield only by 6.2% (Govindarajan *et al.*, 2008).

1.4. Research on coffee endophytic bacteria

Endophytic bacteria were isolated from almost every parts of coffee trees with a very diverse composition (Mekete *et al.*, 2009; Silva *et al.*, 2012; Miguel *et al.*, 2013). The endophytic bacteria strains isolated from coffee mainly belong to the genera: *Pseudomonas, Bacillus, Agrobacterium, Stenotrophomonas and Enterobacter*, of which, mainly Gram-negative bacteria (Mekete *et al.*, 2009).

The majority of endophytic bacteria isolated from coffee and able to re-established their populations inside Robusta coffee tree are belong to the genus *Bacillus*, including: *B. megaterium, B. licheniformis, B. subtilis , B. thuringiensis* and *B. subtilis* (Miguel *et al.*, 2013). Studying on coffee endophytic bacteria, Mekete *et al.* (2009) reported that 33% of coffee root endophytic bacteria isolates are able to suppress root-knot nematode *Meloidogyne incognita*, of which, *Bacillus pumilus* and *B. mycoides* were the most effective in reducing the number of galls and egg masses caused by *M. incognita* by 33 and 39%, respectively (Mekete *et al.*, 2009).

Among the endophytic bacteria strains isolated from coffee trees, *Bacillus* spp. were considered as potential biological agents for controlling *Meloidogyne* spp. because they form endospores which withstand hot and dry conditions (Kloepper *et al.*, 2004). Strains of *B. pumilus* and B. mycoides are the most effective in reducing the number of egg masses and knots in tomato plants caused by M. incognita (33 and 39%, respectively) (Mekete *et al.*, 2009). *B. subtilis* reduced more than 50% of egg masses numbers and tumors caused by M. incognita, M. javanica and M. arenaria (Mahdy *et al.*, 2000, cited by Mekete *et al.*, 2009).

In Vietnam, Nguyen Ngoc My (2012) isolated 30 endophytic nitrogen-fixing bacteria strains from coffee roots. The M15 strain was selected thanks to its highest activities in nitrogen fixation and phosphorous solubilization. The N and P contents in Arabica coffee seedlings treated with this strain increased by 52% and 33.3%, as compared with the control. Initial research results showed that coffee seedling growth parameters including: shoot height, stem diameter, leaf area of treatments applied M15 strain were higher than the control.

Truong Vinh Thoi (2012) isolated 37 endophytic bacterial strains from the Robusta coffee roots. Of which, *B. subtilis EK*17 and *Enterobacter cloace* EK19 were selected thanks to their strong activities in nitrogen fixation and phosphorous solubilization. The seedling growth parameters, such as: shoot height, stem diameter, leaf length and leaf area were higher than the control (Truong Vinh Thoi, 2012). Ngo Van Anh *et al.* (2017) isolated 41 endophytic bacteria strains from Robusta coffee roots. In in vitro conditions, *Bacillus* sp. BMT11 (1,574 μg/ml), *B. pumilus* BMT4 (1,493 μg/ml), *Bacillus* sp. BMT8 (1,474 μg/ml), *Delftia lacustris* BH8 (1,434 μg/ml), *Bacillus* cereus BMT7 (1,399 μgm / l) and *Bacillus* sp. Cu8 (1,372 μg/ml) had the highest nitrogen fixation activity. The highest phosphate solubilization strains were *Bacillus* sp. BMT11 (12.25 μg/ml), *Bacillus* sp. Cu8 (11.46 μg/ml) and Cu2 (11.25 μg/ml). In addition, these strains have a high ability of IAA biosynthesis: *Bacillus* sp. BMT11 (9,048 μg/ml), *Delftia lacustris* BH8 (8,876 μg/ml), *Bacillus* sp. Cu8 (8,153 μg/ml), *B. pumilus* BMT4 (5,624 μg/ml).

In brief, endophytic bacteria play an important role in plant growth and development. Therefore, it is necessary to study them for production of bio-fertilizers and bio-formulation in order to apply in sustainable agricultural production. Numerous studies have shown that the application of multiple microorganism strains on plants were

more effective in stimulating plant growth, development, and productivity, as compared with single strain application. Therefore, attention should be paid to the study of the mixture of potential strains.

Coffee is one of the key crops in Dak Lak province. Initial studies in the world and in Vietnam have shown that the composition of coffee endophytic bacteria is abundant with various good activities such as nitrogen fixation, phosphate solubilization, pathogen antagonism. However, these studies have only been conducted in in vitro and greenhouse conditions. Meanwhile, the composition and activities of bacteria are influenced by various environmental factors and farming techniques. Therefore, it is necessary to conduct research on field conditions to assess their effectiveness in sustainable coffee production.

CHAPTER 2. RESEARCH'S SUBJETS, MATERIALS, CONTENTS AND METHODOLOGIES

2.1. Research subjects

- Robusta coffee (*Coffea canephora* Pierre var. *robusta*) seedlings, young and mature coffee trees grown on ferralitic soil on basalt in Buon Ma Thuot city, Dak Lak province.

- Coffee root endophytic bacteria strains, including: *Bacillus cereus* M15, *Bacillus pumilus* BMT4, *B. subtilis* EK17, *Enterobacter cloace* EK19, *Bacillus* sp. Cu8, *Delftia lacustris* BH8, *Bacillus cereus* BMT7, *Bacillus* sp. BMT8 and *Bacillus* sp. BMT11 has been identified and stored at the Institute of Biotechnology and Environment, Tay Nguyen University. These coffee root endophytic bacteria strains were selected from a collection of more than 100 endophytic bacteria strains which have abilities of N fixation, P solublization and IAA biosynthesis.

2.2. Materials

2.2.1. Media

- Pepton: 7 g Meat extract, 7 g Soya pepton, 5 g NaCl, 15 g Agar, 1 L of distilled water .

- M1 broth culture media:: 2 g yeast extract powder, 6 g Beef extract, 3 g sacharose, 0,3 g $K_2HPO_4.3H_2O$, 0,2 g $MgSO_4.7H_2O$, 0,2 g $FeSO_4.7H_2O$, 3 g NaCl, 1 L of distilled water .

2.2.2. Chemicals and others

- Coffee bean disinfectants: KMnO4 5%, 70^o alcohol, NaOCl 5%, Tween 80.

- Potting materials: TRS1 hybrid coffee beans, basalt red soil, coconut fiber, nylon bags (17 x 25 cm).

- Fertilizers: Phu My Urea (46% N), Phu My SA (21% N + 24% S), Phu My Potassium (61% K_2O), Van Dien Fused Phosphorus (15 – 17% P_2O_5, 28 – 34% CaO; 15 – 18% MgO, 24 – 30% SiO_2, B, Mn, Zn, Cu, Co …), ….

2.3. Research location and period

- ***Research location:*** Invitro assays and greenhouse experiments were conducted at Tay Nguyen University. Field experiments were conducted in Buon Ma Thuot City, Dak Lak Province.

- ***Research period:*** from December 2015 to March 2019

2.4. Research contents

- Effects of selected endophytic bacteria strains on the growth of Robusta coffee seedlings in greenhouse condition.

- Effects of selected endophytic bacteria strains on the growth of vegetative coffee trees in field conditon.

- Effects of selected endophytic bacteria strains on the growth of productive coffee trees in field conditon.

2.5. Research methodology

2.3.1. Evaluate the effects of selected endophytic bacteria strains on the growth of robusta coffee seedlings in greenhouse conditions

The experiment consists of 9 treatments and 2 controls, completely randomized designed, 3 replicates. Each treatment is an endophytic

bacteria strains, including: T1: *Bacillus cereus* M15, T2: *Bacillus subtilis* EK17, T3: *Enterobacter cloace* EK19, T4: *Bacillus* sp. Cu8, T5: *Delftia lacustris* BH8, T6: *B. subtilis* BMT7, T7: *Bacillus pumilus* BMT4, T8: *Bacillus* sp. BMT8, T9: *Bacillus* sp. BMT11, DC control (M1 culture medium), DC0 control (water). These following parameters were investigated: Seedling height (cm), stem diameter (mm), weight of fresh roots (g / root), weight of fresh plants (g / seedling), leaf length (cm), leaf width (cm), leaf area (cm^2 / leaf), leaf content of N%, P%, Chla, Chlb and Ccar.

Based on the obtained results of the Experiment 1, three endophytic bacteria strains showing the most effect on coffee seedling growth in the greenhouse were selected for evaluating their compatibility in vitro. In vitro compatibility test among endophytic bacteria strains of *B. pumilus BMT*4, *B. subtilis EK*17 and *B. subtilis* using Dual culture plate method described by Fukui *et al.* (1994) was employed. Strains that showed negative results (compatible and non-antagonistic) are mixed for field experiments.

2.3.2. Methods for evaluating the effects of selected endophytic bacteria strains on the growth of young robusta coffee trees in field conditions.

a. Research period: from September 2017 to March 2019.

b. Research location: Hòa Thuận commune, Buon Ma Thuot City

c. Research subjects:

- Root offee endophytic bacteria strains: *B. subtilis* M15, *B. subtilis* EK17 and *B. pumilus* BMT4.

- First year Robusta coffee trees grown in basalt red soil.

d. Experimental design: The experiment was Randomized Complete Block Design, 2 factors (bacteria mixtures and suspsension dosage), 3 replications. Each plot size consisted of 9 coffee trees. Experimental plots were seperated by 1 row of coffee tree. The experimental treatmens were as follows:

Mixture / Dosage	B0 (Control)	B1 (*B. subtilis* + *B. subtilis*)	B2 (*B. subtilis* + *B. pumilus*)	B3 (*B. subtilis* + *B. pumilus*)
D1 (10ml/plant)	D1B0 (T1)	D1B1 (T4)	D1B2 (T7)	D1B3 (T10)
D2 (20ml/ plant)	D2B0 (T2)	D2B1 (T5)	D2B2 (T8)	D2B3 (T11)
D3 (30ml/ plant)	D3B0 (T3)	D3B1 (T6)	D3B2 (T9)	D3B3 (T12)

Notes: *D: dosage of bacterial suspension; B: bacteria mixture*

Coffee in the experiment were maintained based on the technical procedure for replanting Robusta coffee (MARD, 2016), with the following chemical fertilizer regime: B0 Control (T1, T2 and T3): Fertilizer application according to the technical procedure for replanting Robusta coffee; Treatments applied endophytic bacteria mixture (T4 to T12): reduced 25% N fertilizer and 25% P as compared to the technical procedure (150 kg urea + 75 kg SA + 412.5 kg of fused phosphate + 150 kg KCl).

f. Monitored parameters: Shoot height (cm), stem diameter (mm), number of primary branch pairs, number of leaf pairs, length of primary branches (cm), number of nodes per primary branches, number of berries/cluster, density of *Pratylenchus* sp. and Meloidogyne sp. in soil (J2/ 50 g of soil), leaf contents of chlorophyll, N and P.

2.3.4. Methods for evaluating the effects of selected endophytic bacteria strains on the growth and development of mature robusta coffee trees in field conditions

a. Research period: from September 2017 to March 2019.

b. Research location: Hoa Xuan commune, Buon Ma Thuot City

c. Research subjects: Coffee endophytic bacteria strains: *B. subtilis* M15, *B. subtilis* EK17 and *B. pumilus B. pumilus* BMT4; 19 years-old-Robusta coffee grown in basalt red soil.

d. Experimental design:

Experiment was designed as similiar as in the experiment of young coffee, with higher bacterial suspension dosage: D1 = 20ml/tree, D2 = 30ml/tree và D3 = 40ml/tree.

f. Monitored parameters: leaf contents of chlorophyll, N and P; length of productive branches (cm), number of nodes/productive branches; Number of nodes with berries; number of berries/cluster; fresh coffee berries: green beans ratio; yields of green beans; percentage of green bean above the sieve of 16; density of *Pratylenchus* sp. and Meloidogyne sp. in soil (J2/ 50 g of soil), and root (con/5 g root).

2.4. Statistical data analysis

Collected data were statistically analyzed by ANOVA, 1 or 2 factors, applying Duncan and LSD test with $P < 0,05$ and $P < 0,01$ to compare the sifnificant differance among treatments. Percantage data were converted into arcsin$\sqrt{x}$ before statistical data analysis.

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Effects of selected endophytic bacteria on growth of coffee seedlings in a greenhouse

3.1.1. Effects of selected endophytic bacteria on leaf chlorophyll and nutrient contents of coffee seedlings

Table 3.1 showed that four months after inoculation of endophytic bacteria into the coffee seedlings, the leaf chlorophyll, N and P contents of all treatments were higher than those of the controls. Remarkably, the treatments inoculated with *B. subtilis* M15 (T1), *B. pumilus* BMT4 (T7) and *Bacillus* sp. BMT11 (T9) had the highest leaf nitrogen contents. Phosphorus contents in leaves were highest in the treatments inoculated with *B. subtilis EK*17 (T2), *B. subtilis* M15 (T1), BH8 (T5), *Bacillus* sp. BMT11 (T9) and *B. pumilus* BMT4 (T7), reaching 0.16 - 0.19% of dry matter of seedling leaves, equivalent to 50.0%, 58.3%, 50.0%, 33.3% and 41.7%, higher than the DC0 control, respectively.

Table 3.1. Effects of selected endophytic bacteria on leaf chlorophyll and nutrient contents of coffee seedlings

Treat-ment	Bacteria strains	Leaf nutrient content *(% dry matter)*		Leaf chlorophyll content *(mg/g fresh leaf)*		
		N	**P**	***Chl$_a$***	***Chl$_b$***	***C$_{car}$***
T1	*B. subtilis* M15	3,18^{a}	0,18^{ab}	1,142^{a}	0,683^{a}	0,529^{bc}
T2	*B. subtilis* EK17	2,90^{b}	0,19^{a}	1,097^{ab}	0,594^{b}	0,542^{b}
T3	*E. cloacae* EK19	2,90^{b}	0,09^{d}	0,957^{bcd}	0,536^{cd}	0,457^{ef}
T4	*Bacillus* sp. Cư8	2,70^{d}	0,10^{d}	0,811^{ef}	0,505^{def}	0,446^{f}
T5	*D. lacustris* BH8	2,84^{bc}	0,18^{ab}	1,037^{abc}	0,519^{de}	0,510^{bcd}
T6	*B. subtilis* BMT7	2,73^{cd}	0,15^{bc}	1,045^{abc}	0,536^{cd}	0,495^{cd}
T7	*B. pumilus* BMT4	3,15^{a}	0,16^{ab}	0,995^{bcd}	0,573^{bc}	0,546^{b}
T8	*Bacillus* sp. BMT8	2,70^{d}	0,12^{cd}	0,931^{cde}	0,485^{ef}	0,498^{cd}
T9	*Bacillus* sp. BMT11	3,15^{a}	0,17^{ab}	1,092^{ab}	0,592^{b}	0,584^{a}
T10	DC	2,63^{d}	0,09^{d}	0,875^{def}	0,490^{def}	0,483^{de}
T11	DC0	2,69^{d}	0,12^{cd}	0,791^{f}	0,464^{f}	0,402^{g}
P		**	**	**	**	**
CV%		2,34	13,35	7,59	4,71	4,07

Notes: *Chla: chlorophyll a; Chlb: chlorophyll b; Car: Carotenoids; **: Significant difference p < 0,01; The same letters on the same column represent no significant difference according to the Duncan's Multiple Rang Test.*

The photosynthesis analysis results showed that most endophytic bacteria strains have a positive effect on the photosynthesis pigments content, increasing the contents of chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoid (Ccar) in coffee seedling leaves as compared with the DC and DC0 control (Table 3.1). The higher the chlorophyll content in leaves, the stronger the photosynthesis ability of the plant leads to increased photosynthetic efficiency, dry matter accumulation, biomass, and plant growth.

In brief, with the same farming practices, the inoculation of endophytic bacteria strains enhanced nitrogen and phosphorus absorption, increased the contents of photosynthetic pigments in coffee

seedling leaves. This result could be the result of endophytic bacteria colonization into coffee roots and thus promoted nitrogen fixation and phosphorus solubilization.

3.1.2. Effects of selected endophytic bacteria on some plant growth parameters of coffee seedlings

Table 3.2. Effects of selected endophytic bacteria on some plant growth parameters of coffee seedlings

Treat-ment	Shoot height *(cm)*	Stem diameter *(mm)*	Number of leaf pairs	Leaf area *(cm^2/leaf)*	Seedling shoot weight *(g)*	Root length *(cm)*	Fresh root weight *(g)*
M15	31,18^{a}	5,61^{ab}	7,11^{a}	76,94^{a}	11,50^{a}	35,49^{b}	5,06^{a}
EK17	28,22^{b}	5,55^{ab}	6,83^{ab}	73,92^{a}	11,22^{a}	27,13^{e}	4,81^{ab}
EK19	24,20^{c}	5,39^{abc}	6,89^{a}	61,85^{b}	7,97^{d}	37,17^{a}	2,76^{de}
C8	21,97^{de}	4,67^{efg}	6,17^{cd}	55,36^{bc}	6,78^{e}	28,79^{d}	2,61^{e}
BH8	23,27^{cd}	5,15^{bcd}	6,50^{abc}	59,79^{b}	8,93^{b}	23,59^{f}	4,14^{c}
BMT7	24,64^{c}	4,79^{def}	6,22^{bcd}	54,71^{bc}	8,29^{cd}	28,04^{de}	3,33^{d}
BMT4	24,22^{c}	5,65^{a}	7,17^{a}	70,45^{a}	8,49^{bcd}	27,17^{e}	4,78^{ab}
BMT8	23,19^{cd}	4,98^{cde}	6,17^{cd}	57,52^{bc}	6,78^{e}	31,69^{c}	1,86^{f}
BMT11	29,69^{ab}	5,41^{abc}	7,00^{a}	71,64^{a}	8,71^{bc}	31,58^{c}	4,34^{bc}
DC	20,62^{e}	4,42^{fg}	6,05^{dc}	49,60^{cd}	5,29^{f}	28,48^{de}	1,51^{f}
DC0	17,89^{f}	4,28^{g}	5,72^{d}	42,14^{d}	4,78^{g}	21,96^{g}	1,38^{f}
P	**	**	**	**	**	**	**
CV%	3,63	4,85	5,42	7,26	3,65	2,90	10,52

The results presented in the Table 3.2 showed that the growth of coffee seedlings treated with *B. subtilis* M15, *B. subtilis* EK17 and *B. pumilus* BMT4 was better than their counterparts. Compared with the DC control, seedling shoot height increased from 17.5 to 51.2%; stem diameter increased by 25.6 - 27.8%, fresh shoot weight increased 60.5 - 117.5%, root length increased 24.6%, and fresh root weight increased up to 235.1%. This is the result of increases in leaf photosynthensis pigment,

nitrogen and phosphorus contents in leaves. These are potential endophytic bacteria strains for study and development of bio-fertilizers applying on sustainable coffee production. Therefore, these strains of bacteria were used for further research in the field conditions on young and mature coffee trees.

Compatibilities of selected endophytic bacteria strains, including: *B. subtilis* M15, *B. subtilis* EK17 and *B. pumilus* BMT4, were tested, followed the method described by Fukui *et al.* (1994). The results showed that *B. subtilis* M15, *B. subtilis* EK17 and *B. pumilus* BMT4 grew normally at the intersection points of two perpendicular bacterial lines. Therefore, these strains can be mixed for testing in field conditions

3.3. Effects of selected endophytic bacteria on plant growth of young coffee

Table 3.6. Effects of selected endophytic bacteria on the leaf nutrient contents of young coffee

Treatment	Combination	N (%)	P (%)
Pretreatment (TXL)		2,98	0,11
T 1	B0D1	3,04	0,10
T2	B0D2	3,04	0,11
T3	B0D3	3,11	0,11
T 4	B1D1	3,58	0,14
T 5	B1D2	3,50	0,14
T 6	B1D3	3,64	0,12
T 7	B2D1	3,35	0,11
T 8	B2D2	3,16	0,10
T 9	B2D3	3,53	0,13
T 10	B3D1	3,16	0,10
T 11	B3D2	3,39	0,11
T 12	B3D3	3,39	0,13

Table 3.6 shows that the leaf N contents of all treatments, including the two control were quite high, ranging from 3.04 to 3.64% of leaf dry matter. The leaf N contents of these treatments were within suitable range for the Robusta coffee growth according to Willson's nutrition scale (1985) and Nguyen Van Sanh (2009). Compared to pretreatment, the leaf N content increased in all treatments, including the controls. However, the leaf N contents of the controls increased only from 2.0 to 4.4%, as compared with pretreatment, increases of endophytic bacteria treatments were 6 - 22%. Notably, the leaf N content of the combination of B1D3, B1D1, B2D3 and B2D2 were highest, increasing 22.1%, 20.1%, 18.5% and 17.4%, as compared with pretreatment, respectively.

The leaf P contents of all treatments applied the B1 mixture were 9.1 - 27.3% higher than pre-treatment, reaching 0.12 - 0.14%. This parameter coincided with the nutrient threshold for Robusta coffee in the Central Highlands, according to the nutrition scale of Nguyen Tri Chiem (1993). Other treatments applied the B2 and B3 mixture, the leaf P content increased only if being treated with 30 ml / tree. The leaf P contents of these two treatments (B2D3 and B3D3) reached 0.13% and were within the suitable nutrient threshold for coffee trees.

Table 3.11. Effects of selected endophytic bacteria on the pair number of primary branch of young coffee trees *(18 MAT)*

Dose of bacterial suspension D (ml/tree)	**Pair number of primary branches per tree**				
	Bacteria mixture				**Average (D)**
	B0	**B1**	**B2**	**B3**	
D1 (10)	13,6 ^{e}	15,3 ^{abcd}	14,9 ^{bcde}	15,0 ^{bcde}	**14,70^{B}**
D2 (20)	14,7 ^{cde}	15,2 ^{abcd}	15,9 ^{abc}	16,0^{ab}	**15,44^{AB}**
D3 (30)	14,1 ^{de}	15,8 ^{abc}	16,7^{a}	15,3 ^{abcd}	**15,47^{A}**
Average (B)	**14,11^{B}**	**15,44^{A}**	**15,81^{A}**	**15,44^{A}**	

Notes: *Averages followed by the same letters show no significant difference, D: p < 0,05; B: p < 0,05; D*B: p > 0,05; CV = 15,6%.*

Table 3.12. Effects of selected endophytic bacteria on the leghth of primary branches of young coffee trees *(18 MAT)*

Dose of bacterial suspension D *(ml/tree)*	**Length of a primary branch *(mm)***				
	Bacterial mixture (B)				**Average (D)**
	Đ/C	**B1**	**B2**	**B3**	
D1 (10)	99,7^{d}	109,6^{c}	115,4^{bc}	108,8^{c}	**108,4^{B}**
D2 (20)	100,5d	110,9^{c}	118,2^{ab}	110,2^{c}	**109,9^{AB}**
D3 (30)	101,3d	115,4^{bc}	122,8^{a}	112,8^{bc}	**113,0^{A}**
Average (B)	**100,5^{C}**	**112,0^{B}**	**118,8^{A}**	**110,6^{B}**	

Notes: *Các Average có cùng kí tự không khác biệt có ý nghĩa thống kê ở mức xác suất với D: p < 0,05; B: p < 0,05; D*B: p >0,05; CV = 13,5%.*

Tables 3.11 and 3.12 shows that the average pair number and length of primary branches treated with mixed bacterial suspensions were significantly higher than those of the Controls ($p < 0.05$). Although the average pair number of primary branches treated with B2 mixture was not significantly different from those treated with B1 and B3 mixture, the average length of treatments applied B2 mixture was significantly higher. The pair number and length of primary branches were proportional with applied bacterial suspension dosage. Average pair number and length of primary branches of the treatment B2D3 were highest, 18.4% and 21.3% higher than the control, respectively. This is the result of increases in chlorophyll content and N and P uptake of coffee leaves after being treated by selected endophytic bacteria suspension. Increasing contents of N, P and chlorophyll led to increases in photosynthesis, cell division and resulted in plant growth promotion. Increasing the pair number and length of primary branches will be resulted in vigorous canopy development and is a premise for high productivity.

It can be seen clearly from the Table 3.14 that the numbers of berries per cluster in the B2 applied treatments were highest and significantly different with the B1 applied treatments and the controls. Although there was no significant difference on the numbers of berries per cluster

between the interactions of bacteria mixtures and doses, the treatments of B2D3, B3D3, B2D2, B2D1 and B3D2 had the most number of berries per cluster and was significantly different as compared with the controls treatment. The average numbers of berries per cluster of these treatments were higher than the corresponding controls: 34.3%, 25.1%, 26.4%, 24.9% and 22.3%, respectively.

Table 3.14. Effects of selected endophytic bacteria on number of berries per cluster of young Robusta coffee trees

Dose of bacterial suspension D (ml/tree)	Number of berries per cluster				
	Bacterial mixture (B)				Average (D)
	B0	B1	B2	B3	
D1 (10)	25,4 ^{d}	29,4 ^{bc}	31,7 ^{ab}	30,6 ^{b}	**29,3**
D2 (20)	25,6 ^{cd}	29,6 ^{bc}	32,4^{ab}	31,3 ^{ab}	**29,7**
D3 (30)	26,2 ^{cd}	30,2 ^{b}	35,1^{a}	32,7^{ab}	**31,1**
Average (B)	**25,7^{C}**	**29,7^{B}**	**33,1^{A}**	**31,6^{AB}**	

Notes: *Averages followed by the same letters show no significant difference D: p > 0,05; B: p < 0,05; D*B: p > 0,05; CV = 17,16%.*

3.4 Effects of selected endophytic bacteria on growth and development of mature Robusta coffee

3.4.1. Effects of selected endophytic bacteria on leaf nutrient contents of mature coffee trees

The results presented in the table 3.18 revealed the role of *B. subtilis* M15, *B. subtilis EK*17 *B. pumilus* BMT4 in increases of N and P uptake. The leaf nutrient contents increased after mature coffee was treated with these bacterial suspensions. This is a result of increases in production of plant growth promoters, root growth stimulation leading to more efficient soil water and nutrient absorption. (Kloepper *et al.*, 1991). This result coincided with the results of greenhouse and field experiment on young coffee.

Table 3.18. Effects of selected endophytic bacteria on nutrient content of coffee leaf in productive stage

Combi-nation	N (%)			P (%)		
	BT	1 YAT	2 YAT	TXL	1 YAT	2 YAT
B0D1	3,26	3,25	3,37	0,11	0,11	0,12
B0D2	3,16	3,17	3,3	0,12	0,12	0,11
B0D3	3,20	3,21	3,34	0,12	0,12	0,12
B1D1	2,88	3,26	3,54	0,11	0,12	0,13
B1D2	3,12	3,4	3,65	0,12	0,13	0,15
B1D3	3,28	3,42	3,54	0,12	0,12	0,13
D2D1	2,90	3,42	3,58	0,12	0,12	0,15
B2D2	3,12	3,64	3,63	0,11	0,13	0,12
B2D3	3,37	3,35	3,61	0,12	0,13	0,13
B3D1	3,04	3,28	3,63	0,12	0,12	0,13
B3D2	3,15	3,25	3,64	0,11	0,12	0,14
B3D3	3,37	3,44	3,56	0,12	0,12	0,14

3.4.2. Effects of selected endophytic bacteria on the control efficiency of Pratylenchus sp. *infesting coffee trees in productive stage*

Table 3.22. Effects of selected endophytic bacteria on the control efficiency of *Pratylenchus* sp. in coffee growing soil

Dose of bacterial suspension D *(ml/tree)*	Control efficiency of *Pratylenchus* sp. in soil (%)				
	Bacterial mixture (B)				Average (D)
	Đ/C	B1	B2	B3	
D1 (20)	0^{b}	$62,2^{a}$	$65,5^{a}$	$69,1^{a}$	**49,2**
D2 (30)	0^{b}	$65,2^{a}$	$74,0^{a}$	$67,9^{a}$	**51,8**
D3 (40)	0^{b}	$67,9^{a}$	$74,5^{a}$	$71,7^{a}$	**53,5**
Average (B)	$\mathbf{0^{C}}$	$\mathbf{65,1^{B}}$	$\mathbf{71,3^{A}}$	$\mathbf{69,6^{AB}}$	

Notes: *Averages followed by the same letters show no significant difference, D: p > 0,05; B: p < 0,05; D*B: p >0,05; CV = 9,3%.*

Table 3.24. Effects of selected endophytic bacteria on the control efficiency of *Pratylenchus* sp. in coffee roots *(the year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Control efficiency of *Pratylenchus* sp. in coffee root (%)**				
	Bacterial mixture (B)				**Average**
	B0 (Đ/C)	**B1**	**B2**	**B3**	**(D)**
D1 (20)	0^{c}	$69,9^{b}$	$78,7^{a}$	$70,7^{b}$	**54,8**
D2 (30)	0^{c}	$69,4^{b}$	$73,7^{ab}$	$71,7^{b}$	**53,7**
D3 (40)	0^{c}	$68,9^{b}$	$78,1^{a}$	$74,4^{ab}$	**55,4**
Average (B)	$\mathbf{0^{C}}$	$\mathbf{69,4^{B}}$	$\mathbf{76,8^{A}}$	$\mathbf{72,3^{B}}$	

Notes: *Averages followed by the same letters show no significant difference D: p < 0,05; B: p < 0,05; D*B: p >0,05; CV = 13,72%.*

It can be seen from the Table 3.22 and 3.24 that the nematode control efficiency of B2 mixture applied treatments (*B. subtilis EK*17 + *B. pumilus* BMT4) was highest, significantly different with the B1 mixture applied ones and controls. There was no significant difference on the nematode control efficiency of the interaction between bacteria suspension mixtures and doses. However, the B2D2 and B2D3 treatments always effectively controlled *Pratylenchus* sp. nematodes in coffee growing soils and roots, reaching over 70% at 18MAT.

3.4.3. Effects of selected endophytic bacteria on some of plant growth and development of coffee trees in productive stage

Length of productive braches were highest in the treatments applied B2 then B1 mixture, 24,3% and 19,7% longer than those of the controls (Table 3.29). Length of productive braches were proportionally increased with applied bacterial suspension.

Although the interactions between the bacterial suspension mixtures and doses were not statistically significant, the length of productive braches treated with B2D3 mixture (T9: 40 ml *B. subtilis EK*17 + *B. pumilus* BMT4) was longest and significantly different from others. The length of productive branch treated with B2D3 mixture (T9) was 26.8% longer than the corresponding controls.

Table 3.29. ***Effects of selected endophytic bacteria on*** **the length of coffee fruiting branch in productive stage** *(year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Length of coffee fruiting branch (cm)**				
	Bacterial mixture (B)				**Average**
	B0	**B1**	**B2**	**B3**	**(D)**
D1 (20)	$35,1^{d}$	$43,1^{bc}$	$43,6^{bc}$	$41,3^{bc}$	**$40,8^{B}$**
D2 (30)	$37,8^{cd}$	$44,5^{ab}$	$46,1^{ab}$	$43,1^{abc}$	**$42,9^{AB}$**
D3 (40)	$38,4^{cd}$	$45,5^{ab}$	$48,7^{a}$	$43,6^{abc}$	**$44,1^{A}$**
Average (B)	**$37,1^{C}$**	**$44,4^{AB}$**	**$46,1^{A}$**	**$42,7^{B}$**	

Notes: *Averages followed by the same letters show no significant difference D: p < 0,05; B: p < 0,05; D*B: p > 0,05; CV = 7,2%.*

Table 3.31. **Effects of selected endophytic bacteria on number of coffee berries per cluster in productive stage** *(year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Number of berries per cluster** *(berries/cluster)*				
	Bacterial mixture (B)				**Average**
	B0 (Đ/C)	**B1**	**B2**	**B3**	**(D)**
D1 (20)	$16,84^{b}$	$21,23^{a}$	$21,62^{a}$	$20,62^{a}$	**20,08**
D2 (30)	$17,10^{b}$	$21,20^{a}$	$21,89^{a}$	$22,35^{a}$	**20,64**
D3 (40)	$17,67^{b}$	$22,05^{a}$	$22,39^{a}$	$22,67^{a}$	**21,19**
Average (B)	**$17,20^{B}$**	**$21,49^{A}$**	**$21,97^{A}$**	**$21,88^{A}$**	

Notes *Averages followed by the same letters show no significant difference D: p > 0,05; B: p < 0,05; D*B: p > 0,05; CV = 18,1%.*

It can be seen clearly from the table 3.31 that application of endophytic bacterial suspension mixture affected number of berries per cluster. Average number of berries per cluster of treatments applied bacteria mixture was significantly higher than the corresponding controls (p <0, 05). However, there were no significant differences between the bacterial suspension mixtures nor the doses. The B3D3 (T12), B2D3 (T9), B3D2 (T11) and B1D3 (T6) treatments had largest average number of berries per cluster and were as many 28.3%, 26.7 %, 30.7% and 24.8%

as corresponding controls, respectively. Increases in the number berries per clusters is a prerequisite for yield increase

The B3D3 (T12), B2D3 (T9), B3D2 (T11) and B1D3 (T6) treatments had highest average number of berries per cluster, 28.3%, 26.7 %, 30.7% and 24.8% as many as corresponding controls, respectively. Increases in the number berries per clusters is a prerequisite for yield increase.

Table 3.32. Effects of selected endophytic bacteria on fresh berry: green bean ratio in coffee productive stage *(year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Fresh coffee berry: green bean ratio**				
	Bacterial mixture (B)				**Average**
	B0 (Đ/C)	**B1**	**B2**	**B3**	**(D)**
D1 (20)	5,02 ^{a}	4,64^{cde}	4,78^{bc}	4,74^{cd}	**4,79**
D2 (30)	5,02 ^{a}	4,54^{de}	4,75^{bcd}	4,85 ^{abc}	**4,79**
D3 (40)	4,95 ^{ab}	4,73^{cde}	4,51^{e}	4,71 ^{cde}	**4,73**
Average (B)	**5,00**	**4,64**	**4,68**	**4,77**	

Notes: *Averages followed by the same letters show no significant difference D: p > 0,05; B: p < 0,05; D*B: p > 0,05; CV% = 12,2%.*

Table 3.32 showed that despite 25% reduction in recommended amount of nitrogen and phosphorus fertilizer, the ratio of fresh berries: green beans of those treated endophytic bacterial suspension mixtures was significantly reduced ($p < 0.05$), as compared with the controls. In the year of 2018, the ratio of fresh berries: green beans of the controls were over 4.95, whereas, of endophytic bacterial suspension applied treatments, the highest ratio was only 4.85 (B3D2: T11). Table 3.32 also showed that lowest ratio of fresh berries: green beans was the B2D3 (T9) and B1D2 (T5) treatments.

Endophytic bacteria increased the yield of green coffee beans, with average yield of the B2 treatment was always highest but not significantly different from those treated with B1 mixture (Table 3.33). Despite 25% reduction in the recommended amount of nitrogen and phosphorus fertilizer, the average green bean yield of B2 Treatment was 21.2% as high as in the controls. This result revealed that application of

these endophytic bacteria strains saved at least 25% amount of the inorganic N and P fertilizers needed for coffee growing.

Table 3.33. Effects of endophytic bacteria on yield of green coffee beans *(the year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Yield of coffee green beans (tons/ha)**				
	Bacterial mixture (B)				**Average (D)**
	B0	**B1**	**B2**	**B3**	
D1 (20)	$2,76^{b}$	$3,21^{a}$	$3,25^{a}$	$3,10^{a}$	**3,08**
D2 (30)	$2,70^{b}$	$3,23^{a}$	$3,25^{a}$	$3,15^{a}$	**3,08**
D3 (40)	$2,77^{b}$	$3,18^{a}$	$3,35^{a}$	$3,12^{a}$	**3,10**
Average (B)	**$2,74^{C}$**	**$3,21^{AB}$**	**$3,28^{A}$**	**$3,12^{B}$**	

Notes: *Averages followed by the same letters show no significant difference, D: p > 0,05; B: p < 0,05; D*B: p< 0,05; CV% = 14,1%.*

Table 3.34. Effects of endophytic bacteria on the percentage of coffee green beans above the sieve of 16 *(the year of 2018)*

Dose of bacterial suspension D *(ml/tree)*	**Percentage of coffee beans above the sieve of 16 (%)**				
	Bacterial mixture (B)				**Average (D)**
	B0	**B1**	**B2**	**B3**	
20	$24,7^{c}$	$37,7^{ab}$	$34,1^{b}$	$36,3^{ab}$	**$33,2^{B}$**
30	$25,0^{c}$	$39,5^{a}$	$36,7^{ab}$	$39,1^{a}$	**$35,0^{A}$**
40	$27,2^{c}$	$39,0^{a}$	$37,2^{ab}$	$38,9^{a}$	**$35,6^{A}$**
Average (B)	**$25,6^{B}$**	**$38,7^{A}$**	**$36,0^{A}$**	**$38,1^{A}$**	

*Averages followed by the same letters show no significant difference, D: p > 0,05; B: p < 0,05; D*B: p > 0,05; CV% = 6,19.*

Table 3.34 revealed that the treated bacterial suspension dose had a significantly different effect on the percentage of green been above the sieve 16, with the ratio proportional to the dosages of treated bacterial suspension. However, the bacterial suspension dose of 30 ml / tree was as effective as those of 40 ml / tree. Although the interactions between the bacteria mixtures and the treated dosage were not statistically significant, the B1D2 treatment (T5: 30 ml *B. subtilis* M15 + *B. subtilis*

*EK*17) and B1D3 treatment (T6: 40 ml *B. subtilis* M15 + *B. subtilis EK*17) always had the highest percentage of green been above the sieve 16, ranging from 38 to 39.5% in the second (2017) and the third (2018) crops.

B. *subtilis*, *B. pumilus* and *B. subtilis* are common Gram-positive bacteria, non-toxic and harmless to humans, animals and the environment (Janarthine, 2010; de-Bashan, 2010; Huang *et al.*, 2011). Many studies have shown that B. *subtilis*, *B. pumilus* and *B. subtilis* are closely associated with plants, able to stimulate plant growth by producing plant growth promoters, enhancing nutrient absorption and protect plants from some harmful agents (Oka *et al.*, 1993; Fabio and Gabriel, 2009; Murugappan, 2013). In addition, these bacteria are able to form spores, thus, they can be formulated in dusts, wettable powders and flowables while retaining viability as they can remain dormant for long periods in environmental conditions (Turner and Backman, 1991).

The results of this study reconfirmed that the bacteria strains of *B. subtilis* M15, *B. subtilis EK*17 and *B. pumilus* BMT4 used in this study are able to suppress nematode density of *Pratylenchus* sp. and promote growth and development of Robusta coffee grown on basalt red soil in Buon Ma Thuot.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Among the 9 studied endophytic bacterial strains, *Bacillus cereus* M15, *B. subtilis* EK17, *B. pumilus* BMT4 enhanced the growth of Robusta coffee, the obtained results showed that these bacteria increased in the leaf chlorophyll content of 9.5 – 39.4%; N content of 10.3 to 20.9%, P content of 77.8 to 111.1%, plant height of 17.5 – 51.2%; stem diameter of 25.6 to 27.8%; seedling fresh weight of 60.5 -

117.5%; seedling fresh root weight of 218.5 - 235.2%; root length up to 24.6% as compared to the DC control.

2. The B1 combination *(B. subtilis* M15 + *B. subtilis EK*17) and B2 combination (*B. subtilis EK*17+ *B. pumilus* BMT4) showed the best effect on N and P nutrient uptake thus enhancing the growth of young Robusta coffee trees when applied at the dosage of 20 - 30 ml of bacterial suspension (10^9 CFU/mL) per tree.

3. The B1 combination *(B. subtilis* M15 + *B. subtilis EK*17) and B2 (*B. subtilis EK*17+ *B. pumilus* BMT4) had positively affected on the mature coffee leaf chlorophyll content, N and P nutrient uptake, that lead to promoting the growth and development of mature coffee trees, increasing the number of fruits/clusters. These results increased the coffee productivity of 14.8 – 20.9%.

4. Applying the B2 combination (*B. subtilis EK*17+ *B. pumilus* BMT4) and B3 (*B. subtilis* M15 + *B. pumilus* BMT4) with the dosage of 20 - 30 ml/plant for coffee seedlings or 30 - 40 ml/plant for mature coffee effectively reduced the density of *Meloidogyne* sp. and *Pratylenchus* sp. down to 80%.

Recommendations

1. Bacteria strains *B. subtilis* M15, *B. subtilis EK*17 and *B. pumilus* BMT4 have great potential for application in sustainable coffee production, contributing to reducing the amount of inorganic fertilizer and chemical pesticides needed for coffee production. These are important materials for conducting research and development of bacteria formulations applied in coffee trials.

2. Further research on bacteria mechanism in plant growth promotion and nematode suppression needed to be conducted.

3. It is necessary to study the effect of these bacterial suspension mixtures on the growth promotion of other important crops in the Central Highlands.