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Study of the ability of biduri plants in bioremediation of pesticides with indigenous bacteria and biochar in shallot planting soil, Brebes, Indonesia

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Abstract. The use of pesticides can worsen the climate, while climate change itself increases pest attacks, thus increasing pesticide use. The use of pesticides in Brebes has caused pollution to the environment because it left a residue of 4.05 mg/kg, which has exceeded the maximum residue limit on soil, 0.2 mg/kg. This study aims to determine the effect of treatments on the bioremediation of soil contaminated with pesticides in shallot planting soil. This type of research was an experiment in a greenhouse with a factorial experimental design and using a completely randomized design (CRD) consisting of 2 factors; Indigenous Bacteria (I): without bacteria (I0), *Pseudomonas sp.* (I1), Consortium of bacteria (I2) and Biochar (B): without biochar (B0), with biochar (B1). There were six combinations, each of which was repeated 3 times to obtain 18 treatment units. The results showed that the levels of pesticides in the soil after treatment decreased compared to the control treatment. The highest reduction in pesticide levels was in I2B1 treatment, which was 3.97 mg/kg or 96.7 % higher than the control. Treatments can reduce pesticide levels in the soil so that it can be used as a method of bioremediation to achieve food security.

1 Introduction

Good agricultural management needs to be done to increase crop yields. One of them is efforts to control the amount of damage to plants caused by pests and plant diseases using pesticides. Pesticides are chemicals whose use plays a role in eradicating plant-disturbing organisms in agriculture. The use of pesticides can reduce losses due to pest attacks and increase agricultural production from the land. On the other hand, the use of pesticides can cause harmful residues [1,2].

Pesticide use and climate change have a mutually reinforcing two-way relationship. Chemical-based pesticide production requires significant energy, releasing greenhouse gas emissions, while residues can damage soil fertility and reduce its ability to store carbon. On

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the other hand, climate change, such as rising temperatures, extreme rainfall, and unstable weather patterns, triggers an explosion of pests and plant diseases as insects reproduce more rapidly and their distribution expands. This situation makes farmers increasingly dependent on pesticides, even in higher doses, as their effectiveness decreases in hot temperatures. Consequently, a negative feedback loop occurs: climate change increases pesticide use, and excessive pesticide use exacerbates climate conditions [2].

Based on data from the World Health Organization (WHO) in 2020, there are more than 1,000 different types of pesticides used around the world. The use of this pesticide is usually intended to kill pests, including insects, rats, and weeds. One of the most common pesticides used in agriculture is organophosphate pesticides [1]. One of the active ingredients of organophosphate pesticides is chlorpyrifos. Pesticides are intended to increase plant productivity, but over time, they will accumulate in plant parts, water, soil, air, and biota [2]. The extensive use of pesticides pollutes soil and water, thereby posing a threat to humans. This is reinforced by the results of previous research, which showed 385 million cases of pesticide poisoning and 11,000 deaths due to the impact of pesticides per year, especially for farmers and farm workers.

Brebes Regency is the largest shallot producer in Central Java, Indonesia, and supplies around 37.8% of the national shallot market demand [3]. Most shallot farmers in Brebes Regency still use excessive fertilizers and pesticides to increase yields. Based on the results of the analysis by the Agricultural Environmental Research Center on pesticide levels in shallot planting soil in Brebes Regency, it was found that the level of chlorpyrifos pesticides in Wanasari District was 2.819 mg/kg, in Bulakamba District was 7.496 mg/kg, and in Larangan District was 1.921 mg/kg. The Residue Maximum Limit of pesticides on soil is 0.2 mg/kg [4]. This is one of the causes of environmental damage and an increase in the number of pesticide poisonings. Therefore, it is necessary to carry out remediation efforts on shallot planting land so that productivity is sustainable.

Biduri (*Calotropis gigantea*) is a plant capable of producing fiber and medicinal medicine. Biduri can grow on marginal land. Utilization of Biduri combined with inorganic fertilizers and bioremediation agents has been shown to be able to reduce lead levels in the soil [5]. In addition, the Biduri has also shown its potential in remediating soil contaminated with heavy metals, Cadmium (Cd) and Chromium (Cr). This shows the potential of Biduri as a phytoremediation agent. This research will be combined with bacteria and biochar to determine its ability as a phytoremediation agent in pesticide bioremediation. Indigenous bacteria, which are local bacteria in the soil, are an alternative to remediation techniques that are environmentally friendly. This bacterium is the result of an evolutionary process that has adapted to pesticide pollutants, so it has the potential as a bioremediation agent [6]. Indigenous bacteria used are isolated *Pseudomonas* sp. and the bacterial consortium, which has been tested to be capable of degrading pesticides in artificial media, so further tests are needed to assess its ability as a bioremediation agent in the field. Bacterial isolates such as *Pseudomonas fluorescens, Bacillus subtilis, Brucella melitensis*, and *P. aeruginosa* have succeeded in degrading the pesticide chlorpyrifos after 30 days [7]. The use of biochar is intended to support the bioremediation process. The biochar used in this study is made from coconut shells. Coconut shell biochar can remediate soil from heavy metal contamination. Therefore, research on the potential of the Biduri plant in combination with indigenous bacteria and biochar in the bioremediation of pesticides in shallot planting soil is essential to conduct.

2 Materials and methods

2.1. Experiment area

The experiment was conducted in March – November 2022 and was located in the Greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret, using soil from the Shallots plantation in Brebes Regency. The soil used comes from shallot fields in Brebes Regency, which is then composited. Laboratory analysis was carried out at the Soil Biology and Biotechnology Laboratory, Soil Physics and Conservation Laboratory, Chemistry Laboratory, Soil Fertility Faculty of Agriculture, Universitas Sebelas Maret, and the Agricultural Environmental Research Center Pati.

2.2. Materials

The materials used in this study were shallot planting soil from Wanasari District (6°58'31,3" S dan 109°00'23,9"E), Bulakamba District (6°52'23,8" S dan 108°59'31,9" E), dan Larangan District (6°58'16,8" S dan 108°58'00,3" E), Indigenous bacteria, namely isolate *Pseudomonas* sp. and the bacterial consortium, Biduri seeds from the Bandung Textile Center, coconut shell biochar produced by Aulia Jaya Mandiri, Dursban 200 EC pesticide, "Segartan" vegetable pesticide from the Agricultural Environmental Research Institute, inorganic fertilizers and manure produced by Putra Mandiri. The tools used are gas chromatography, autoclaves, microscopes, hemacytometers, shakers, vortexes, spectrophotometers, petri dishes, test tubes, Erlenmeyer flasks, etc.

2.3. Experimental design

This type of research was an experiment in a greenhouse with a factorial experimental design and using a completely randomized design (CRD) consisting of 2 factors, namely Indigenous Bacteria (I): without Indigenous Bacteria (I0), *Pseudomonas sp.* (I1), Consortium of bacteria (I2) and Biochar (B): without biochar (B0), with biochar (B1). There were six combinations, each of which was repeated 3 times to obtain 18 treatment units.

2.4. Research preparation

2.4.1 Soil preparation

The composite soil is wind-dried first, then the soil is sieved using a multilevel sieve. The filtered soil is then weighed at 7 kg for each pot. The next step is to sterilize the soil using the evaporation method for 3 hours on three consecutive days.

2.4.2 Liquid bacterial inoculum preparation

The stage of making this liquid bacterial inoculum includes the multiplication of indigenous bacterial inocula. The bacterial isolates used were *Pseudomonas* sp. and previously isolated bacterial consortia. Propagation of bacterial inoculum begins with the preparation of NA (Nutrient Agar) media on slanted media, which is then isolated with *Pseudomonas* sp. and the bacterial consortium. Indigenous bacterial isolates that had been incubated in slanted media were then grown in 100 ml of liquid NB (Nutrient Broth) media. The starter was shaken for 3 days, then liquid NB medium was poured with a volume of 400 ml as a scaleup. The media was then shaken until the density of the bacterial cells was 109 cells/ml. Measurement of its density using a hemacytometer and the aid of a microscope.

2.4.3 Fertilizer preparation

The basic fertilizer will then be applied to the planting medium. Basic fertilizer is weighed first according to the specified dose. The dosage of inorganic fertilizer for Biduri is 60 kg/ha of urea, 20 kg/ha of SP-36, and 30 kg/ha of KCl.

2.4.4 Plant seed preparation

Biduri seeds were obtained from the Bandung Textile Center. The seeds are soaked in water for 4 hours, then sown until 4 leaves appear. Seeding is done in seedling trays with soil and manure media. Biduri was then transferred to a polybag for 2 weeks.

2.4.5 Cultivation

2.4.5.1. Liquid bacteria inoculum application

The application of liquid bacterial inoculum was carried out a week before planting. The dose of bacteria given was 106 cells/g of soil, and those applied to the planting medium were 109 cells/ml and 70 ml.

2.4.5.2. Biochar application

The application of coconut shell biochar was carried out a week before planting. The dose of biochar application is 15 tons/ha, which, after conversion, to 5.25 grams per pot.

2.4.5.3. Fertilizer application

Basic fertilizer application is done the day before planting. After conversion, the basic fertilizer dose for each pot is 0.47 g urea, 0.195 g SP-36, and 0.175 g KCl.

2.4.5.4. Biduri planting

Planting is done using Biduri, which is already in a polybag for 2 weeks. Biduri is then transferred to a pot containing the prepared planting medium. Planting Biduri in pots lasts for 60 days.

2.4.5.5. Plant maintenance

Plant maintenance includes watering, weeding, pest control by installing insect glue, and spraying organic pesticides.

2.4.6 Laboratory analysis and observation parameters

The implementation of this research includes analysis and field and laboratory observations. The parameters of the soil analysis are a) soil pH using the electrometric method; b) Cation Exchange Capacity (CEC) with ammonium saturation; c) C Organic with the Walkey and

Black method; d) Bacterial colonies with plate count method; e) Pesticide residues on soil by Quechers method; f) the effectiveness of bioremediation with the formula:

$$\text{Effectiveness (\%)} = \frac{\text{Initial Levels} - \text{Final Levels}}{\text{Initial Levels}} x\ 100\% \quad (1)$$

Where the initial levels are the levels of pesticides in the soil before treatment, and the final levels are the levels of pesticides in the soil after treatment. The parameters of plant analysis are a) plant dry weight by weighing; b) Pesticide uptake by plants with the Quechers method.

2.4.7. Data analysis

The research data were analyzed statistically using ANOVA (Analysis of Variance) and continued with the Duncan Multiple Range Test (DMRT) to find out the differences between treatments and which treatment was best.

3 Results and Discussion

3.1. Early soil characteristics

The results of the analysis of the initial soil characteristics on shallot farming land in Brebes Regency are shown in Table 1.

Table 1. Early Soil Characteristics

Number	Parameters	Value	Units	Class
1	pH	7.47	-	Neutral
2	CEC	13.00	me/100g of soil	Low
3	C Organic	1.29	%	Low
4	Pesticide Residues	4.05	mg/kg	-

Source: Primary Data (2022)
Notes: Class based on Bogor Soil Research Institute 2009

Based on Table 1, the initial soil characteristics are known to have a pH value of 7.47 and a neutral level. C-organic and soil CEC have low values with respective values of 1.29 % and 13 me/100g of soil, respectively. Most of the soil types in Brebes Regency are Alluvial Grey, and the Larangan, Wanasari, and Bulakamba areas have Typic endoaquepts soil classification. Typic endoaquepts have soil characteristics with a strong acid-to-neutral pH, low to moderate organic carbon, and low CEC values. Soils with loam, dusty loam, and clay loam textures have moderate soil permeability so the potential to pass the pesticide is also moderate. Pesticide residues in the shallot planting soil in Brebes Regency were 4.05 mg/kg, which exceeded the maximum residue limit.

3.2. Final Soil Characteristics

3.2.1. Soil pH

Table 2. Effect of treatments on soil pH

Treatments	Soil pH
I0B0	7.80bc
I1B0	7.60a
I2B0	7.80bc
I0B1	7.90c
I1B1	7.95c
I2B1	7.70a

Notes: I0: Without indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial Consortium, B0: Without biochar, B1: With biochar

The results of ANOVA showed that the treatment of Indigenous bacteria and biochar had a significant effect on soil pH values ($p<0.05$). The interaction of the two treatments also significantly affected the soil pH value ($p<0.05$). Table 2 shows that the soil pH has increased after the bioremediation process, with the highest pH in the *Pseudomonas sp.* + biochar (I1B1), which is 7.95 or 2.01 % higher than the control treatment. DMRT results showed that the treatment of *Pseudomonas sp.* + biochar (I1B1) had no significant difference with the control treatment, bacteria consortium + without biochar (I2B0), and without indigenous bacteria + biochar (I1B1). This study uses the actual pH observation variable. Actual pH using H_2O solution. Actual pH is the value of soil acidity, which indicates the concentration of H^+ in the soil solution according to the actual conditions at that time. An increase in soil pH can be attributed to the addition of biochar and biochar pH, as well as to the breakdown of hydroxide and carbonate present in biochar [8]. Pesticide reduction rates are higher in alkaline pH conditions. Increasing soil pH can reduce the mobility of pesticide residues so that the decomposition of residues by microbial activity is better. The degradation of chlorpyrifos pesticides at pH 4.7 – 6.7 requires 35 days, while at pH 7.7 – 8.4 the degradation of chlorpyrifos pesticide residues only requires 16 days. In soils with high pH, the level of persistence of pesticide residues decreases.

3.2.2. Cation Exchange Capacity (CEC)

Table 3. Effect of treatments on soil CEC

Treatments	Soil CEC (me/100g)
I0B0	19.23b
I1B0	21.15bc
I2B0	21.22bc
I0B1	23.23cd
I1B1	15.33a
I2B1	25.65d

Notes: I0: Without Indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial Consortium, B0: Without biochar, B1: With biochar

The results of the ANOVA showed that the treatment with indigenous bacteria and biochar had a significant effect on soil CEC values ($p<0.05$). The interaction of the two treatments also had a significant effect on soil CEC values ($p<0.05$). Table 3 showed the highest mean in the consortium treatment of bacteria + biochar (I2B1) of 25.65 me/100g soil, or 49.38% higher than the control. DMRT results showed that the consortium treatment with bacteria + biochar (I2B1) had no significant difference from the treatment without indigenous bacteria + biochar (I0B1). The bioremediation process using the Biduri plant combined with Indigenous bacteria and biochar has a significant effect on increasing soil CEC. The increase in soil CEC occurred in all treatments when compared to the initial soil CEC. The primary mechanism for adding biochar can increase CEC, namely through a larger surface area, negative surface charge, and charge density of biochar. The cation exchange capacity (CEC) of soil is the number of cations that soil colloids can exchange. After the application of biochar, the phenomenon of toxicity is reduced due to the presence of macro and micropores that strengthen the functional groups, which provides adequate space to increase the breakdown of available pollutants by the microbial community.

3.2.3.C Organic

Table 4. Effect of treatments on soil C-Organic

Treatments	C-Organic (%)
I0B0	1.26c
I1B0	0.87ab
I2B0	0.81ab
I0B1	0.68ab
I1B1	3.14c
I2B1	0.47a

Notes: I0: Without Indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial Consortium, B0: Without biochar, B1: With biochar

The results of the ANOVA showed that the treatment with Indigenous bacteria and biochar had a significant effect on the C-Organic value of the soil ($p<0.05$). The interaction of the two treatments also had a significant effect on the C-Organic value of the soil ($p<0.05$). Table 4 shows that the C-Organic content in the soil after the bioremediation process has decreased except for the treatment of *Pseudomonas* sp. + biochar (I1B1). The highest decrease in C-Organic occurred in the bacteria consortium + biochar (I2B1) treatment, which was 0.47% or 61% higher than the control treatment. DMRT results showed that the consortium treatment of bacteria + biochar (I2B1) had no significant difference with the treatment of *Pseudomonas* sp. + without biochar (I1B0), Consortium of bacteria + without biochar (I2B0), and without indigenous bacteria + biochar (I0B1). Soil is the result of the weathering of mineral materials contained in the soil. The addition of organic matter was further developed for biostimulation of pesticide reduction in groundwater [9]. High organic matter content has the potential to increase the availability of pesticide residues in soil, while dissolved organic matter can facilitate their leaching.

3.2.4. Bacterial colonies

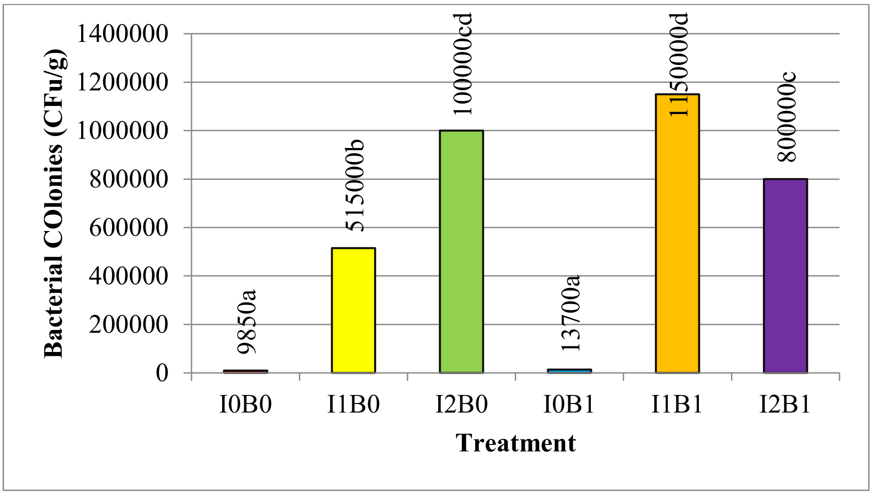

Fig. 1. Bacterial colonies in soil after treatment. Notes: I0: Without indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial Consortium, B0: Without biochar, B1: With biochar

Based on the results of the 5% level ANOVA, the treatment of indigenous bacteria and biochar had a significant effect on the total value of bacterial colonies ($p < 0.05$). The interaction of the two treatments also significantly affected the total bacterial colonies ($p<0.05$). Fig. 1 showed the highest mean in the *Pseudomonas* sp. + biochar (I1B1) of 1,5 x 105 CFu/g soil or 93,4% higher than the control. DMRT results showed that the consortium treatment with bacteria + biochar (I2B1) had no significant difference from the treatment consortium bacteria + without biochar (I2B0). The application of indigenous bacteria can increase the number of bacterial colonies. The surviving bacterial colonies are bacteria that are able to adapt and tolerate pesticide-contaminated soil conditions. This was indicated by an increase in the number of bacterial colonies in the good treatment with *Pseudomona*s sp. and the bacterial consortium. The highest average of total bacterial colonies was in the treatment of *Pseudomonas* sp. + with biochar (I1B1). In the treatment of *Pseudomonas sp.* + with biochar (I1B1), total bacterial colonies were 1.5 x 105 CFu/g. The mechanism of pesticide bioremediation with bacteria shows that hydrolysis can reduce pesticide levels by cleavage of the ester bond of the pesticide and breaking of the diaryl bond, followed by degradation of the aromatic ring [10]. Bacterial degradation of chlorpyrifos by showing that its metabolites, namely 3,5,6-trichloro-2-pyridinol (TCP) and diethyl phosphate (DEP) into non-toxic compounds such as CO_2 and H_2O. Combining microbial and phytoremediation is an approach that ensures more efficient cleanup of contaminated sites. The combined interaction between microbes and plants is beneficial for bioremediation by releasing flavonoid compounds by roots to stimulate the activity and growth of contaminant-degrading bacteria.

3.2.5. Pesticide levels in soil

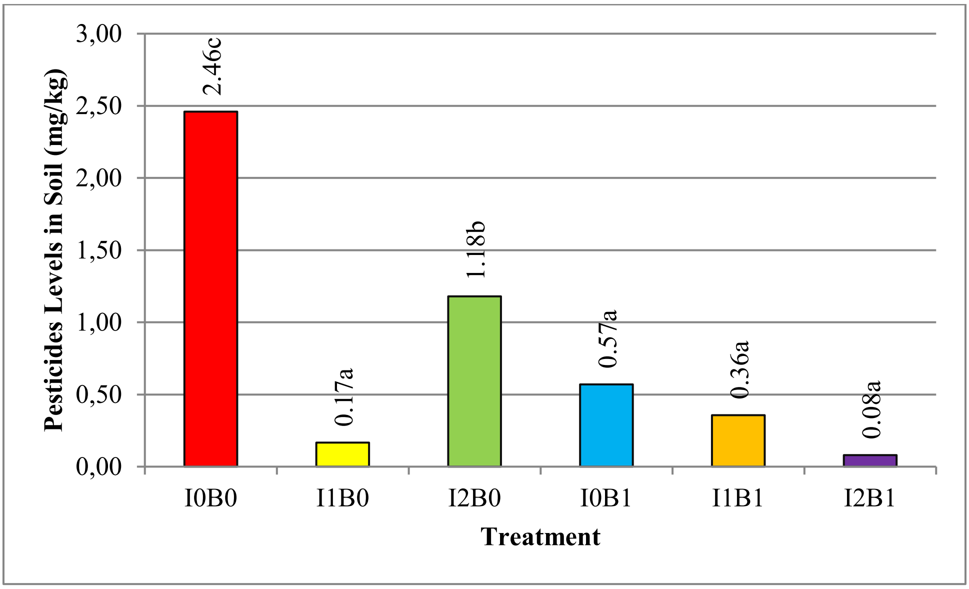

Fig. 2. Pesticide Levels in Soil after Treatment. Notes: I0: Without indigenous bacteria, Control, I1: *Pseudomonas,* sp., I2: Bacterial consortium, B0: Without biochar, B1: With biochar

Based on the results of ANOVA, treatment with indigenous bacteria and biochar significantly affected the levels of pesticides in the soil ($p<0.05$). The interaction of the two treatments also significantly affected the levels of pesticides in the soil ($p<0.05$). Based on Fig. 2, the highest reduction in pesticide levels was in the bacterial consortium + biochar (I2B1) treatment, which reached 3.97 mg/kg (initially 4.05 mg/kg) or 96.7 % higher than the control treatment. DMRT results showed that the consortium treatment of bacteria + biochar (I2B1) had no significant difference with the treatment of *Pseudomonas sp.* + biochar (I1B1). Bioremediation using Biduri plants combined with indigenous bacteria and biochar resulted in reduced pesticide levels in all treatments when compared to pesticide levels in the initial soil. Plants extract pesticide pollutants through their root systems, transfer them from roots to shoots, and then accumulate or mineralize them in metabolic processes. And root exudates facilitate the formation of rhizosphere microorganisms and increase the diversity of bacterial communities, both of which promote pesticide degradation. The microbial consortium can degrade pesticides in a shorter span of time, unlike the axenic strain. Microorganisms support plant resistance to pollutants by releasing active substances such as plant hormones and ion carriers, or reducing organic pollutants that are unfavorable to plant growth. Biochar can stimulate soil microbiota while increasing pesticide degradation, nutrient content, and water-holding capacity, improving acid soil and aeration properties, and increasing soil fertility and crop yields [11].

3.2.6. Plant characteristics

3.2.6.1. Dry weight

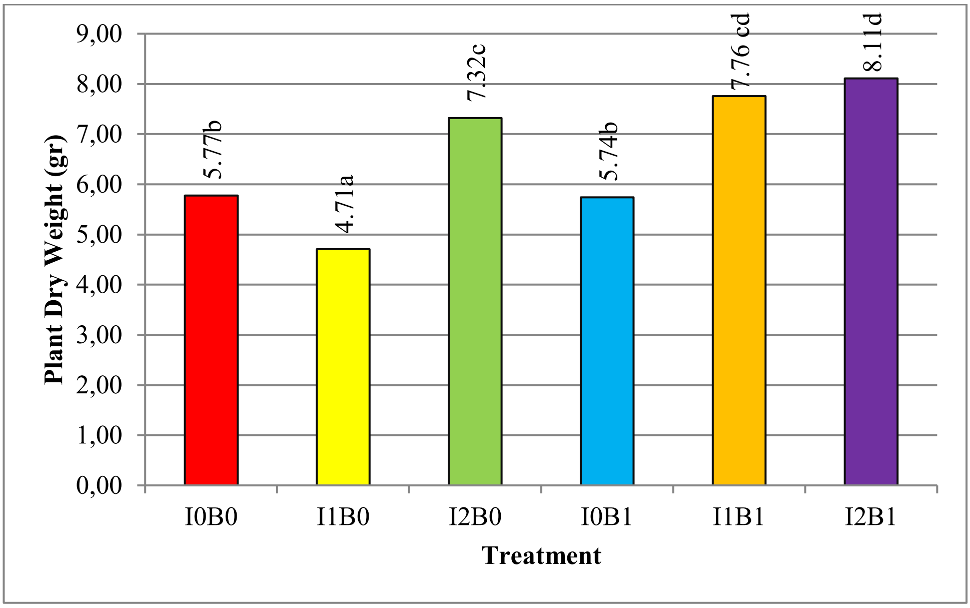

Fig. 3. Dry Weight of Biduri after treatment. Notes: I0: Without indigenous bacteria, Control, I1: *Pseudomonas,* sp., I2: Bacterial consortium, B0: Without biochar, B1: With biochar

Based on ANOVA results. indigenous bacteria and biochar treatment had a significant effect on plant dry weight ($p<0.05$). The interaction of the two treatments also had a significant effect on the dry weight of Biduri ($p<0.05$). Based on Fig. 3, the highest average was in the bacterial + biochar (I2B1) consortium treatment of 8.11 g, or 2.34 g higher than the control treatment. DMRT results showed that the consortium treatment of bacteria + biochar (I2B1) had no significant difference with the treatment of *Pseudomonas sp.* + biochar (I1B1). Most plants generally accumulate metals, for example, nickel at 10 mg/kg dry weight (equivalent to 0.001%), but metal hyperaccumulator plants can accumulate up to 11% dry weight. The growth response of the Biduri plant shows that the Biduri plant can adapt to polluted soil conditions, primarily when supported by Indigenous bacteria and biochar treatment. The addition of microorganisms can dissolve soil nutrients to become available to plants so as to increase plant growth [12]. The addition of biochar increases growth because the surface area can increase the availability of nutrients for plants.

3.2.6.2. Pesticide uptake by Biduri

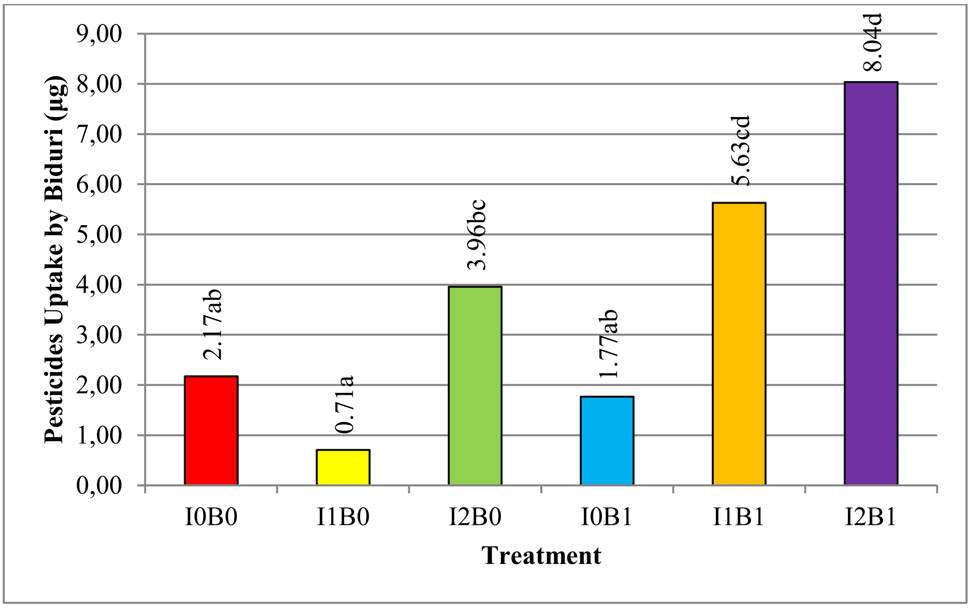

Fig. 4. Pesticide uptake by Biduri after treatment. Notes: I0: Without indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial consortium, B0: Without biochar, B1: With biochar

Based on the results of ANOVA, treatment with Indigenous bacteria and biochar had a significant effect on pesticide uptake in plants ($p<0.05$). The interaction of the two treatments also had a significant effect on pesticide uptake in plants ($p<0.05$). Based on Fig. 4, the Bacterial Consortium + biochar (I2B1) treatment had the highest average absorption, namely 8.04 µg, or 73 % higher than the control. DMRT results showed that the consortium treatment of bacteria + biochar (I2B1) had no significant difference with the treatment of *Pseudomonas* sp. + biochar (I1B1). The mechanism of absorption of pesticides is to pass through the membrane between the root symplast and the xylem apoplast by diffusion, and their entry depends on passive movement through the membrane for their absorption into the plant. Absorption of pesticides by Biduri through the roots and then translocation of metals occurs with the xylem and phloem to other plant parts. Biochar helps provide support for beneficial microbial life and nutrient supply for plants. Organic components promote metabolic activity within the plant and increase the flow of metabolites from roots to leaves, potentially increasing the nutrient content of the plant. Plants that have an adequate supply of nutrients will be more vigorous in absorbing contaminants. A consortium of degrading bacteria can strengthen the metal reduction phytoremediation process using plants because the beneficial bacterial community can support the host in overcoming stress, increasing plant growth and health, and maintaining plant productivity [13]. The function of bacteria for other phytoremediators is to encourage the antagonism of host plants to disease.

3.2.6.3. Bioremediation effectiveness

Table 5 shows the effectiveness of bioremediation using Biduri with a combination of Indigenous bacteria and biochar treatment.

Table 5. Effect of treatments on Bioremediation effectiveness (%)

Treatments	Bioremediation effectiveness (%)
I0B0	38.653
I1B0	95.843
I2B0	70.573
I0B1	85.785
I1B1	91.105
I2B1	98.005

Notes: I0: Without Indigenous bacteria, Control, I1: *Pseudomonas*, sp., I2: Bacterial Consortium, B0: Without biochar, B1: With biochar

Based on Table 5, it is known that the bacterial consortium + biochar (I2B1) treatment was able to reduce pesticide levels by up to 98.005%, while the control treatment without indigenous bacteria + without biochar reduced pesticide levels only by 38.654%. The effectiveness of bioremediation is the success of plants in absorbing contaminants in the soil, which is calculated from the difference between the initial and final levels after being treated. The success of bioremediation in this study is the percentage of the amount of pesticide reduction in the soil. Plants accumulate contaminants from the soil and distribute them to plant tissues. Chelating agents are known to enhance metal accumulation by increasing their solubility even in non-hyperaccumulator plant species and thus may play an important role in heavy metal remediation [14].

4 Conclusions

The conclusion from this study is that the combination of Biduri, indigenous bacteria, and biochar can reduce pesticide levels in the soil and increase pesticide absorption by Biduri. The bacteria consortium + biochar (I2B1) treatment had the highest average in reducing pesticide levels in the soil, namely 03.97 mg/kg or 96.7 % higher than the control. The highest average absorption was obtained by the bacterial consortium + biochar (I2B1) treatment, which was 8.04 µg or 73 % compared to the control.

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