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Effect of *Padina minor* powder extract as biostimulant and black soldier fly fertilizer on growth and yield of soybean (*Glycine max* L. Merrill)

Abstract. Soybean production has not met domestic demand, causing dependence on imported soybeans. Increasing soybean production can be done by giving organic materials such as fertilizer and biostimulants. Applying *Padina minor* extract as a biostimulant and black soldier fly (BSF) fertilizer can be an alternative to increase the growth and production of soybean. The research aims to determine the effect of *Padina minor* powder extract, BSF fertilizer, and the combination of *Padina minor* powder extract and BSF fertilizer on the growth and yield of soybean. This research was carried out from April to July 2021 at the Plant Physiology Laboratory and Greenhouse, Department of Biology, Andalas University, Padang. The experiment was arranged in a Completely Randomized Design with four treatments (control or without extract and fertilizer), *Padina minor* extract, BSF fertilizer, and the combination of *Padina minor* extract and BSF fertilizer) and six replications. The results showed that the extract of *Padina minor* did not significantly affect the growth and yield of soybean. BSF fertilizer significantly increased the number of leaves, branches, leaf area, chlorophyll b, and total chlorophyll of soybean. The combination of *Padina minor* extract and BSF fertilizer gave similar effects as BSF fertilizer on increasing the number of leaves, leaf area, chlorophyll b, and total chlorophyll of soybean.

Keywords: Biostimulant · Black Soldier Fly · Organic fertilizer · *Padina minor*

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Introduction

Soybean is the third strategic food crop commodity after rice and corn, making them a target commodity in food self-sufficiency (Handriawan et al., 2016). Soybean consumption and demand increase along with population growth, but domestic soybean production have yet to meet the increasing demand for soybeans. It causes domestic soybean needs to be met from imports (Nuhung, 2013). Over the last decade, soybean imports reached 67% of the national soybean demand, indicating that domestic production can only meet 33% of the demand (Swastika, 2015). Soybean imports until 2020 have reached 2.48 million tons (Statistics Indonesia, 2020), while MOA estimates that soybean imports in 2021 will reach 2.6 million tons. Therefore, increasing the growth and production of soybean in Indonesia needs to be done using biostimulants and organic fertilizers.

Biostimulants are formulations of bioactive compounds applied to plants to improve nutrient absorption efficiency, abiotic stress tolerance, and plant quality (Calvo et al., 2014; Du Jardin, 2015). Biostimulants can provide nutrients, increase availability (Kesaulya et al., 2015), and modify plant physiology processes such as respiration, photosynthesis, nucleic acid synthesis, and ion absorption (Abbas, 2013). Sources of biostimulants include microbial inoculum, humic acid, fulvic acid, amino acids, seaweed extracts, and plant extracts (Calvo et al., 2014).

Seaweed can be used as a biostimulant because of the high diversity of seaweed in Indonesia and its suboptimal use. The seaweed in Indonesia reaches 12 million hectares but has only been utilized around 281,474 ha (Ministry of Marine Affairs and Fisheries, 2019). The use of seaweed extract as a biostimulant has been widely studied and proven to affect the growth and development of roots and shoots, photosynthesis, increase plant vigor and delay fruit aging (Zodape et al., 2011; Pise & Sabale, 2010). Hadi et al. (2016) reported that there are five types of seaweed in Nirwana Beach, Padang, West Sumatra, that have the potential to be used as biostimulants. Noli et al. (2021; 2022) reported the screening results of seaweed from West Sumatra and showed that *Padina minor* extract gave the best results in spurring germination and vegetative growth of soybeans.

In addition to seaweed, using organic matter as a booster for plant growth and development can be obtained through the bioconversion of organic waste by bioconversion agents (bacteria, fungi, and insect larvae). One of the insect larvae used as a bioconversion agent is the *Hermetia illucens* or Black Soldier Fly (BSF) species (Kinasih et al., 2018), which is commonly found in palm oil waste. The *Hermetia illucens* larvae are known as maggot.

BSF larvae or maggots have been widely used as bioconversion agents. They can overcome the problem of organic waste because of their ability to reduce 50-80% of organic waste per day from the amount of food they get (Balitbangtan, 2016; Diener et al., 2011). In addition, BSF larvae can process organic matter into products that can be used as fertilizer. Yuwono and Mentari (2018) reported that the analysis of organic waste content decomposed by BSF larvae showed that it could be declared as compost and relatively well function like compost within 30 days of the composting process. Another study by Nirmala et al. (2020) reported that the results of waste decomposition derived from 100% vegetables, 100% fruits, and a mixture of 80% vegetables + 20% fruits aged 15 days met the requirements of good compost.

Reswita et al. (2021) reported that maggot bioconversion fertilizer could improve the physical and chemical properties of Ultisol soil and increase the grain weight of 100 upland rice seeds. Pratama (2020) reported that solid fertilizer from former BSF larvae could increase stem height, root length, number of leaves, and leaf area of chili at a ratio of 1:3 for BSF fertilizer and soil. The concentration ratio has the highest average value compared to compost and NPK fertilizer.

In this study, the effect of *Padina minor* extract as a biostimulant and Black Soldier Fly fertilizer was tested to increase the growth and yield of soybean. This study aims to determine the effect of *Padina minor* extract, BSF fertilizer, and the combination of both on the growth and yield of soybean (*Glycine max*).

Materials and Methods

This study was conducted from April 2021 to July 2021 at the Plant Physiology Laboratory and Greenhouse, Department of Biology, Andalas University, Padang. The tools used in this study were polybags (60 x 40 cm), collection plastic, a grinder, analytical scales, measuring cups, sprayers, filter paper, label paper, stationery, cameras, meters, shakers, centrifuge, and spectrophotometers. The materials used in this study were *Padina minor* seaweed, BSF fertilizer, soybean seeds (Anjasmoro var.), aquadest, 80% acetone, Ultisol soil, manure, Urea fertilizer, KCl fertilizer, and TSP fertilizer.

This experiment used a Completely Randomized Design with four treatments such as control (without the application of extract and fertilizer), *Padina minor* extract (25 mL), BSF fertilizer (300 g), and the combination of *Padina minor* extract and BSF fertilizer, with six replications for each treatment.

Fertilizer obtained from Maggot Farming Business in Mungka, 50 Kota Regency, a place for waste management and BSF maggot cultivation. Nutrient analysis has been carried out, and it is known that BSF fertilizer has the main nutrient content, where the concentration of N is 3.219%, P is 1.705%, K is 0.534%, and C/N content is 6.445%. *Padina minor* collected at Nirwana Beach, Padang, West Sumatra. *P. minor* cleaned of sand and mud attached to seawater, then put it in a labeled plastic bag. The collected *P. minor* was then rewashed with tap water to remove any remaining salt and sand, air-dried the samples for four days, then pulverized to obtain a coarse powder. The coarse powder was then weighed and soaked with hot water in a ratio of 1:20 (w/v) for sample and water, stirred for 24 hours at room temperature then filtered. Dissolved the resulting filtrate in 1 liter of water and put it into a storage bottle (Norra et al., 2016).

The extract was sprayed for 25 ml when the soybeans were 2, 4, and 5 weeks old after planting (Kalaivanan et al., 2012; Grabowska et al., 2012; Zakiah et al., 2017). BSF fertilizer was applied for 300 g at the beginning of seed planting and when soybeans were four weeks after planting (WAP).

Soybean seeds were planted in a mixture of Ultisol soil and manure (5:1), as much as 10 kg/polybag. Maintenance included watering, weeding, and fertilizing. Fertilizer application was carried out based on the recommended dosage for soybean, which was 50 kg/ha for urea fertilizer, 100 kg/ha for TSP, and 100 kg/ha for KCl or equivalent to 0.45 g Urea, 0.9 g TSP and 0.9 g KCl. Half of Urea dosage was applied at the beginning of seed planting and when the plant was 30 days old, while 1 part of KCl and TSP was applicated at the beginning of seed planting (Rukmana & Yudirachman, 2014; Zakiah et al., 2017).

The parameters observed were height, number of leaves, number of branches, leaf area, wet weight, dry weight, leaf chlorophyll content, number of pods, the weight of the entire seed, and weight of 100 seeds per plant. The data were tested with Analysis of Variance (ANOVA) and continued with the Duncan New Multiple Range Test (DNMRT) at 5%.

Results and Discussion

The results of the vegetative parameters analysis of soybean are presented in Table 1. Based on the table, it is known that *Padina minor* extract alone had not been able to provide a significant effect on all vegetative growth parameters of soybean; however, BSF fertilizer provides a significant effect in increasing the parameters of the number of leaves, the number of branches and leaf area. A combination of *P. minor* and BSF fertilizer also significantly influenced the parameters of leaf number and leaf area. It is shown that applying BSF fertilizer was an effective treatment to increase the growth of the number of leaves, leaf area, and the number of branches of soybean.

The vegetative growth of plants is affected by the nutrients absorbed. In this case, the macro-nutrient nitrogen (N) contained in BSF fertilizer is thought to play a role in increasing the number and area of leaves and branches on soybean plants. Nitrogen is needed for the vegetative growth of plants, mainly stems, branches, and leaves, and is a constituent component of amino acids, proteins, and the formation of cell protoplasm, which can stimulate plant growth (Safei et al., 2014; Lingga and Marsono, 2013). The results of Zahn's research (2017) showed that applying 5 g of BSF fertilizer (kasgot) increased *Allium cepa*.

Table 1. Number of leaves, number of branches, leaf area, height, wet weight and dry weight of soybeans with the application of *P. minor* extract and BSF fertilizer

Treatment	Number of Leaves (strands)	Number of Branches	Leaf Area (cm^2)	Height (cm)	Wet Weight (g)	Dry Weight (g)
Control	50.33 a	5.33 a	53.51 a	53.04 a	133.63 a	37.89 a
P. minor extract	58.66 a	5.66 a	49.48 a	53.58 a	150.83 a	38.35 a
BSF fertilizer	70.40 b	7.00 b	76.09 b	54.11 a	158.36 a	39.58 a
P. minor extract + BSF fertilizer	74.40 b	6.00 ab	85.62 b	54.98 a	167.90 a	40.59 a

Note: Numbers followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Plant height did not show significant results with the application of BSF fertilizer, allegedly because BSF fertilizer plays more role in vegetative growth processes that lead to the increasing growth of leaves and branches. The significant results on the number and area of leaves and the number of branches were in line with the weight of seed parameter (Table 3), which showed that the application of BSF fertilizer tends to increase the weight of soybean seeds. The number of branches and leaves correlated with the increase in photosynthetic product, which will also enhance the metabolism of plants to grow and produce properly (Syaifudin et al., 2019; Ermawati et al., 2018). A large number of branches tends to be followed by increased pods, resulting in more seed yield (Dwiputra et al., 2015).

A combination of *P. minor* extract and BSF fertilizer also showed significant results on the number of leaves and leaf area. However, giving *P. minor* extract alone did not significantly affect all growth parameters. It showed that BSF fertilizer was more dominant in affecting the improvement of some growth parameters of soybean than *P. minor* extract. It is shown that *P. minor* extract alone could not increase photosynthetic and other metabolic activities, leading to the increase of various plant metabolites responsible for cell division and elongation (Kanwal et al., 2016). It did not have a significant effect on the vegetative growth of soybean plants.

The effect of *P. minor* extract, BSF fertilizer, and the combination of both on chlorophyll levels of soybean plants is presented in Table 2. Based on the table, it is known that chlorophyll b and total chlorophyll of soybean leaves showed markedly different results against the application of BSF fertilizer and a combination of extracts and fertilizers. While the application of *P. minor* extract did not differ markedly on chlorophyll a, chlorophyll b, and total chlorophyll levels of soybean plants showed that *P. minor* extract exerted the same effect as BSF fertilizer and did not differ markedly from controls. This showed that BSF fertilizer and the combination of fertilizer with *P. minor* extract could influence the increase in chlorophyll levels of soybean plants, *and P. minor* extract had not been able to exert a significant influence in increasing chlorophyll levels of soybean plants on Ultisol soils.

Table 2. Chlorophyll levels of soybean plants treated with *P. minor* extract and BSF fertilizer

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Control	0.5138 a	0.9348 a	1.4487 a
P. minor extract	0.5458 a	1.0398 ab	1.5858 ab
BSF Fertilizer	0.5578 a	1.1257 b	1.6837 b
P. minor extract+BSF fertilizer	0.5615 a	1.0694 b	1.6311 b

Note: Numbers followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

BSF fertilizer and the combination of *P. minor* extracts and fertilizers were able to affect the chlorophyll levels of soybean plants allegedly because of the availability of Mg nutrients in fertilizers which were known to play an essential role in the process of chlorophyll formation in plants, where Mg acts as the central atom of the chlorophyll molecule (Lingga and Marsono, 2013). Mg deficiency in plants will interfere with electron transport in photosynthesis, resulting in a decrease in chlorophyll content and CO2 fixation and impaired carbon metabolism (Farhat et al., 2014). Research by Wu et al. (2019) reported that BSF organic fertilizer significantly increased chlorophyll levels in tomato plants. Similar results were reported by Reswita et al. (2022), that upland rice plants treated with BSF bioconversion fertilizer had higher chlorophyll levels.

Table 2 shows that the application of *P. minor* extract did not show statistically significant differences in the chlorophyll content of soybean plants. However, there was a tendency for higher chlorophyll levels in applying *P. minor* extract compared to the control. This indicates that *P. minor* extract could increase plants' chlorophyll levels. Similar results were reported by Noli et al. (2021), where the application of *P. minor* seaweed extract had no effect statistically on soybean plants' chlorophyll levels but tended to increase in frequency treatment and different applications when compared with controls.

Table 3. Number of pods, weight of seeds and weight of 100 seeds of soybean plants treated with *P. minor* extract and BSF fertilizer

Treatment	Number of Pods	Weight of Seeds (g)	Weight of 100 Seeds (g)
Control	96.33 a	52.71 a	29.35 a
P.minor extract	91.33 a	58.22 ab	32.51 a
BSF fertilizer	101.93 a	60.99 b	30.08 a
P.minor extract+ BSF fertilizer	104.76 a	63.24 b	30.39 a

Note: Numbers followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

The result showed in Table 3 that the treatment of BSF fertilizer and the combination of *P. minor* extract with BSF fertilizer had a significantly different effect than *P. minor* extract on the weight of seeds of the soybean plant. Meanwhile, the application of *P. minor* extract alone had not been able to exert a markedly different effect on all generative parameters of soybean plants compared to the control treatment. It showed that BSF fertilizer could increase soybean yield on the weight of seeds parameter. BSF fertilizer provided sufficient nutrients for plants that had a role in seed synthesis and other metabolism resulting in the increase of seed weight in soybean plants. Dinariani et al. (2014) stated that organic fertilizers would be well decomposed during tillage, so that plant roots quickly absorb nutrients in the soil.

Phosphorus (P) and potassium (K) contained in BSF fertilizer had 1.705% for P content and 0.534% for K content. These two elements had an essential role in the formation of soybean seeds. The effect of BSF fertilizer on crop production was reported by Tanga et al. (2021); the combination of BSF larvae fertilizer and NPK in corn (*Zea mays*) produced grains 23-68% higher than other fertilizer treatments, and BSF fertilizer alone provided higher grain yields than crops given commercial organic fertilizer (Evergrow). Research by Menino et al. (2021) added that BSF fertilizer significantly affected the overall production of ryegrass (*Lolium multiflorum*).

Providing sufficient P nutrients that plants can absorb will increase the weight of soybean seeds. P nutrients play a role in plant cell division, strengthening rooting and accelerating the flowering and ripening of seeds (Supartha et al., 2012). Hayati et al. (2012) added that the benefits of P fertilizer are to support the beginning of root growth, the growth of flowers and seeds, and the increase in the percentage of flower formation into seeds. Potassium is an activator of many enzymes that are important for the photosynthesis process; it also helps form starch and protein, so it plays an essential role in increasing the number of pods and seeds in plants. Potassium has a low ion exchange capacity and is often replaced by aluminum ions in acidic soils, so potassium ions can potentially be lost (Subandi, 2013; Puspitasari and Elfarisna, 2017).

Conclusion

Based on the research that has been carried out, it can be concluded that:

1. *P. minor* powder extract did not affect the growth and production of soybean (*Glycine max*).
2. Black Soldier Fly fertilizer increased the number of leaves, branches, leaf area, chlorophyll b, and total soybean chlorophyll (*Glycine max*).

3. The combination of *P. minor* powder extract and black soldier fly fertilizer increased the number of leaves, leaf area, chlorophyll b, and total soybean chlorophyll (*Glycine max*).

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