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The ability of indigenous *Bacillus* spp. consortia to control the anthracnose disease (*Colletotricum capsici*) and increase the growth of chili plants

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Abstract. *Yanti Y, Hamid H, Reflin, Warnita, Habazar T. 2019. The ability of indigenous* Bacillus *spp. consortia to control the anthracnose disease* (Colletotricum capsici) *and increase the growth of chili. Biodiversitas 21: 179-186.* Anthracnose disease caused by *Colletotrichum capsici* can reduce yields of chili up to 80%. The control of anthracnose disease is generally carried out using synthetic fungicides. However, the use of these fungicides can pollute the environment and harm human health. One alternative control that needs to be developed is the use of *Bacillus* spp. as a biocontrol agent. The study was conducted to obtain an indigenous *Bacillus* spp. consortium that compatible as a biological agent for the control of the anthracnose diseases and promoting the growth of chili plants. The experiment was arranged as a completely randomized design, consisted of three stages, namely: 1) inhibitory test of indigenous *Bacillus* spp. against *C. capsici* in vitro, 2) compatibility test of indigenous *Bacillus* spp. and 3) Test of indigenous *Bacillus* spp.consortium for controlling *C. capsici* and promoting the growth of chili. The results showed that eight species of indigenous endophytic bacteria could inhibit the growth of *C. capsici* in vitro. All combinations of indigenous *Bacillus* spp. were compatible for controlling *C. capsici*. Four combinations of *Bacillus* spp. were able to increase the growth of chili plants. K1 consortium (*Bacillus pseudomycoides* strain SLBE 3.1 AP, *Bacillus thuringiensis* strain SLBE 2.3 BB, *Bacillus toyonensis* strain AGBE 2.1 TL) was the best consortium for controlling *C. capsici*.

Keywords: *Bacillus* spp., *Colletotrichum capsici*, compatibility, consortium

INTRODUCTION

Anthracnose disease caused by *Colletotrichum capsici* can attack seeds, leaves, twigs, and fruits of infected both at pre-harvest and post-harvest stages. The yield loss of chili due to this disease attack can reach 80% if not appropriately controlled (Than et al. 2008). Generally, the management and control of anthracnose disease are still being extensively researched, however, commercial cultivars of chili that are resistant to the anthracnose have not yet been developed (Than et al. 2008). Meanwhile, the control of anthracnose disease using synthetic fungicides can cause environmental damage and adversely affect human health, both directly and indirectly (Zhang et al. 2011). Therefore, it is necessary to develop controls with the Integrated Pest Management (IPM) approach through the use of biological agents, including endophytic bacteria. Amaresan et al (2014) found that most endophytic bacterial isolates from chili show antagonistic activity in vitro against *Pythium* sp. (37.8%), *Fusarium oxysporum* and *Colletotrichum capsici* (35.1%), and *Sclerotium rolfsii* (21.6%). *Bacillus* sp. is the dominant antagonist. Three endophytic bacterial isolates show multiple attributes that can promote growth and yield of chili and reduce disease incidence (Amaresan et al. 2014). Yanti et al. (2018) showed that three endophytic bacterial species (*Bacillus pseudomycoides* strain SLBE1.12N, *B. thuringiensis* strain SLBE3.1BB, and *B. mycoides* strain SLBE1.1AP) were useful for controlling *Ralstonia syzygii* subsp. *indonesiensis* and *Fusarium oxysporum* f. sp. *capsici*.

The direct mechanism of endophytic bacteria for controlling plant pathogens is by producing an antimicrobial, siderophore, and chitinase enzymes, while the indirect mechanism is through the induction of systemic resistance in plants (Wang et al. 2010). Biofilm formation around the root surface by *Bacillus* spp. and their secretion of toxins (surfactin, iturin, macrolactin, bacillomycin, and fengycin) destroy the pathogenic bacterial populations and reduce disease incidence in plants (Chen et al. 2013; Hinarejos et al. 2016). The secretions of *Bacillus* spp. degrade the pathogenic bacterial cell walls and change the cell morphology to kill the pathogen (Elshakh et al. 2016). The antagonistic activity of *Bacillus* spp. controls the mycelial growth of fungi, preventing plant fungal disease (Abdalla 2014; Chowdhury et al. 2015; Aydi Ben Abdallah et al. 2016). *Bacillus* spp. attach to the mycelial cell walls, and the chitosanase (EC 3.2.1.123), protease (EC 3.4.21.112), cellulase (EC 3.2.1.4), glucanase (EC 3.2.1.21), siderophores, and cyanide acid of the bacteria crack and deform the hyphae, which leads to altered cell structure and functions due to vacuolation and protoplast leakage (Khedher et al. 2015; Babu et al. 2015). Bacterially synthesized antifungal peptides, such as iturin, fengycin, mixirin, pumilacidin, and surfactin, are involved in the

destruction of the pathogenic fungi in rhizospheres (Han et al. 2015; Yamamoto et al. 2015). The systemic resistance to diseases induced in plants by *Bacillus* spp. is made possible by increasing salicylic acid content, the gene, and protein expression of proteinase inhibitor II (Pin2) and pathogen-resistant 1 (PR1) (Jiang et al. 2015; Fousia et al. 2016; Hinarejos et al. 2016).

Emerging strategies for plant disease management involve biological and integrated biological control by applying antagonistic microorganisms either alone or in combination (Paulitz and Belanger 2001). A microbial consortium is a group of species of microorganisms that acts together as a community. In a consortium, the organisms work together in a complex and synergistic way (Sudharani et al. 2014). Each endophytic bacterial species has different mechanisms so that the endophytic bacterial consortium can provide various control mechanisms simultaneously, thereby it is more effective in controlling pathogens (James et al. 2003). According to Bashan (2005), a consortium of bacteria that interacts synergistically can give better results than a single bacterial application. Rajasekar and Thaiyalnayagi (2018) also revealed that the consortium of plant growth-promoting bacteria effect was better than the single inoculant effect on the growth of periwinkle. Many other researchers also found that consortium of PGPR could be better options as plant disease control such as in *Phytophthora* on chili pepper (Zhang et al. 2019), *Macrophomina phaseolina* on mungbean (Sharma et al. 2018), and *Ralstonia syzygii* subsp. *indonesiensis* on tomato (Yanti et al. 2018). The study aimed to obtain endophytic bacterial isolates that can inhibit the growth of *C. capsici* in vitro, to test the compatibility of selected stage 1 isolates, and to obtain an effective endophytic bacterial consortium for controlling anthracnose disease.

MATERIALS AND METHODS

Study area

The study was conducted in March-August 2019 at the Laboratory of Microbiology and Experimental Fields, Faculty of Agriculture, Andalas University, Padang, Indonesia.

Procedures

The research experiment was arranged in a completely randomized design. The study consisted of 3 stages, namely: (I) the inhibitory test of *Bacillus* spp. against *C. capsici* in vitro using the dual culture method, (II) compatibility test between indigenous *Bacillus* spp. by the cross-scratch method. The design and treatment were the same as those in stage I, and (III) the test of the ability of the indigenous *Bacillus* spp. to control anthracnose disease in chili in-planta. All the *Bacillus* strains used for treatments in this study were from the previous research (Yanti et al. 2017) which were isolated from chili root tissues and selected for their ability to both promote growth and control *R. syzygii* subsp. *indonesiensis.*

In the stages I and II, the experiment consisted of 9 treatments and 5 replications. The treatments consisted of 9 species of *Bacillus*, namely: *Bacillus cereus* strain AGBE3.3.BB, *B. cereus* strain SLBE1.1.SN, *B. cereus* strain AGBE1.2.TL, *B. cereus* strain SLBE3.1.BB, *B. cereus* strain SLBE1. 1.BB, *B. cereus* strain SLBE1.1.AP, *B. pseudomycoides* strain SLBE3.1.AP, *B. toyonensis* strain AGBE2.1.TL, *B. thuringiensis* strain SLBE2.3.BB and control. In the stageIII, the experiment consisted of 5 consortiums of *Bacillus* spp. (results of stage II, Table 1) and control.

Procedures

Rejuvenation and confirmation of indigenous *Bacillus* spp.

Bacillus spp.were obtained from Yanti's collection (2017). The bacteria were rejuvenated by the scratch method on Nutrient Agar (NA) medium and incubated for 2x24 hours. Furthermore, the bacteria were characterized by Gram test with 3% KOH solution and hypersensitive reaction on *Mirabilis jalapa* leaves.

Inhibitory test of *Bacillus* spp. indigenous against *C. capsici* in vitro

The growth inhibition of *C. capsici* by *Bacillus* spp. was tested by the dual culture method. *C. capsici* culture on 14 days old Potato Dextrose Agar (PDA) was taken with a 5 mm diameter cork borer and transferred to PDA and mixed media (PDA + NA). *C. capsici* pieces were placed 3 cm from the edge of the Petri dish, then each *Bacillus* spp culture was streaked lengthwise 3 cm from the side of the Petri dish opposite the direction of *C. capsici.* For control, *C. capsici* cultures were moved 3 cm from the edge of the petri dish without *Bacillus* spp.

Table 1. Name and origin of selected indigenous endophytic bacterial (IEB) isolates

Bacillus spp. consortium	*Bacillus* species								
	BpAP	**BthBB**	**Bto2TL**	**BcSN**	**Bc31BB**	**Bc1BB**	**Bto1TL**	**Bc33BB**	**BcAP**
K1	+	+	+						
K2					+	+		+	
K3				+		+			
K4					+		+		
K5								+	+

Note: BpAP = *B. pseudomycoides* strain SLBE 3.1 AP; BthBB = *B. thuringiensis* strain SLBE 2.3 BB Bto2TL = *B. toyonensis* strain AGBE2.1TL; BcSN = *B. cereus* strain SLBE 1.1 SN; Bc31BB = *B. cereus* strain SLBE 3.1 BB; Bc1BB = *B. cereus* strain SLBE 1.1 BB; Bto1TL = *B. toyonensis* strain AGBE 1.2 TL; Bc33BB = *B. cereus* strain SLBE 3.3 BB; BcAP = *B. cereus* strain SLBE1.1AP (BcAP)).

Hemolysis test

Bacillus spp. was tested for its ability to hydrolyze red blood cells. The 48-hour-old *Bacillus* spp. bacterial culture was grown on the Blood Agar medium, incubated for 24 hours, and the hemolysis zone formed was observed (Beutin 1991).

Compatibility test of indigenous *Bacillus* spp.

Compatibility between *Bacillus* spp. was tested by crossing the endophytic bacteria on NA medium in a petri dish, which were incubated for 2x24 hours at room temperature. Furthermore, the inhibition zones that appeared between isolates were observed.

Test of the ability of the indigenous *Bacillus* spp. to control anthracnose in chili

Preparation of Bacillus spp. consortium

The *Bacillus* spp. consortium was prepared by combining 2-3 compatible species (from the results of stage II). For pre-culture, the bacteria were cultured in 5 mL Nutrient Broth (NB) incubated in a rotary shaker at 150 rpm for 24 hours. Furthermore, for the main culture, 1 mL pre-culture suspension was put into 24 mL of sterile coconut water and incubated in the same way for 2x24 hours.

The introduction of Bacillus spp.

Bacillus spp. consortium was introduced into the seeds and chili seeds two times. The chili seeds were sterilized with 1% NaOCl-distilled water-each distilled for 1 minute. The seeds were immersed in the consortium of *Bacillus* spp. for 15 minutes then sowed in the seed tray. Chili seeds were maintained for 21 days.

Next, chili seedlings were planted in 10 kg-polybags containing soil mixed with manure (2: 1, v/ v). Before the chili seedlings were planted, they were first soaked in a *Bacillus* spp. for 15 minutes, and the control was soaked in sterile distilled water. The maintenance of chili was carried out by following the recommended cultivation techniques.

Propagation of Colletotrichum capsici

The symptomatic anthracnose chili was isolated by direct planting method on PDA media. The growing *C. capsici* were then purified on a PDA medium. One piece of the fungal mat was inoculated on PDA medium and incubated for two weeks. Conidial suspension was prepared by adding 10 ml of sterile distilled water to culture; conidia was removed using a soft brush. The suspension was transferred into the test tube and homogenized with vortex. Then, 1 ml of the suspension was taken with a dropper, and the number of conidia was counted with a hemocytometer. The density of conidia inoculum to be inoculated in chili plants was 10^6 conidia/ml (Putro et al. 2014).

Inoculation of C. capsici

C. capsici was inoculated on chili one month after flowering. Chili fruit was injured with a sterile needle at the base, middle, and bottom, then sprayed with a suspension of *C. capsici* (10^6 conidia/ml) by spraying it to the entire surface of the fruit until it was wet. The fruit was covered with clear plastic for 14 days to maintain moisture.

Observation

Inhibitory test of indigenous *Bacillus* spp. against *C. capsici* was performed by observing the inhibition zone or the transparent area (the space between the growth of *C. capsici* and *Bacillus* spp.) on PDA and mixed PDA-NA (1:1). Clear zone observations were conducted by measuring the distance between the tip of the *C. capsici* hyphae and a scratch of *Bacillus* spp. Compatibility test of indigenous *Bacillus* spp. was observed to the occurrence of lysis at the intersection of vertical and horizontal scratches.

The development of anthracnose diseases observed in the study was the incubation period (days after inoculation/dai), disease incidence, and disease severity.

Disease severity was calculated using the following formula:

$$S = (\sum (ni \times vi))/ (N \times V) \times 100\%,$$

Where : S = Severity of disease, n = Number of fruits ineach attack category, v = Scale value of each attack category, N = Number of fruit observed, V = highest numerical score in the attack category.

The anthracnose severity scoring is shown in Table 2.

Table 2. Anthracnose severity scores on chili fruits, chili resistance levels, and symptom description

Score	Resistance level	Symptom details
0	Highly resistant	No infection
1	Resistant	1-2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site
3	Moderately resistant	>2-5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit surface
5	Moderately susceptible	>5-15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface
7	Susceptible	>15-25% of the fruit area shows necrotic lesion with acervuli
9	Highly susceptible	>25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli

Source: Montri et al. (2009).

The effectiveness of each treatment for the incubation period was calculated using theformula 1 of *E = (p-kn)/ kn x 100%*, where: E = Effectiveness, P = Treatment, Kn = negative control (Sivan and Chet 1989). Meanwhile, the incidence and severity of the disease were calculated using the formula 2 of *E = (kn-p)/ kn x 100%*, where E = Effectiveness, P = Treatment, Kn = negative control (Sivan and Chet 1996).

The growth of chili seedlings was observed for field emergence, seedling height, number of leaves, and root length. Seedling height and number of leaves were observed from the first week after sowing to 21 days after sowing at 7-day intervals. Root length was measured at 21 days after sowing. Vigor index of seedlings was calculated using the formula by Maji and Chakrabartty (2014) as follows: [(mean of root length + mean of seedlings height) x germination rate]. The growth variables of chili plants at 21 days after sowing observed were plant height, number of leaves, and number of branches. Observation of plant growth started one week after planting until the growth was constant (7 weeks). The effectiveness of each view was calculated by formula 2.

RESULTS AND DISCUSSION

Inhibitory effect of *Bacillus* spp. on *C. capsici* in vitro

Assessment of in vitro antagonism among the biocontrol agents is one of the most important pre-requisites to assess their compatibility in any biocontrol strategy. In the present study, it was observed that both the *Bacillus* strains inhibited the growth of the *C. capsici* in vitro, though at different degrees. The *Bacillus* strains produced zones of inhibition in the dual culture plate assay on PDA and NA plates (Fig. 1) suggesting that metabolites secreted by the *Bacillus* strains may inhibit the growth of the pathogenic fungus.

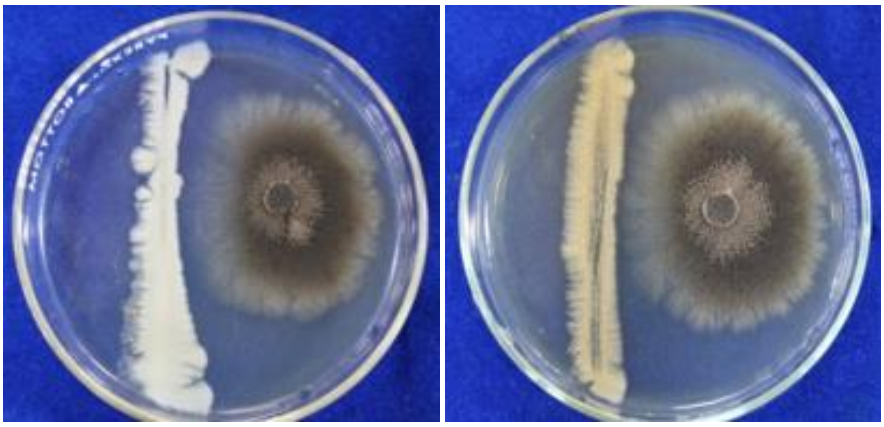
All *Bacillus* spp. could inhibit the growth of *C. capsici* with varying abilities (Table 3). The inhibition capacity of *Bacillus* spp. against *C. capsici* was indicated by the inhibition zone formed between *C. capsici* and *Bacillus* spp. (Figure 1). Inhibition of *C. capsici* growth by *Bacillus* spp. was higher on PDA and NA mixed medium than on PDA medium with an increase of 2.28-76.55%. However, in three *Bacillus* species (*B. cereus* strain SLBE 1.1 SN, *B. cereus* strain SLBE 1.1 BB and *B. toyonensis* strain AGBE1.2TL) with relatively high inhibition, the difference was relatively small, which was 88.89-94.02% on the PDA medium and 90.92-100.00% on the mixed medium.

Hemolysin test of *Bacillus* spp.

Hemolysis activity testing was conducted to obtain *Bacillus* spp. that was safe and not pathogenic in animals and humans. Hemolysis activity is shown in Table 3.

Compatibility between indigenous *Bacillus* spp.

Not all *Bacillus* species combinations were compatible with each other to develop on the same medium (33 combinations or 45.83%) (Table 4). *B. pseudomycoides* strain SLBE 3.1 AP (BpAP) was compatible with 7 other *Bacillus* species; *B. cereus* strain SLBE 1.1 BB (Bc1BB) with 6 *Bacillus* species; *B. cereus* strain SLBE 3.1 BB (Bc31BB) with 5 *Bacillus* species; *B. thuringiensis* strain SLBE 2.3 BB (BthBB), *B. toyonensis* strain AGBE 2.1 TL (Bto2TL) and *B. cereus* strain SLBE 1.1 SN (BcSN) with 4 *Bacillus* species; *B. toyonensis* strain AGBE 1.2 TL (BtoTL) with 3 species of *Bacillus*; *B. cereus* strain SLBE 3.3 BB (Bc33BB) and *B. cereus* strain SLBE 1.1 AP (BcAP) with 2 species of *Bacillus*. In this case, it was also seen that the interactions between strains in the same species were generally incompatible, namely between *B. toyonensis* strain AGBE 2.1 TL (Bto2TL) and *B. toyonensis* strain AGBE 1.2 TL (Bto1TL); *B. cereus* strain SLBE 1.1 SN (BcSN) with *B. cereus* strain SLBE 3.1 BB (Bc31BB), *B. cereus* strain SLBE 1.1 BB (Bc1BB) and *B. cereus* strain SLBE 3.3 BB (Bc33BB).



A **B**

Figure 1. Inhibitory activity of *Bacillus toyonensis* strain AGBE1.2TL in: A. Mixed medium, B. PDA medium

Table 3. Inhibitory effects of *Bacillus* spp. on *C. capsici* in 2 types of medium in vitro (14 days after incubation) and its hemolysin activity

Bacillus species	Inhibition (%)		Increment (%)	Hemolysis test
	PDA Medium	Mixed medium (PDA and NA)		
B. cereus strain SLBE 1.1 BB	91.67	97.44	6.29	-
B. cereus strain SLBE 1.1 SN	94.02	100.00	6.36	-
B. cereus strain SLBE 3.1 BB	44.44	62.09	39.72	-
B. cereus strain SLBE1.1AP	36.84	54.05	46.72	-
B. cereus strain SLBE3.3BB	25.00	42.40	69.60	-
B. pseudomycoides strain SLBE 3.1 AP	38.46	67.90	76.55	-
B. thuringiensis strain SLBE 2.3 BB	60.00	82.55	37.58	-
B. toyonensis strain AGBE 2.1 TL	36.67	50.35	37.31	-
B. toyonensis strain AGBE1.2TL	88.89	90.92	2.28	-

Note: (-) is not pathogenic to humans

Table 4. Compatibility between indigenous *Bacillus* spp. isolates

Bacillus	BpAP	BthBB	Bto2TL	BcSN	Bc31BB	Bc1BB	Bto1TL	Bc33BB	BcAP
BpAP		+	+	+	+	-	+	+	+
BthBB	+		+	+	-	+	+	-	-
Bto2TL	+	+		-	+	+	-	-	-
BcSN	+	+	-		-	+	-	-	-
Bc31BB	+	-	+	-		+	+	-	-
Bc1BB	-	+	+	+	+		-	-	-
Bto1TL	+	+	-	-	+	-		-	-
Bc33BB	+	-	-	+	+	+	-		+
BcAP	+	-	-	-	+	+	-	+	

Note: +: compatible, -: incompatible

Based on compatibility between *Bacillus* spp. growth on the NA, 5 consortia obtained consisted of K1 (*B. pseudomycoides* strain SLBE 3.1 AP (BpAP), *B. thuringiensis* strain SLBE 2.3 BB (BthBB), and *B. toyonensis* strain AGBE2.1TL (Bto2TL), K2 (*B. cereus* strain SLBE 2.3 BB (BthBB), and *B. toyonensis* strain AGBE2.1TL (Bto2TL), K2 (*B. cereus* strain SLBE 2.3 BB (BthBB) 3.1 BB (Bc31BB), *B. cereus* strain SLBE 1.1 BB (Bc1BB), *B. cereus* strain SLBE 3.3 BB (Bc33BB), K3 (*B. cereus* strain SLBE 1.1 SN (BcSN), *B. cereus* strain SLBE 1.1 BB (Bc1BBB)), K4 (*B. cereus* strain SLBE 3.1 BB (Bc31BB), *B. toyonensis* strain AGBE 1.2 TL (Bto1TL) and K5 (*B. cereus* strain SLBE3.3BB (Bc33BB), *B. cereus* strain SLBE1.1AP (BcAP).

The ability of the *Bacillus* spp. consortium to control anthracnose disease

All chili plants introduced with the *Bacillus* spp. showed the inhibition of anthracnose disease development (Table 5). The incubation period of *C. capsici* was slower in chili plants introduced with four consortiums of *Bacillus* spp. (10.55-16.25 days after inoculation, dai) compared to controls (10.30 dai). The best consortium of *Bacillus* spp. able to slow down the incubation period of *C. capsici* in chili plants was K1 (*B. pseudomycoides* strain SLBE 3.1 AP, *B. thuringiensis* strain SLBE 2.3 BB, and *B. toyonensis* strain AGBE2.1TL). The same condition was also found in the occurrence and severity of anthracnose disease in chili plants. The occurrence of anthracnose disease in chili plants introduced withall consortia of *Bacillus* spp. was lower (5.00-30.00%) compared to controls (70.00%) with effectiveness/ reduction of 57.14-92.86%. The severity of anthracnose disease was also lower in chili plants introduced with all the *Bacillus* spp. (5.00-25.00%), compared to controls (40%) with effectiveness from 37.50 to 87.50%.

Table 5. Development of anthracnose disease in chili plants introduced with the consortium of *Bacillus* spp.

Bacillus spp. consortium	Incubation period		Disease incidence (30 day)		Disease severity (30 day)	
	Day	Effectiveness (%)	%	Effectiveness (%)	%	Effectiveness (%)
K1	16.25	57.80	5.00	92.86	5.00	87.50
K2	12.90	25.24	10.00	85.71	9.00	77.50
K3	11.25	9.22	20.00	71.43	15.00	62.50
K4	10.55	2.43	30.00	57.14	11.50	71.25
K5	10.25	-0.50	10.00	85.71	25.00	37.50
Control	10.30	-	70.00	-	40.00	-

Table 6. Growth of chili seedlings introduced with the consortium of *Bacillus* spp. (3 weeks after sowing)

Bacillus spp. consortium	Field emergence		Seedling height		Number of leaves		Root length		Vigor index	
	%	Effecti-veness (%)	cm	Effecti-veness (%)	Sheet	Effecti-veness (%)	cm	Effecti-veness (%)	i	Effecti-veness (%)
K1	95.80 a	64.32	4.09	58.52	3.83	64.38	6.12	32.92	978.12	134.65
K2	77.05 b	32.16	3.79	46.90	3.08	32.19	5.35	17.07	704.24	68.94
K3	81.55 b	39.87	3.54	37.21	2.97	22.47	4.65	1.75	667.89	60.22
K4	70.80 bc	21.44	3.37	30.62	2.89	24.03	4.07	-10.94	526.75	26.36
K5	60.40 cd	3.60	2.91	12.79	3.60	54.51	3.87	-15.32	409.51	-1.76
Control	58.30 d	0.00	2.58	0.00	2.33	0.00	4.57	0.00	416.85	0.00

Table 7. The growth of chili plants introduced with the consortium of *Bacillus* spp. (49 days after planting)

Bacillus spp. consortium	Plant height		Number of leaves		Number of branches	
	Cm	Effectiveness (%)	Sheet	Effectiveness (%)	Piece	Effectiveness (%)
K1	88.50	31.11	111.75	39.25	18.00	80.00
K2	79.00	17.04	100.25	24.92	15.00	50.00
K3	69.25	2.59	87.25	8.72	12.00	20.00
K4	69.25	2.59	81.75	1.87	11.50	15.00
K5	63.00	-6.67	69.25	-13.71	11.00	10.00
Control	67.50	0.00	80.25	0.00	10.00	00.00

The ability of the *Bacillus* spp. consortium to promote the chili growth

All *Bacillus* spp. were able to improve the growth of chili seeds compared to control (Table 6). The field emergence of the plants introduced with the consortium of *Bacillus* spp. was higher (60.40-95.80%) than the control (58.30%). The best consortium of *Bacillus* spp. in increasing the field emergence was K1. The same thing could also be seen in the ability of the consortium of *Bacillus* spp. to increase the number of leaves and the length of roots of chili seedlings. Chili seedlings introduced with the *Bacillus* spp. were taller (2.91-4.09 cm) than the control (2.58 cm) with effectiveness of 12.79-58.52%. The best *Bacillus* spp. consortium in increasing the seedling height was also K1. The number of leaves in the seedlings introduced by the *Bacillus* spp. was higher (2.91-3.83 strands) compared to controls (2.33 strands) with the effectiveness of 22.47-64.38%. The roots of chili seedlings introduced with three *Bacillus* spp. consirtia were longer (4.65-6.12 cm) than the control (4.57 cm) with effectiveness of 1.75-32.92%.

Chili plants introduced with four *Bacillus* spp. consortia were taller (69.25-88.50 cm) than the control (67.50 cm) with effectiveness of 2.59-31.11%. The best consortium of *Bacillus* spp. in increasing plant height was K1. The same effects could also be seen on the number of chili leaves and the number of chili branches. The number of leaves was higher in the chili plants introduced with four *Bacillus* spp. consortia (81.75-111.75 strands) compared to that in the control (80.25 strands) with the effectiveness of 1.87-39.25%. The chili plants introduced with all the *Bacillus* spp. showed a higher number of branches (11.00-15.00) compared to the control (10.00 pieces) with the effectiveness of 10.00-50.00%.

Discussion

High inhibitory effects of 3 species of *Bacillus* spp. on PDA and mixed media (PDA and NA) on the growth of *C. capsici* in vitro showed that the effect of the three species in inhibiting the growth of *C. capsici* was stable. Changes in the composition of the medium did not affect the inhibitory effects of the three species. Generally, the inhibitory testing of *Bacillus* spp. against plant pathogenic fungi is carried out using PDA medium. According to Ozaktan et al. (2013), more than 53% of EB isolates inhibited the mycelial growth of FOC at the rate of 20-64% on PDA plates, producing inhibition zones or showing cell wall lysis. Therefore, the information about using a PDA media mixture with NA for bacterial testing as a biocontrol agent for fungi in vitro is still limited.

In this study, the *Bacillus* spp. consortium was based on the compatibility of each line of *Bacillus* to obtain nine sequences of *Bacillus* spp. and five consortia of *Bacillus* spp. Evaluation of the compatibility and synergism of microbial components is essential in the ability of a microorganism consortium of as biocontrol agents and biofertilizers (Kumar and Jagadesh 2016). Likewise, according to Bashan (2005), a bacterial consortium that interacts synergistically results in better control of plant pathogens compared to a single bacterial application. Some researchers compile a consortium of biocontrol agents based on their ability to control target pathogens. Ouhaibi-Ben Abdejalil et al. (2016) used a consortium of 3 types of selected rhizobacteria to control wilting of tomato plants by *Sclerotinia sclerotiorum* and to promote the growth of tomato plants.

There were five consortia of *Bacillus* spp., consisting of 2-3 compatible *Bacillus* species. The isolate used was *Bacillus* spp., which was useful in controlling bacterial wilt and fusarium wilt with the effectiveness of 100%, which

was singly introduced (Yanti et al. 2018). The introduction of mixed culture (consortium) will have a better effect when compared to the single introduction because each endophytic bacterial candidate has various advantages which, if introduced in a mixture (consortium) give better results.

All the consortium combinations introduced in chili seeds were able to increase field emergence compared to controls. *Bacillus* spp. consortium could also increase the growth of chili both in the nursery stage and after planting. As many as 4 of 5 combinations of *Bacillus* spp. were effective in suppressing the incubation period, the incidence of disease, and the severity of anthracnose disease in the chili plants. The *Bacillus* spp. consortium is more profitable than a single isolate because of its more varied species so that the colonization and domination of the rhizosphere and roots are faster, and the mechanism of action of biocontrol agents is more diverse. The study of Shanmugam et al. (2013) showed that the PGPR strain consortium treatment could promote the plant growth and enhanced production of ginger rhizome by 45.8%. In field experiments, the PGPR strain consortium could also reduce yellow and rhizome rot incidences on ginger by about 50.5%, which was comparable to that of a carbendazim and mancozeb fungicide mixture.

In conclusion, the results showed that almost all isolates were able to inhibit the growth of *Colletotrichum capsici*. Combination 1 (*Bacillus cereus* AGBE3.3.BB, *B. cereus* SLBE1.1.SN and *B. cereus* AGBE1.2.TL) was the best combination in suppressing the development of anthracnose disease with 5% disease incidence.

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