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Humic Acid and Biofertilizer Applications Enhanced Pod and Cocoa Bean Production during the Dry Season at Kaliwining Plantation, Jember, East Java, Indonesia

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Abstract

Cocoa (*Theobroma cacao* L.) is an important crop in Indonesia, but many farmers still face problem in improving bean production. This research aimed to evaluate the effect of humic acid and biofertilizer applications on pod growth and yield of cocoa. The research was conducted at Kaliwining Plantation managed by Indonesian Coffee and Cocoa Research Institute at Jember District, East Java, Indonesia from June 2017 to February 2018. The experiment used mature tree of Sulawesi from one clone. Treatment used were humic acid at level of 0, 1000, 2000, 3000 and 4000 ppm in combination with biofertilizer at level of 0, 500, 1000, 1500 and 2000 ppm that were applied through soil and foliar respectively. Results of this experiment showed that there was an interaction between humic acid and biofertilizer on beans number per plant and photosynthesis rate. Plants treated with 1000 ppm humic acid produced the highest number of small cherelle. Biofertilizer applied at 1500 ppm increased cherelle number, healthy cherelle, number of young pods, number of harvested pods, bean weight per plant and bean yield. The combination of 1000 ppm humic acid and 1500 ppm biofertilizer increased bean production by 39.7%. The high bean production was in line with the high photosynthetic rate. Thus, humic acid and biofertilizer applications could be a way to increase cocoa bean production in this area.

Keywords: *Theobroma cacao*, cherelle wilt, climate change, cocoa bean, photosynthesis.

Introduction

Cocoa (*Theobroma cacao* L.) is an important crop in Indonesia, but production sustainability faces challenges related to poor soil fertility, pest and disease attacks and climate change effects (ICCRI, 2015; Santosa et al., 2018). From 2012 to 2016, cocoa bean production in Indonesia decreased to 658,399 tons indicating a 11.09% decline grown in an area of 1.72 million hectares indicating a 3.03% decrease in area under production (Ditjenbun, 2017).

In Indonesia, cocoa cultivation generally relies on NPK fertilizers from inorganic sources; although cocoa intercropping to optimize fertilizer input is also common (Santosa et al., 2005). Santosa et al. (2018) speculated that high rainfall will have a detrimental effect on cocoa plantation because of high nutrient leaching and disease outbreak. Therefore, there is a need to improve fertilizer management in relation to climate change in order to conserve soil nutrients in the plantation. Many researchers have reported that the use of inorganic fertilizers in a long term has negative impacts on soil structure, cause water pollution and disturb soil micro-organism (Massah and Azedagan, 2016; Zhou et al., 2017).

Recently, the application of organic fertilizers such as humic acid and biofertilizer is getting popular in the plantation. Humic acid is an active substance derived from organic matter that has functional group of –COOH, phenolic –OH, carboxylic group and –C=O (Darmokoesoemo et al., 2014). Humic acid could improve soil permeability, aggregation, water holding capacity, cation exchange capacity, form complex compounds with heavy metals and increases nitrogen, phosphorus, potasium and sulfur availability (Tan, 2009; Hermanto et al., 2012). Humic acid could stimulate plant growth (Dariah and Nurida, 2011), and according to Suwardi and Wijaya (2013), increase in plant growth after humic acid treatment is due to enhancement activities of endogenous hormones such as auxin, gibberellin and cytokinin. However, organic fertilization application in cocoa plantation using humic acid has rarely been studied.

Biofertilizer application stimulates plant growth and production through mechanisms of phosphate mineral dilution, nitrogen fixation and phytohormones production (Ramakrishnan and Selvakumar, 2012; Siagian et al., 2014; Khan et al., 2016; Sahu et al., 2017; Setyadi et al., 2017). Siagian et al. (2014) stated that biofertilizers contain microorganisms such as *Azospirillum* sp., *Bacillus* sp., *Pseudomonas* sp. and *Trichoderma* sp; and *Azospirillum* sp. which could fix nitrogen air to stimulate plant growth. The existence of *Bacillus* sp. and *Pseudomonas* sp. according to Sivasakthi et al. (2014), was able to synthesize IAA, dissolve phosphate and increase N, P, and K absorption. Pratama et al. (2013) reported that microorganisms in biofertilizers could also improve plant resistance to *Phytophtora palmivora*, an important disease in cocoa plantation. The study aimed to evaluate the effect of humic acid and biofertilizer applications on cocoa pod growth and bean production.

Methods

Site and Materials

Research was conducted from June 2017 to February 2018 at Kaliwining Plantation, a farm managed by Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember District, East Java, Indonesia. The peak of the rainy season at the study site was November to May, thus the research was conducted predominantly during the dry season. The climatic data during the course of the research were as follows: average monthly rainfall was 129 mm, average daily minimum temperature (T) was 20.95 °C and maximum was 32.51 °C, air relative humidity was 89.98%, wind velocity was 0.53 km hour^{-1}, sunshine duration was 65.21%, and evaporation was 3.27 mm. Soil had pH (H_2O) 4.27 and CEC 15 cmol.kg^{-1} with C-organic content, N, P, K, Ca, Mg and Na were 3.79%, 0.26%, 147.36 ppm, 1.46 cmol.kg^{-1}, 12.74 cmol kg^{-1}, 8.69 cmo. kg^{-1} and 0.95 cmol. kg^{-1}, respectively.

Experiment was conducted on one clone of cocoa from Sulawesi aged 5–15 years with 3–5 m in height. Treatment was arranged in blocks that represented replications. The blocks had different trees aged 5, 10 and 15 years, represented as block 1, 2 and 3, respectively. Cocoa planting space was 3 x 3 m using the standard population of 1,111 plants per hectare. Plants were derived from top grafting using scion plagiotropic branch, height of jorquette was 10–60 cm from the soil surface. Shade tree used was lamtoro (*Leucaena leucocephala*) in block 1 and 2, while block 3 used mixed of lamtoro and teak trees (*Tectona grandis*). Population of *L.leucocephal* was 555 plants for all blocks, while *T. grandis* was 84 plants per hectare along the edge of the block 3.

Humic acid (Humatop®, Indonesia) according to the manufacturer's description was from leonardite rocks with solubility of 99%, pH 8.6, 38.81% C-organic content, 10.31% K_2O, 6,503 ppm Fe, 20 ppm Mn, 23 ppm Zn and water content of 16.56%. Biofertilizer (Bactoplus ®, Indonesia) according to the manufacturer's description contained *Azospirillum* sp. 2.35 x 10^6, *Bacillus endophiticus* 1.12 x 10^7, *Pseudomonas flourescens* 8.40 x 10^6 and *Tricoderma* sp. 1.50 x 10^5 cfu.g^{-1}. Both materials were purchased from the commercial stores.

Treatments

Research was conducted using complete randomized block design with two factors. The first factor was humic acid concentration, i.e., 0, 1000, 2000, 3000 and 4000 ppm obtained by dissolving 0, 1, 2, 3 and 4 g.L^{-1} of powder in water, respectively. The second factor was biofertilizer, i.e., 0, 500, 1000, 1500 and 2000 ppm obtained by dissolving i.e. 0, 0.5, 1.0, 1.5 and 2.0 g. L^{-1} of powder in water, respectively. Each combination was repeated three times, so there were 75 experimental units. Each experimental unit consists of 16 trees, and 4 trees at the middle of block was selected as samples.

Humic acid was applied in the soil at 75 cm from the stem while the biofertilizer was sprayed onto the canopy. The control treatment used was water. Treatment application was conducted three times after every four weeks starting on the 19th of June 2017. The application of inorganic fertilization (N, P, K, Mg) was based on the Indonesian Coffee and Cacao Research Institute (ICCRI) standard, where annually a tree received urea (46% N) 220 g, TSP (36% P_2O_5) 180 g, KCl (60% K_2O) 170 g and Kieserit (60% MgO) 115 g, which were applied one third each in January, May and November. Pest and disease were controlled using Nordox 86 WG ® and Azure 200 EC ® at 500 L. ha^{-1} of each, applied in November.

Observation and Measurement

Observations and measurements were made on pods in the main stem and branches located 75 cm from jorquette for 24 weeks after treatment (WAT) at 2-week intervals (Figure 1). Number of harvested pods, number of beans per plant, single bean weight, bean weight per plant and productivity data were observed from 24 to 28 WAT in a weekly interval.

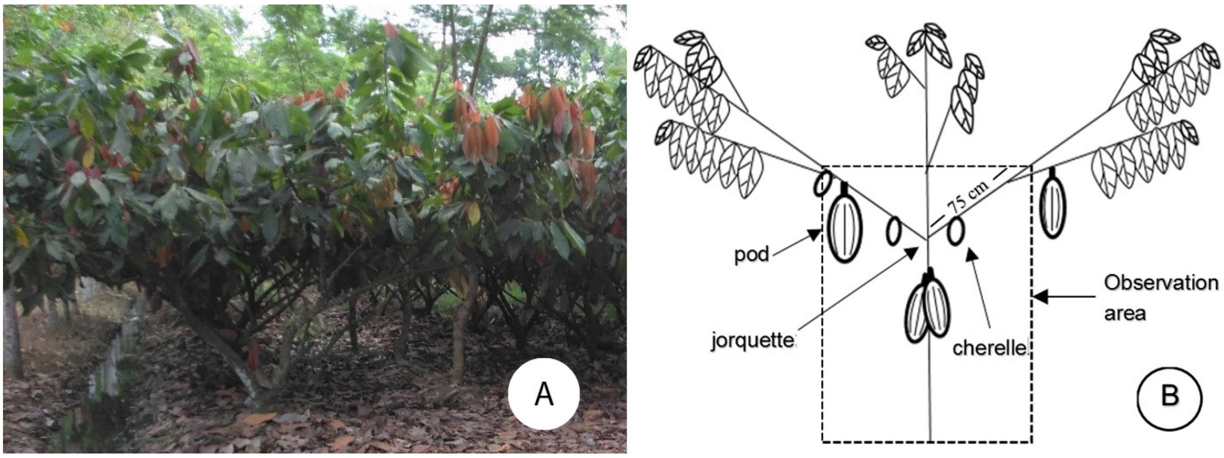

Figure 1. Ten-year-old ocoa trees in the field (A); shade area for cherelles and pods measurement (B). Drawings are not to scale.

Pods were classified based on their length into tiny-sized cherelle (TC) <5cm, small-sized cherelle (SC) 5–10 cm, medium-sized pod (MP) 10–15 cm and large-sized pod (LP) >15 cm. Photosynthetic rate, transpiration rate, stomatal conductance, Ci/Ca ratio and water use efficiency (WUE) were observed using LICOR 6400 XT at 24 WAT. Pod growth and production data were analyzed using ANCOVA, while physiological data were analyzed using ANOVA. Significant means were further analyzed using Duncan's Multiple Range Test (DMRT) at α=5% using SAS software 9.4.

Results

Plant Growth

Plant growth could be observed visually from flush emergence and the intensity of shoot formation. Cocoa tree treated with humic acid and biofertilizer had more flush than those trees that did not receive any treatments. The high number of flushes was followed by new shoot (chupons) growth that were also higher than those of the control. Compared to the control, the number of chupons in the treated plants were approximately 34% higher, but the presence of the new shoot were not observed further. Extensive new shoot was detrimental on to pod development, therefore, during tree maintenance the undesirable shoots were removed.

Tiny-sized (TC) and Small-sized Cherelle (SC)

Cocoa tree produces flowers and cherelles throughout the year and they do not depend on tree age, as observed in all blocks. Total cherelles per observation reached 2.4–6.4 and 1.4–4.4, consecutively for tiny cherelle (TC) and small cherelle (SC). However, not all cherelle developed into pod as some cherelles wilted. Number of TC was affected by biofertilizer, while number of SC was affected by single treatment of humic acid and biofertilizer. On average, TC developed into SC was 55% and SC developed into medium pod then large pod was 62%. The percentage of survived TC and SC were not affected by humic acid but affected by biofertilizer application. The higher concentration of biofertilizer produced more number of healthy cherelles.

Humic acid application on cocoa tree had no significant effect on total TC at 24 WAT (Table 1). On the other hand, biofertilizer spraying significantly affected total TC and SC, percentage of healthy TC and SC. Interaction between humic acid and biofertilizer had no effect on total and percentage of healthy cherelles.

Plants treated with 1000 ppm biofertilizer significantly produced higher total TC than control and 500 ppm, but were not significantly different with 1500 and 2000 ppm biofertilizers (Table 2). At 4–24 WAT, the highest total TC were obtained after application of 2000 ppm biofertilizer (Figure 2). Plants sprayed with 1500 biofertilizer significantly produced higher number of healthy TC than the other treatments (Table 2). Application level of 1500 ppm biofertilizer consistently showed the highest percentage of healthy TC at 12–24 WAT (Figure 2).

At 24 WAT, total SC of plants treated with 2000 ppm humic acid was significantly higher than the other treatments. Biofertilizer application at the same level produced SC 43% higher than the control (Table 2). The combination of humic acid and biofertilizer at 2,000 ppm each showed the most favor for SC production at 8–24 WAT (Figure 3). Nevertheless, percentage of healthy SC did not corelate to the number of SC especially on the application of humic acid (Table 1). Percentage of healthy SC of plants

Table 1. Summary of covariance and variance analysis of pod growth and physiological variables at 24 week after treatment (WAT) and harvest variables at 28 WAT

Variable	Humic Acid	Biofertilizer	Interaction
Total tiny cherelle (TC)	0.99 ns	3.39 *	1.42 ns
Healthy TC (%)	1.14 ns	9.61**	0.56 ns
Total small cherelle (SC)	5.53 **	4.86 **	0.81 ns
Healthy SC (%)	0.70 ns	2.91 *	1.81 ns
Medium-sized pod (MP)	0.45 ns	10.81 **	1.00 ns
Large-sized pod (LP)	0.43 ns	1.60 ns	0.66 ns
Harvested pods number	0.53 ns	3.86 **	1.74 ns
Beans number per plant	0.81 ns	7.65 **	1.98 *
Single bean weight	0.33 ns	2.47 ns	0.81 ns
Beans weight per plant	0.74 ns	5.94 **	1.82 ns
Yield per harvest	0.80 ns	5.71 **	1.72 ns
Photosynthetic rate	1.41 ns	26.38 **	1.87 *
Transpiration rate	0.38 ns	0.36 ns	1.37 ns
Stomatal conductance	0.74 ns	0.40 ns	0.88 ns
Ci/Ca	0.61 ns	17.83 **	1.45 ns
WUE	1.27 ns	19.81**	1.37 ns

Note: * = P<0.05, ** = P<0.01 and ns = not significant according to Fisher test α 5%; where Ci/Ca = ratio of CO_2 intercellular to CO_2 atmosphere; WUE = water use efficiency.

treated with 1500 ppm biofertilizer was significantly higher than the control and the biofertilizer applied at 500 ppm (Table 2). The highest percentage of healthy SC at 12–24 WAT was produced by plants treated with 1500 ppm biofertilizer (Figure 3).

Medium-sized (MP) and Large-sized Pod (LP), and Bean Production

Number of medium-sized pod (MP) was not affected by humic acid application (Table 1). Spraying of 1500 ppm biofertilizer significantly increased MP number

Table 2. Number of tiny-sized cherelles (TC), small-sized cherelle (SC), medium-sized pod (MP) and large-sized pod (LP) on humic acid and biofertilizer treatments at 24 WAT

Treatment (ppm)	Number of TC		Number of SC		Number of pod	
	Total	Healthy (%)	Total	Healthy (%)	MP	LP
Humic acid						
0 (control)	5.3 a	41.23 a	2.7 c	58.82 a	1.8 a	1.2 a
1000	5.8 a	41.30 a	4.2 a	53.43 a	1.9 a	1.3 a
2000	5.4 a	42.49 a	3.5 b	56.13 a	2.0 a	1.3 a
3000	5.6 a	43.62 a	3.5 b	56.75 a	2.0 a	1.3 a
4000	5.5 a	46.32 a	3.3 bc	59.97 a	2.0 a	1.2 a
Biofertilizer						
0 (control)	5.1 b	37.57 b	2.8 c	50.61 b	1.6 b	1.2 a
500	5.0 b	38.42 b	3.0 bc	52.93 b	1.7 b	1.2 a
1000	5.8 a	42.31 b	3.5 ab	59.16 ab	1.9 a	1.2 a
1500	5.7 a	53.92 a	3.9 a	64.42 a	2.4 a	1.4 a
2000	5.8 a	42.71 b	4.0 a	57.92 ab	2.0 a	1.3 a

Note: Values followed by different letters within a column are significantly different according to DMRT α=5%. Interactions among treatments are shown in Table 1.

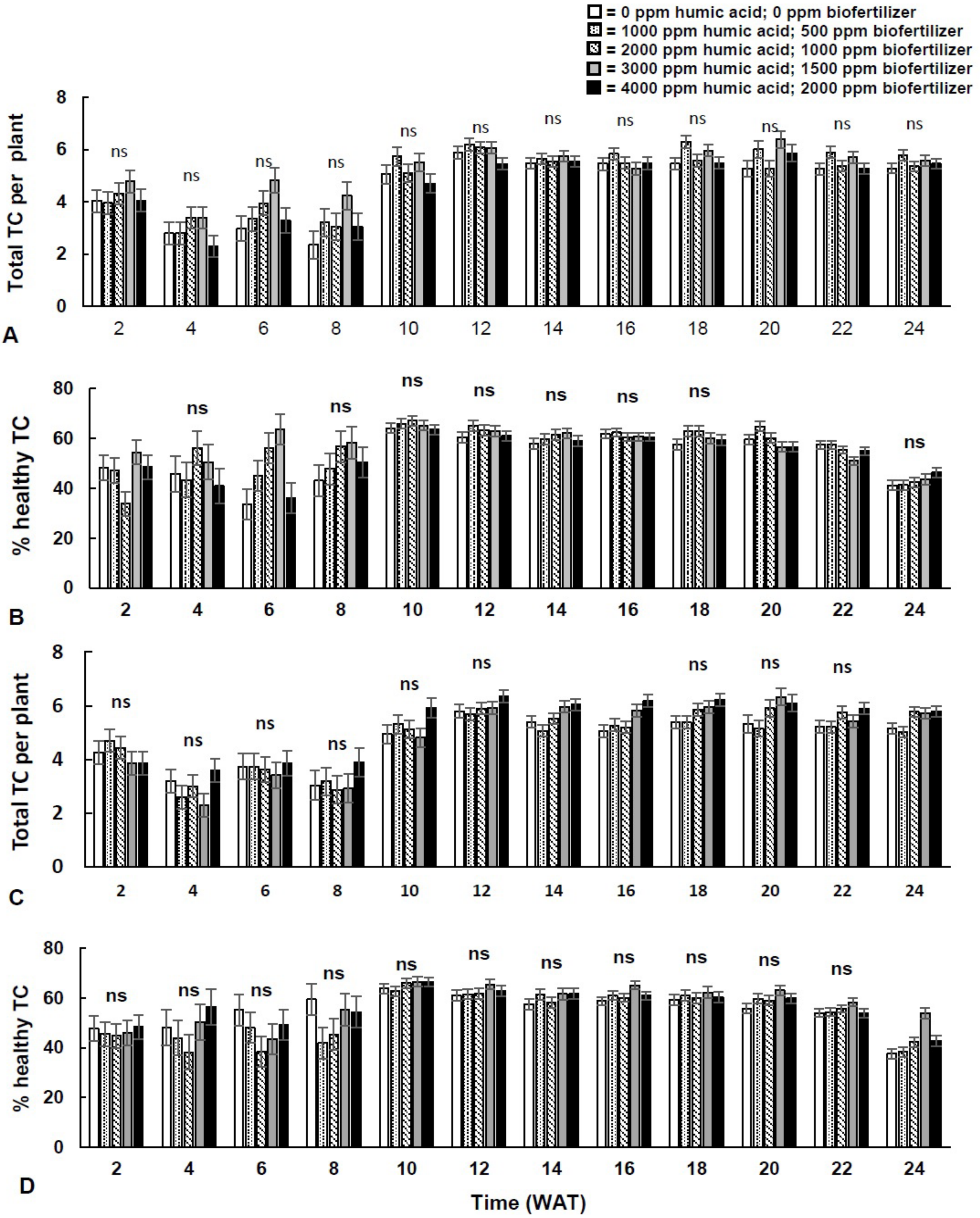

Figure 2. Total and percentage of tiny-sized cherelle (TC) on different level of humic acid (A-B) and biofertilizer applications (C-D) at different weeks after treatment (WAT); ns = not significant according to Fisher test α = 5%.

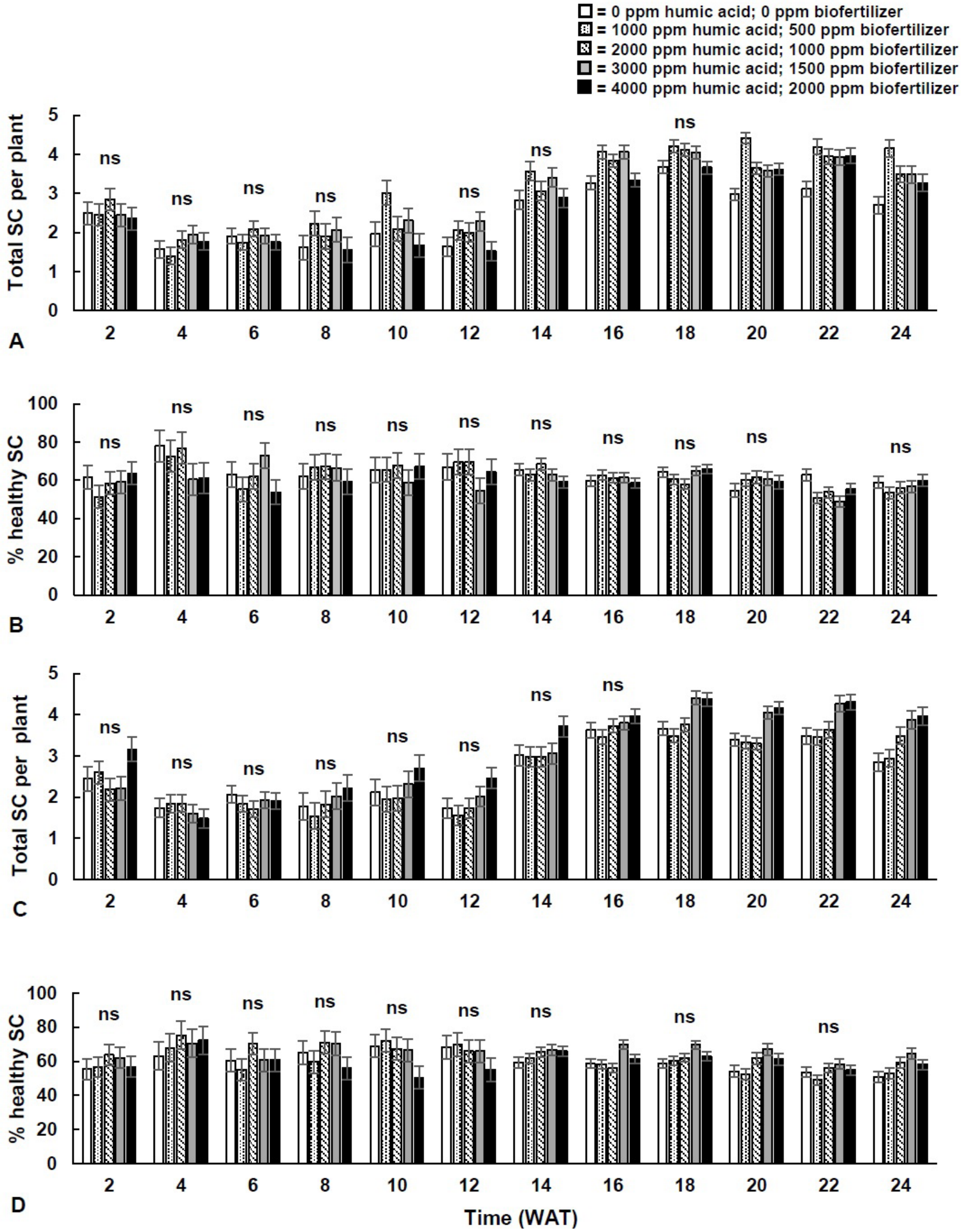

Figure 3. Total and percentage of small-sized cherelle (SC) of different level of humic acid (A-B) and biofertilizer applications (C-D) at 2 to 24 weeks after treatment (WAT); ns = not significant according to Fisher test α = 5%.

in 24 WAT as compared to the control and 500 ppm (Table 2). Plants treated with 1500 ppm biofertilizer produced the highest MP at 12–24 WAT (Figure 4).

the higher level of biofertilizer stimulated higher photosynthesis rate. However, photosynthesis rate was relatively constant, irrespective of humic acid

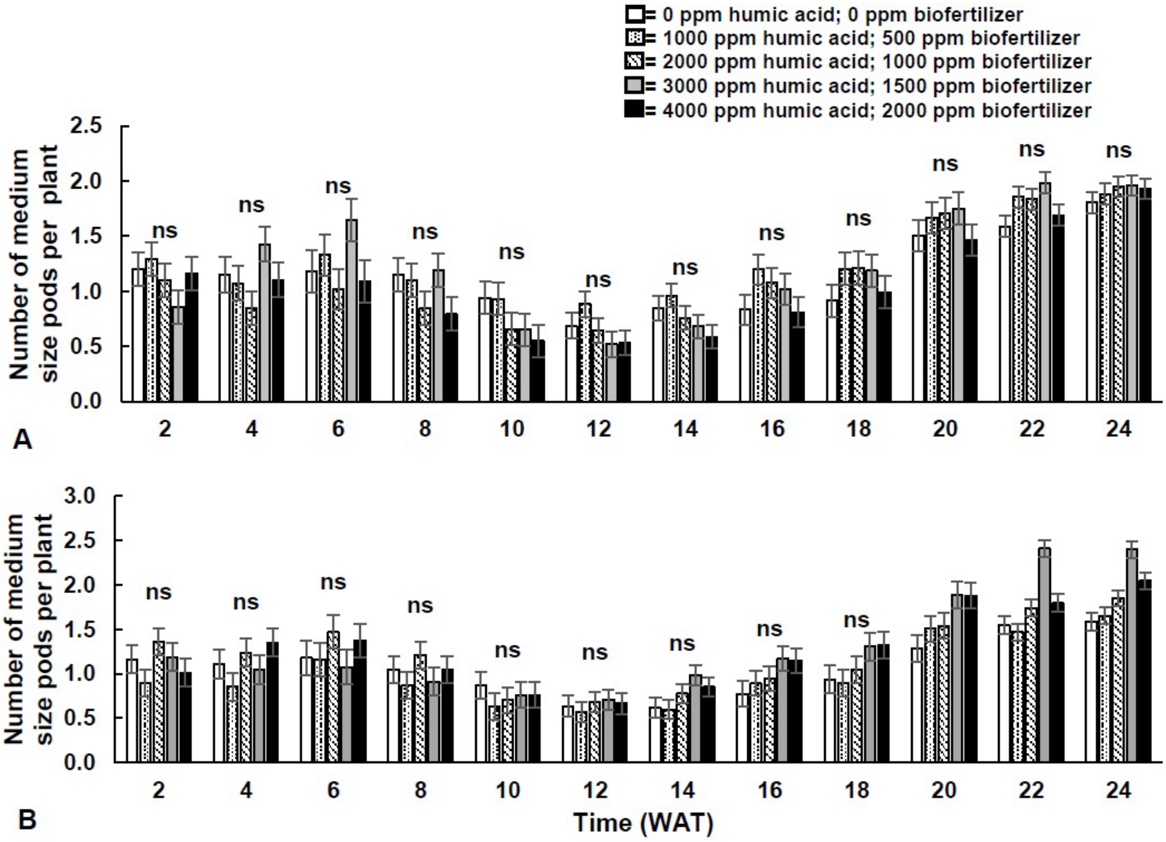

Figure 4. Number of medium-sized pods (MP) at different level of humic acid (A) and biofertilizer application (B) at 2 to 24 weeks after treatment (WAT); ns = not significant according to Fisher test α = 5%).

Application of 1500 ppm biofertilizer showed the highest number of harvested pods, cocoa bean weight per plant and yield per harvest at 27 and 28 WAT. Increasing number of harvested pods, bean weight per plant and yield per plant at 28 WAT were 32.8%, 39.7% and 39.4%, respectively, as compared to the control (Figure 5). Figure 5C showed that single bean weight was relatively constant among harvesting times and among treatments, i.e., 1.04–1.10 g.

Interaction of humic acid and biofertilizer significantly affected number of beans per plant at 28 WAT (Table 1). Cocoa tree that received a combination of humic acid and biofertilizer at 1000 ppm each produced the higherst number of cocoa beans per plant (Table 3).

Photosynthesis

The interaction of humic acid and biofertilizer significantly affected photosynthesis rate in 24 WAT (Table 1). The combination of 3000 ppm humic acid and 1500 ppm biofertilizer showed the highest photosynthesis rate (Table 4). Table 4 shows that levels, i.e. 41.2–44.7 µmol CO_2 $m^{-2}.s^{-1}$.

Humic acid application had no effect on Ci/Ca ratio of cocoa tree at 24 WAT (Table 1). On the other hand, application of 1,500 ppm biofertilizer significantly decreased Ci/Ca ratio compared to the control, 500 and 1000 ppm, but it was not significantly different with 2000 ppm (Table 5). Table 5 shows that transpiration rate and stomatal conductance were not affected by fertilizer treatment. Water use efficiency (WUE) was 50-54%, irrespective of humic acid treatment at 24 WAT, while increasing level of biofertilizer higher than 500 ppm significantly increased WUE. Spraying biofertilizer at the rate of 1500 ppm significantly increased WUE of cacao plants by 17–41% as compared to 0–1000 ppm (Table 5).

Discussion

The application of biofertilizer increased cocoa production (Table 2), and at 1000–2000 ppm significantly resulted in a higher bean weight per plant and yield relative to the control. The increase in

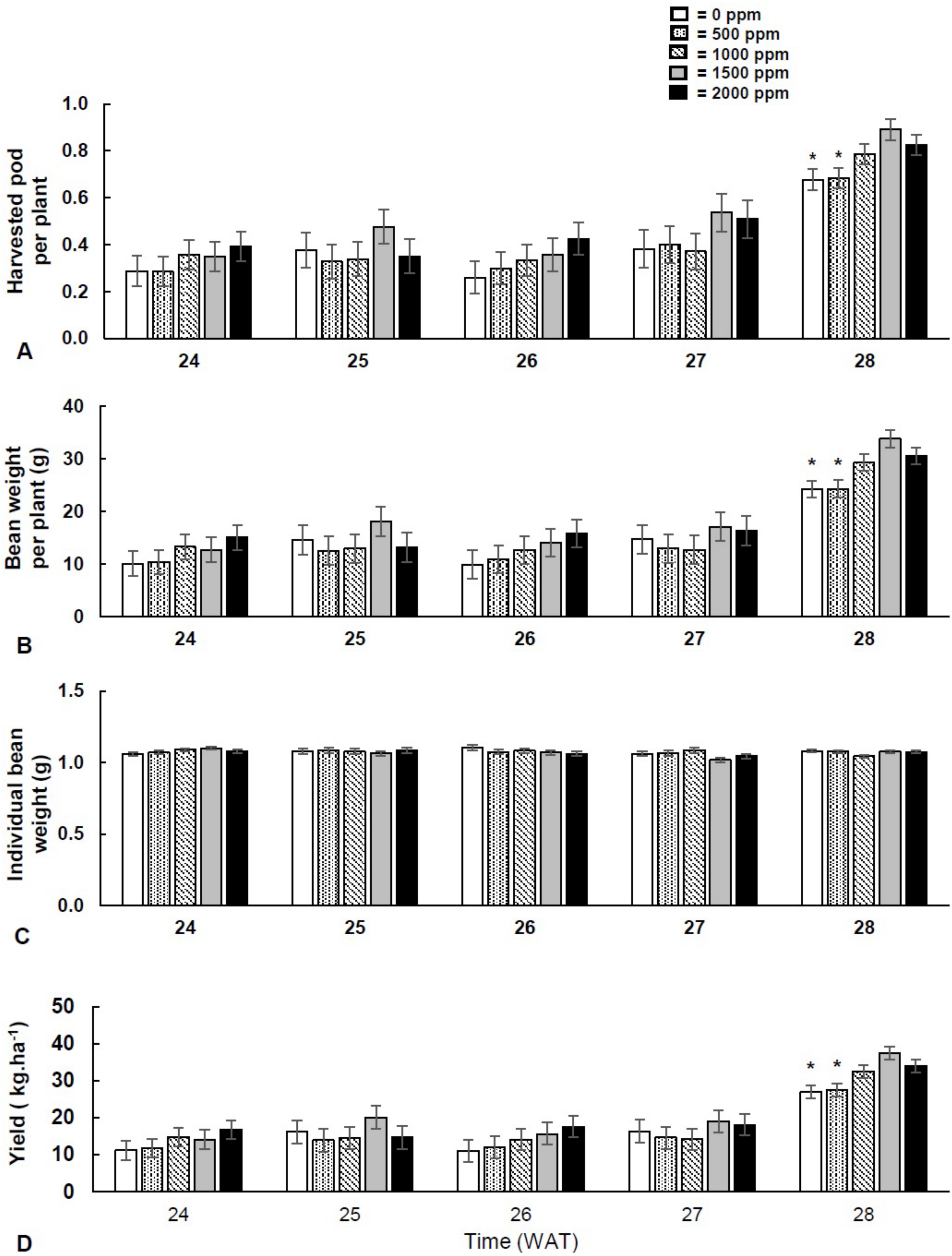

Figure 5. Number of harvested pod (A), beans weight per plant (B), individual bean weight (C) and yield per harvest (D) at different level of biofertilizer at 24 to 28 weeks after treatment (WAT); where * = significant different according to DMRT α 5%.

Table 3. Number of beans per plant as affected by different level of humic acid and biofertilizer treatments at 28 weeks after treatment (WAT)

Biofertilizer level (ppm)	Humic acid level (ppm)										Average
	0		1000		2000		3000		4000		
0	17.3	ef	23.1	cdef	24.8	cdef	22.1	cdef	25.3	cdef	22.5
500	22.8	cdef	16.5	f	22.4	cdef	26.0	cdef	25.7	cdef	22.7
1000	28.5	abcd	37.9	a	26.4	bcdef	20.7	def	24.4	cdef	27.6
1500	29.9	abcd	29.2	abcd	27.8	abcde	37.4	ab	32.3	abc	31.3
2000	28.7	abcd	28.7	abcd	23.7	cdef	31.5	abcd	30.6	abcd	28.6
Average	25.4		27.1		25.0		27.5		27.7		26.5

Note: Values followed by different letters within a column and a row are significantly different according to DMRT α=5%.

Table 4. Photosynthetic rate (μmol CO_2 $m^{-2}.s^{-1}$) of cocoa tree with different level of humic acid and biofertilizer treatments at 24 WAT

Biofertilizer level (ppm)	Humic acid level (ppm)										Average
	0		1000		2000		3000		4000		
0	36.12	fgh	33.72	h	33.27	h	39.09	defgh	39.09	defgh	36.3
500	37.28	efgh	33.24	h	34.39	gh	41.13	cdefgh	40.21	defgh	37.3
1000	41.28	cdefgh	50.26	abc	43.26	cdefg	36.73	efgh	44.86	bcdef	43.3
1500	48.16	abcd	53.72	ab	47.42	abcd	56.03	a	48.55	abcd	50.8
2000	46.06	bcde	48.55	abcd	47.56	abcd	50.31	abc	43.43	cdefg	47.2
Average	41.8		43.9		41.2		44.7		43.2		42.9

Note: Values followed by different letters within a column and a row are significantly different according to DMRT α=5%.

Table 5. Transpiration rate, stomatal conductance, Ci/Ca ratio and water use efficiency (WUE) as affected by different levels of humic acid and biofertilizer treatments at 24 WAT

Fertilizer level (ppm)	Transpiration rate(mmol H_2O $m^{-2}.s^{-1}$)	Stomatal conductance (mol H_2O $m^{-2}.s^{-1}$)	Ci/Ca	WUE
Humic acid				
0	8.42 a	0.30 a	0.33 a	0.50 a
1000	8.22 a	0.29 a	0.30 a	0.54 a
2000	8.31 a	0.30 a	0.34 a	0.50 a
3000	8.28 a	0.30 a	0.30 a	0.54 a
4000	8.33 a	0.29 a	0.31 a	0.52 a
Biofertilizer				
0	8.33 a	0.30 a	0.42 a	0.44 c
500	8.41 a	0.30 a	0.41 a	0.45 c
1000	8.29 a	0.29 a	0.31 b	0.53 b
1500	8.30 a	0.30 a	0.19 c	0.62 a
2000	8.23 a	0.30 a	0.25 bc	0.58 a

Note: Values followed by different letters within a column are significantly different according to DMRT α=5%. Interaction among treatments are shown in Table 1.

production is likely due to an increase in the number of harvested pods and bean weight per plant. It is also noted that spraying with 1500 ppm biofertilizer increases the number of total cherelles and the percentage of healthy cherelle (Table 2). The high percentage of healthy cherelle means high chance to obtain harvested pods. Khattab et al. (2012) stated that application of humic acid on pomegranate plants increased fruit number per tree and production by 10.8% and 24.6%, respectively. In apple, Hidayatullah et al. (2018) revealed that the application of humic acid increases fruit number by 470%.

Increasing bean weight per plant on the tree that received biofertilizer spraying is likely related to an increase in the photosynthetic rate (Tabel 4); higher photosynthesis means higher photosynthate that presumably directed into cacao pods. Khan et al. (2016) and Sahu et al. (2017) demonstrated that application of *Trichoderma* sp. dan *Azospirillum* sp. increased photosynthesis rate of tomato through increases in nitrogen uptake as the main component of chlorophyll.

The results of this research clearly shows that application of humic acid and biofertilizer could increase cacao productivity. Phenologically, cacao tree is able to produce pods throughout the year. However, cocoa production might fluctuate depending on water availability (ICCRI, 2015; Santosa et al., 2018). In general, it takes 5 – 6 months or about 143 – 170 days from anthesis to pod ready to be harvested (ICCRI, 2015), at which first phase (75 days) started from fertilization followed by a 40-day of slow growth and a 35-day of fast growth resulted in pod length about 11 cm, and the second phase (120 days) characterized by pods enlargement (ICCRI, 2006). Mature pods is indicated by a change in pod color and beans separate from pod. Therefore, the humic acid and biofertilizer is likely need to be applied regularly following the cycle of pod production. It is important to evaluate the effectiveness of humic acid and biofertilizer application in both dry and rainy season, including the replacement of inorganic fertilizers in order to develop more sustainable cocoa production and better application for farmers.

Conclusion

The application of humic acid and biofertilizer benefit pod growth and bean production during the dry season. Plants treated with 1000 ppm humic acid produced the highest number of small-sized cherelle whereas biofertilizer applied at 1500 ppm increased cherelles number, percentage of healthy cherelle, number of medium-sized pod and harvested pods, and yield of cacao beans. The application of 1000 ppm humic acid and 1500 ppm biofertilizer increased cocoa bean production by 39.7%. Based on this results application of humic acid and biofertilizer is recommended to increase cocoa production in the dry season.

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