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Research article

Effect of Seaweed Combined With an Endophytic Microbial Consortium on Two Varieties of Chili

Chotimatul Azmi[1]*, Hadis Jayanti[2], Rini Murtiningsih[1], Helmi Kurniawan[1], Surono Surono[3], Rafika Yuniawati[4], Karden Mulya[4], and Dwi Ningsih Susilowati[4]

[1]Indonesian Vegetables Research Institute, Indonesia
[2]Bali Assessment Institute for Agricultural Technology, Indonesia
[3]Indonesian Soil Research Institute, Indonesia
[4]Indonesian Center for Agricultural Biotechnology and Genetic Resources and Development, Indonesia

ORCID
Chotimatul Azmi: https://orcid.org/0000-0001-7780-6756

Abstract.
In Indonesia, the chili (*Capsicum annuum* L.) is popular. Unfortunately, its current productivity is lower than its potential yield. The low productivity is due to the cultivation methods or varieties used in the field. Seaweed is a known bio-stimulant and combining it with a microbial consortium could be an effective way to boost crop productivity. The goal of this study was to examine how a combination of bio stimulants enriched with a microbial consortium can affect the productivity of two chili varieties, Tanjung-2 and Ciko. The experiment used a complete randomized block design with a single factor, namely a combination of bio-stimulants (B1-B6). In comparison to other formulas, the bio-stimulant B4 formula was found to be the most effective.

Keywords: bio stimulant; chili; endophytic microbial consortium; seaweed

Corresponding Author: Chotimatul Azmi; email: chotimazmi@yahoo.com

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1. Introduction

As the main horticultural commodity in Indonesia due to its high economic value, chili (*Capsicum annum* L.) is set to be prioritized for development. This product is needed both in fresh vegetables market and food industry. The chili could be cultivated in the lowlands until highlands. Unfortunately, its productivity is commonly below its potential yield due to pest attacks or poor technical culture applied during the production.

The yields of chili can be increased by modifying the technical cultures, for example by applying bio-stimulants to the crops. In addition to fertilizers and microorganisms, bio-stimulants is categorized as materials that if applied in small amount could improve plant growth [1]. Bio-stimulant is able to stimulate and adjust plant physiological processes such as respiration, photosynthesis, nucleic acid synthesis and ion absorption [2].

According to [3] there are several sources of bio-stimulants that have been developed including agricultural microbial inoculants (bacteria, fungi), humic acid [4], fulvic acid, amino acids, seaweed extracts, and plant extracts. These bio-stimulants can be used individually or in combination with more than one type of bio-stimulant.

Previous research showed that bio-stimulants developed from seaweed extracts contain nutrients, amino acids, cytokinins, auxins, laminaran, fucoidan, alginate, and betain which stimulate plant metabolism and increase plant growth and yield [5]. Those chemicals could act as fertilizers and play important role in germination, the formation of new plants, and better crop development [6]. The use of seaweed to stimulate plant growth has been implemented in various crops, for instance okra [7], Tagetes [8], chickpeas [9], [10], and chili [11]. Additionally, beneficial bacteria have been widely used as bio-stimulants in various plants, including lettuce [12], rice, corn [13], strawberry [14], rice [15], and chili [16]. While beneficial fungi have been applied to rice [17] and chili [18], [19]. However, there were limited studies done on the use of bio-stimulant made from a combination of seaweed and endophytic microbes. Therefore, this research was conducted to evaluate the effect of bio-stimulants combination on chili crops.

2. Methodology

This research was conducted at the Microbiology Laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRD), Bogor and the Indonesian Vegetable Research Institute (IVEGRI), Lembang from March to August 2020. Bio-stimulant B1, B2, B3, B4, B5, and B6 were formulated in the Microbiology laboratory of ICABIOGRD and applied on the chili crops cultivated in the research field station of IVEGRI.

2.1. Bio-stimulant formulation

The bio-stimulant was formulated by mixing bacterial suspension or dark septat endophytes (DSE) and carrier made from *Sargasum* or *Ulva* seaweed extracts. Some organic and mineral ingredients were added into the bio-stimulant formula. The seaweed extract was prepared to follow the extraction method developed by Hernandez-Herrera et al. (2014) [20] with some modifications. Two seaweeds i.e., *Ulva* sp. and *Sargasum* sp. collected from the Bayah seashore of Banten were put into a heat-resistant plastic containers and then dried in oven at 60°C temperature for 72 hours. The dried seaweed was powdered by using blender. Finally, a total of 200 g of seaweed powder was diluted in 400 mL distilled water (1:2 w/v) and centrifuged for 15 minutes at a speed of 10,000 rpm to obtain seaweed extract.

The composition of the six bio-stimulant formulations used in the study included

B1 = *Sargasum* seaweed extract enriched with a consortium of selected endophytic bacteria

B2 = *Sargasum* seaweed extract enriched with a consortium of selected dark septate endophytic

B3 = *Ulva* seaweed extract enriched with a consortium of selected endophytic bacteria

B4 = *Ulva* seaweed extract enriched with a consortium of selected dark septate endophytic

B5 = commercial seaweed extract (*Ascophyllum nodosum*) as control

B6 = water as control

In this study, the consortium of selected endophytic bacteria consisted of five ICABI-OGRD Culture Collection (ICABIOGRD CC) bacteria collections i.e., *Bacillus subtilis* strain DBS2, *B. cereus* strain Filos-8, *B. subtilis* strain 30, *B. subtilis* strain Kal-47, and *B. thuringiensis* strain FBE-79. While the dark septate endophytic isolates consisted of LKM 2BTR 2B, *Curvularia* sp. strain TKC 22a, *Rhizopus* sp. strain TKH 1.1.1, *Curvularia* sp. strain PP 23, and LKM 2B GR 43. Each isolate used in the consortium has been tested for its synergism. Additionally, the phytohormonal content of each bio-stimulant formula has been previously measured by using HPLC and presented in Table 1.

Table 1: The Concentration of Phytohormones (IAA, Giberellin, Zeatin, Kinetin) in The Tested Bio-stimulants.

Treatments	Application dose (ml/L)	Concentration (ppm)			
		IAA	Gibberellin	Zeatin	Kinetin
B1	7.5	3.94	4.89	1.32	0.91
B2	7.5	4.29	5.58	1.56	0.97
B3	7.5	3.95	6.47	1.46	0.91
B4	7.5	3.54	6.23	1.53	0.81
B5	3.0	42.05	93.12	41.66	38.40
B6 (water)	-	-	-	-	-

The evaluation of bio-stimulant formula in chili crops

A complete randomized block design (RCBD) with single factor, six bio-stimulants treatments (B1-B6) and 4 replications was performed in this study. This design was implemented in the plot of two chili varieties, i.e., Tanjung-2 and Ciko to made two adjacent experimental plots. Each experimental unit consisted of 120 plants and 12 plants from each unit were used as sample plant.

Chili seeds were sown in the nursery, and 30 days after sowing (DAS), seedlings were transplanted on to soil bed mulched with black silver plastic in the field. A 7.5 mL L-1 of bio-stimulant were applied on chili plant accordingly to follow the designed treatment. The bio-stimulant solution was sprayed on the underside of the leaves at the 30, 45, and 60 days after transplanting (DAT) [21].

2.2. Observation and analysis

Observations were conducted during the vegetative and harvest crop stages. The plant height, number of healthy and broken fruit, weight of healthy and broken fruit, fruit length, fruit diameter, peduncle length, peduncle diameter, number of locules and fruit weight were recorded during the observation. The data were then analyzed for variance using the R program.

3. Result and Discussion

In general, chili plants treated with bio-stimulants showed good growth. Plant height was recorded at 4, 6, 8, 10 and 12 days after transplanting (DAT). Chili fruits were harvested once they reached fully reddish color stage. Harvested fruits were sorted into two groups i.e., healthy fruits and broken fruits result of wilting, fruit flies attack or anthracnose disease. The number and weight of fruits in each group were recorded. Additionally, harvested fruits were sampled for individual fruit examination including the fruit's length and diameter, peduncle's length and diameter, fruit weight and the number of locules. Chili variety of Tanjung-2 were harvested earlier (16 weeks after transplanting/WAT) than the Ciko (18 WAT), and it considered as early variety. The variance analysis showed the different data trend of all observed characters in both tested varieties, while the bio-stimulant treatments did not have significant effect in all observed characters.

Figure 1 shows that variety of Tanjung-2 has higher posture than Ciko. However, in both varieties, there was no significant different among plant height in all tested treatments. Previous study on cayenne found the increasing height of plant that been treated with bio-stimulants made from *Bacillus vallismortis* [22]. However, similarly to the current study, there was no significant difference among treatments.

Chili fruits were harvested once they reached fully reddish color stage. Harvested fruits then being sorted to two groups i.e., healthy fruits and broken fruits either due to wilting, fruit flies or anthracnose disease. The number and weight of fruits in each group were recorded. Table 2 showed the number and weight of healthy and damaged

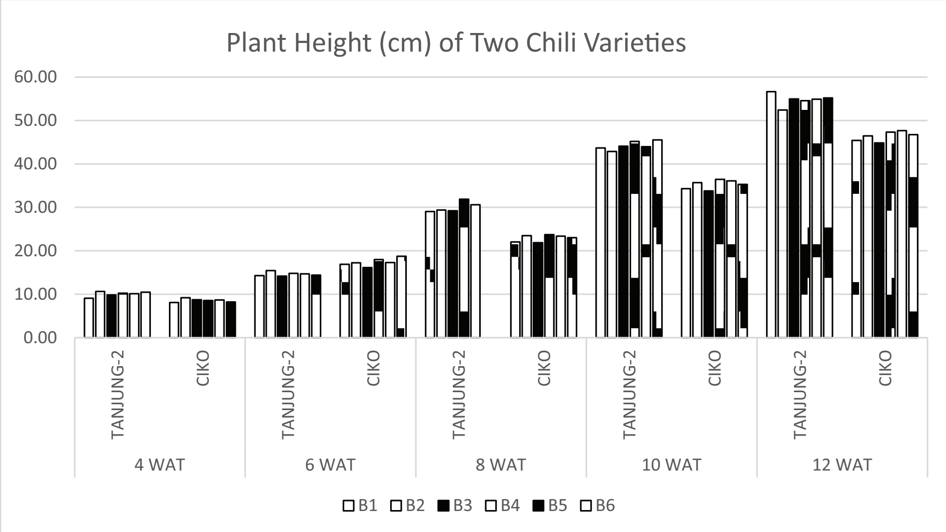

Figure 1: Plant Height of Chili Variety of Tanjung-2 and Ciko at 4, 6, 8, 10, and 12 WAT.

fruit per plant and per plot of the Tanjung-2 variety. There were no significant different among all treatments in all observed variables. The highest number of healthy fruits per plant and per plot were experienced by the crops treated with bio-stimulant B4 (i.e., 29.90 and 1,878 respectively). The lowest number of damaged fruits per plant was in treatment B6, while the lowest number of damaged fruits per plot was found in by treatment B5 (i.e., 0.31 and 87.25 respectively). The highest weight of healthy fruit per plant and per plot occurred in crops treated with bio-stimulant B3 (417.09 g) and bio-stimulant B2 (26,704.33 g) respectively. Application of bio-stimulant B1 and B5 caused the lowest broken fruit weight per plant and per plot (i.e., 3.47 g and 841.95 g respectively). Chili crops treated with bio-stimulant B1 produced the highest average weight per healthy fruit (14.53 g) and the lowest average of broken fruit weight per fruit (6.06 g). Table 3 showed that there was no significant difference of total number and weight of harvested fruits in all tested treatments. The highest fruit number and weight per plant and number of fruits per plot were occurred on crops treated with bio-stimulant B4 (30.63; 2.013.50 g; 424.06 respectively). While crops in treatment B2 produced the highest fruit weight per plot (27,961.68 g).

Note: JBSATS = the number of healthy fruits per plant; JBSATPP = the number of healthy fruits per plot; JBSITS = the number of damaged fruits per plant; JBSITPP = the number of damaged fruits per plot; BBSATS = the weight of healthy fruit per plant; BBSATPP = the weight of healthy fruit per plot; BBSITS = the weight of damaged fruit per plant; BBSITPP = the weight of damaged fruit per plot; BPBSA = the average weight of healthy fruit; BPBSI = the average weight of damaged fruit

Table 2: Characteristics of Tanjung-2 Harvested Fruits in Six Bio-stimulant Applications.

Treatments	JBSATS	JBSATPP	JBSITS	JBSITPP	BBSATS	BBSATPP	BBSITS	BBSITPP	BPBSA	BPBSI
							g			
B1	24.81	1,730.25	0.42	110.25	362.07	24,626.85	3.47	1,162.75	14.53	6.06
B2	25.79	1,730.25	0.42	134.25	350.51	26,704.33	4.71	1,257.35	13.63	9.60
B3	29.73	1,706.50	0.54	148.50	417.09	25,533.48	5.49	1,415.37	14.03	9.88
B4	29.90	1,878.00	0.73	135.50	416.76	25,642.52	7.30	1,133.80	13.90	10.36
B5	28.92	1,720.50	0.54	87.25	405.76	25,098.92	5.42	841.95	14.04	8.82
B6	26.21	1,695.50	0.31	107.50	355.29	24,933.12	3.61	1,087.52	13.57	12.63

Table 3: The Total Tanjung-2 Fruit Number and Weight per Plant and per Plot.

Treatments	JBPT	BBPT (g)	JBPP	BBPP (g)
B1	25.23	1,840.50	365.54	25,789.60
B2	26.21	1,864.50	355.22	27,961.68
B3	30.27	1,855.00	422.58	26,948.85
B4	30.63	2,013.50	424.06	26,776.32
B5	29.46	1,807.75	411.18	25,940.87
B6	26.52	1,803.00	358.90	26,020.64

Note: JBPT = The total fruit number per plant; BBPT = The total weight per plant; JBPP = The total fruit number per plot; BBPP = The total weight per plot

Characteristics of Ciko harvested fruits in six bio-stimulant applications are shown in Table 4. There was no significant difference of all the observed variables in different treatments. The highest number of healthy fruits per plant and per plot was produced by crops in treatment B1 (i.e., 31.29 and 2.215 respectively). While the lowest number of damaged fruits per plant and per plot occurred in crops treated with bio-stimulants B5 (i.e., 0.23 and 43 respectively). The highest healthy fruit weight per plant was experienced by crops sprayed by bio-stimulant B4 (451.92 g). The crops treated with bio-stimulants B1 produced the highest healthy fruit weight per plot (30,876.78 g). The crops treated with bio-stimulants B5 produced the lowest diseased fruit weight per plant and per plot (i.e., 3.06 g and 501.38 g respectively). The highest average weight of healthy fruit was produced in crops sprayed by bio-stimulant B3 (14.36 g). While the lowest average weight of broken fruit was occurred in treatment B6 (10.8 g). Table 5 shows the data on the number and weight of the total fruit harvested from each treatment. Treatment B4 produced the highest number and weight of fruits per plant and number of fruits per plot (33.32; 2,186.50 g; 457.26). Meanwhile, the highest fruit weight per plot was occurred in treatment B1 (31, 563.66 g). These results are in line with the study conducted by Susilowati (2020) which found that the number and weight of harvested cayenne fruits increased due to the application of *Bacillus vallismortis* but it was not significantly different from the control [22].

TABLE 4: Characteristics of Ciko Harvested Fruits in Six Bio-stimulant Applications.

Treatments	JBSATS	JBSATPP	JBSITS	JBSITPP	BBSATS	BBSATPP	BBSITS	BBSITPP	BPBSA	BPBSI
					g					
B1	31.29	2,152.75	0.81	63.75	423.04	30,876.78	9.08	686.88	13.58	11.65
B2	27.15	1,840.50	0.31	80.25	351.02	24,447.28	4.65	615.53	12.93	16.89
B3	27.79	1,779.75	0.42	51.75	401.11	23,248.90	4.84	571.43	14.36	10.81
B4	32.88	2,106.25	0.44	80.25	451.92	29,698.63	5.34	795.20	13.68	11.00
B5	29.38	1,901.50	0.23	43.00	389.16	25,923.60	3.06	501.38	13.16	12.33
B6	30.44	2,073.50	0.65	77.00	396.6	29,593.83	6.28	769.50	12.92	10.80

Note: JBSATS = the number of healthy fruits per plant; JBSATPP = the number of healthy fruits per plot; JBSITS = the number of damaged fruits per plant; JBSITPP = the number of damaged fruits per plot; BBSATS = the weight of healthy fruit per plant; BBSATPP = the weight of healthy fruit per plot; BBSITS = the weight of damaged fruit per plant; BBSITPP = the weight of damaged fruit per plot; BPBSA = the average weight of healthy fruit; BPBSI = the average weight of damaged fruit

The length of fruit was measured from the base of the fruit to the tip of the fruit, while the length of the peduncle was measured from the base of the peduncle to the end of the peduncle. The digital caliper was used to measure the fruit diameter in biggest part of the fruit and peduncle diameter in the center of the peduncle. The locule is described as the cavity where the placenta located. Table 6 shows the non-significant difference of all Tanjung-2 fruit observed variables in all treatments tested. The highest fruit length and peduncle length were occurred in treatment B5 (18.94 cm) and treatment B2 (4.28 cm) respectively. While the fruits in treatments B1 and B6 have the highest fruit diameter and peduncle diameter i.e., 20.11 mm and 3.92 mm respectively. The highest number of locule was occurred in the crops treated with bio-stimulant B6 (2.39).

TABLE 5: The Total Ciko Fruit Number and Weight per Plant and per Plot.

Treatments	JBPT	BBPT (g)	JBPP	BBPP (g)
B1	32.10	2,216.50	432.12	31,563.66
B2	27.46	1,920.75	355.67	25,062.81
B3	28.21	1,831.50	405.95	23,820.33
B4	33.32	2,186.50	457.26	30,493.83
B5	29.61	1,944.50	392.22	26,424.98
B6	31.09	2,150.50	402.88	30,363.33

Note: JBPT = The total fruit number per plant; BBPT = The total weight per plant; JBPP = The total fruit number per plot; BBPP = The total weight per plot

Table 7 shows the characters of Ciko fruits in six bio-stimulant treatments and similar to the Tanjung-2 variety. This table shows that there were no significant differences of all observed fruit characters in all treatments. The highest fruit length and peduncle length were occurred in treatment B1 (12.76 cm) and treatment B6 (2.82 cm) respectively. While the highest fruit diameter and peduncle diameter were found in the crops treated with bio-stimulant B4 (20.11 mm) and B5 (3.24 mm) respectively. The highest number of locule was produced by crops in treatment B5 (2.16).

Table 6: Characters of Tanjung-2 Fruits in Six Bio-stimulant Treatments.

Treatments	Fruit height	Peduncle	Fruit diameter	Peduncle diameter	Locule
	cm		mm		
B1	15.49	3.42	20.11	3.89	2.27
B2	15.49	4.28	19.66	3.38	2.20
B3	14.90	3.52	19.51	3.71	2.23
B4	15.07	3.42	20.00	2.90	2.27
B5	18.24	3.53	19.60	3.54	2.27
B6	15.19	3.54	19.82	3.92	2.39

Table 7: Characters of Ciko Fruits in Six Bio-stimulant Treatments.

Treatments	Fruit height	Peduncle height	Fruit diameter	Peduncle diameter	Locule
	cm		mm		
B1	12.76	2.70	18.41	3.00	2.14
B2	12.00	2.61	18.23	2.99	2.14
B3	12.59	2.63	18.91	2.93	2.14
B4	12.25	2.63	18.94	3.20	2.13
B5	12.43	2.69	18.78	3.24	2.16
B6	12.67	2.82	18.79	3.13	2.07

In general, it appears that the application of bio-stimulants did not significantly improved crop characters. This is presumably due to the availability of endogenous phytohormones within the crops that is sufficient for promoting the chilies growth and development. Thus, the crops did not respond to the addition of bio-stimulant extracts. According to [23] the availability of optimal amounts of phytohormones in fruit during fruit formation could ensure the continuity of growth, development, and perfect fruit formation. The contents of phytohormones (IAA, gibberellins, zeatin, and kinetin) in the seaweed extract formula of B1, B2, B3, B4 and B5 are quite similar but they are lower compared to content commercial seaweed extracts (Table 1). The effects of bio-stimulant B1, B2, B3, B4, B5 and B6 application were not significantly different in all parameters in both varieties, only the B4 treatment performed the best result compared to other treatments. Susilowati (2020) found similar result in the study of cayenne crops treated with *Bacillus vallismortis* in which the length and diameter of cayenne fruit were increasing due to the treatment but not significantly different to the control [22]. The insignificant effect was probably caused by the improper spraying method. The crops were not covered during the application; thus, the sprinkling of seaweed extract broadly diffused and less absorbed by the plant's tissues. The phytohormones are unstable chemical highly affected by temperature, humidity, and direct sunlight. Therefore, special treatment is needed to maintain the effication of phytohormones both in storage and application in the field.

The three times application of 7.5 ml L-1 bio-stimulant solution were unable to significantly trigger the chili plants growth. The inaccurate doses used in the study could be responsible for this ineffective application. An excessive amount of phytohormones can inhibit plant growth. As mentioned by [24] there is a correlation between the response of plants to auxin and its concentration. The high concentration of auxin might inhibit plant growth. The application of both *Ulva* sp. and *Sargasum* sp. at concentration of 2.5%, 5%, and 7.5% on tomato seed did not significantly increase the root (radicle) length of tomato sprouts, but the radicle length was greater at the concentration of 2.5% than in other concentrations [21]. According to Du Jardin (2015)[1], one chemical could be categorized as bio-stimulant if it is applied in small amounts. In their study [11] found that 7 times application of 1 mL L-1 seaweed and compost to chili plants could increase the weight of marketable chili fruits. A dose of 20% seaweed extract solution can increase the growth of okra sprouts [7]. Bio-stimulant could be applied in different methods including seed soaking and plat drenching. The study found that vegetative growth, crop yield and crops resistance against plant diseases (damping off and anthracnose) increased when chili seeds were soaked for 30 minutes in 1 mL of bio-stimulant (microbial consortium) and 10 mL of bio-stimulant was applied to the plant at the time of flowering [16].

Furthermore, the response of Tagetes to the bio-stimulant dosage formed a quadratic shape [8]. The application of bio-stimulant increased crops yield, however, after reaching a certain point, the addition of bio-stimulant reduced the crop yield. Sridhar & Rengasamy (2010) [8] further recommended the optimum dose of 1% seaweed extract and 50% dose of chemical fertilizers to obtain maximum yields of Tagetes. It is necessary to determine the optimum dosage of both single and combined bio-stimulants. The optimum single dosage might be similar or different to the optimum combination dosage. Bio-stimulant commonly contains of bacteria and fungi as living things that both interact with each other in the carrier medium. Çakmakç (2019) [25] stated that the bio-stimulants is better used in combination rather than in single form. However, for the successful use of bio-stimulants, formulation (either single or compound), the carrier characteristics, and its interaction with the type of bio-stimulant, and the effective method of application should be carefully considered. Thus, the interaction pattern among bio-stimulant made from seaweed, fungi and bacteria must be observed in order to obtain an effective formulation.

In this study, both varieties of Tanjung-2 and Ciko examined were categorized as big chili. The result shows that both varieties performed different responses to the treatments. This is similar to the previous study on sweet pepper. Different varieties of sweet pepper responded differently to the application of bio-stimulant made from seaweed extract and compost [11]. Bio-stimulants induces plant target for plant to effectively absorb nutrient needed to increase growth, quality and quantity of yield [1]. The induction effect caused by the combination of bio-stimulants depends on the characteristics of each variety. In the current study, treatment B4 consistently promote the highest yield compared to other treatments.

Previous study on the effect of seaweed extracts on tomato seed germinations shows that radicle length of tomato seed germinated in seaweed extract made from *Ulva* sp. were longer (2.94 cm) than in bio-stimulants made from *Sargassum* sp. (1.92 cm) [21]. The *Ulva* sp. is a type of green seaweed (Chlorophyta) which contains more chlorophyll rather than other types of seaweed. This chlorophyll can act as bio-pigment and bio-active when applied on soil and plants. In addition, Ulva seaweed also contains rhamnose (glucose deoxy) which is able to increase plant growth through the mechanism of increasing hormonal activity [6].

4. Conclusion

The Tanjung-2 and Ciko varieties responded differently to various bio-stimulants application in all observed parameters. Bio-stimulant B4 was considered as the best formula compared to other formulas.

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