The effectiveness of biofertilizer biofilm formulation in promoting the growth of Pak choi (Brassica rapa L.) plant

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Abstract. One of the climate changes is the El Nino phenomenon, causing the soil to dry out, so it needs to be addressed because it will impact plant growth. Farmers use a lot of inorganic fertilizers (NPK), and in the long term, it can cause soil damage. One alternative treatment is to use organic fertilizers or biological fertilizers. Biofilm biofertilizer is a form of biological fertilizer. This study aims to determine the effect of dosing biofilm biofertilizer on the growth of Pak choi plants so that it can help reduce the use of inorganic fertilizers. This type of research was an experiment in a greenhouse using a completely randomized design (CRD) with six treatments, consisting of A (100% inorganic fertilizer), B (75% inorganic fertilizer and 25% biofilm biofertilizer), C (50% inorganic fertilizer and 50% biofertilizer biofilm), D (25% inorganic fertilizer and 75% biofertilizer biofilm), E (100% biofertilizer biofilm), and F (no inorganic fertilizer and no biofertilizer biofilm). Each treatment was repeated four times. Parameters used in this study included plant height, number of leaves, dry weight and wet weight, and leaf diameter. Data analysis used the ANOVA test with a 95% confidence level, followed by Duncan's Multiple Range Test (DMRT). The results showed that the combination of 25% biofertilizer biofilm treatment with 75% inorganic fertilizer gave the best results compared to the control and other treatments. The treatment of 25% Biofilm biofertilizer with 75% inorganic fertilizer was able to increase plant height by 21.175 cm or 74.24% higher than control, number of leaves by 17.25 strands or 65.62% higher than control, leaf width by 7.125 cm or 61.80% higher than control, wet weight by 28.083 g or 97.29% higher than control, and dry weight by 1.869 g or 95.88% higher than control so that this treatment can be an alternative to reduce the use of inorganic fertilizer.

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1 Introduction

Climate changes such as rainfall trends, temperature trends, and changes in average rainfall significantly affect crop production results. One of the phenomena resulting from climate change is El Nino, where deficient rainfall causes the land to become dry. El Nino is a phenomenon of warming sea surface temperatures in the central and eastern Pacific Ocean. Warming sea surface temperatures increase the potential for cloud growth in the Pacific Ocean. This condition causes reduced rainfall in Indonesia. El Nino also causes drought conditions and hot weather in parts of Indonesia [1]. This needs to be addressed because it impacts plant growth and production. One of the leaf vegetable plants that is popular with many people is Pak choi. Pak choi belongs to the Brassicaceae family which originates from China and has high economic value [2]. These vegetables contain high nutritional value: the body needs, such as protein, fat, carbohydrates, calcium, phosphorus, iron, vitamin A, vitamin B, vitamin C, minerals, and fiber [3]. The production potential and market demand for this vegetable is very large so it needs to be managed appropriately [4].

Pak choi plants (Brassica rapa L.) can reach a 15-30 cm height with oval-shaped, green leaves and light green or white stalks. Pak choi can grow optimally at 100-200 meters above sea level [5]. The air temperature needed for Pak choi growth ranges from 15°C-30°C with air humidity of 80%-90% [4]. These plants can be planted yearly and are even better if planted in loose soil, rich in organic matter, and good drainage with an acidity degree (pH) of 6 to 7 [6].

The availability of nutrients in the form of nitrogen, phosphorus, and potassium is needed in large quantities to form Pak choi biomass. Fertilization is an effort to increase Pak choi productivity by replacing lost nutrients and increasing the supply of nutrients required by plants to increase crop production and quality [7]. However, in the practice of cultivating vegetables, especially Pak choi, they still depend on high doses of inorganic fertilizers [8]. Excessive use of inorganic fertilizers will harm the environment. The structure will harden soil given excessive inorganic fertilizers, making it difficult for roots to grow and absorb nutrients. Applying inorganic fertilizers also leaves chemical residues that can inhibit performance and reduce the diversity of microorganisms beneficial to plants [8]. Therefore, an alternative is needed to reduce the use of inorganic fertilizers. One way is to fertilize using biological fertilizers [9]. Biofilm Biofertilizer is a natural fertilizer that contains various kinds of microbes that can increase the availability of nutrients for plants [10]. Biofertilizers are classified as environmentally friendly fertilizers and can play a dual role by producing phytohormones beneficial to plants [11]. Applying Biofilm Biofertilizer to rice plants (Oryza sativa L.) is proven to replace about 50% of inorganic fertilizers while increasing crop yields by 24%. Application of BFBB is an effective method to reduce the use of inorganic fertilizers by half in C. asiatica cultivation, thereby minimizing the negative impact of inorganic fertilizers [12]. The addition of biological fertilizers is expected to substitute inorganic fertilizers so that the use of inorganic fertilizers can be reduced.

2 Materials and methods

2.1 Study area

This research was conducted from May to August 2023 in the Greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret (UNS). Plant analysis was carried out at the UNS Soil Biology and Biotechnology Laboratory.
2.2 Materials and equipment

The materials used include Nauli f1 Pak choi plant seeds, Andisol soil, inorganic fertilizer (Urea, Sp-36, and KCl), bacteria and fungi isolated from previous research [13], alcohol, aquadest, spiritus, PDB (Potato Dextrose Broth), NB (Nutrient Broth), YEMA (Yeast Extract Mannitol Agar), Pikovskaya medium, Jansen medium, crystal violet 0.1%, dan PBS (Phosphate Buffer Saline). The equipment needed is a pot, seedling tray, scales, hand colony counter, spectrophotometer, hemacytometer, autoclave, measuring cup, Erlenmeyer, cotton pads, plastic wrap, microscope, rack and test tubes, Petri dishes, tube needles, bunsen, pipette, micropipette, chips, tissue rolls, incubators, shakers, rulers, ovens and vortexes.

2.3 Research methods

The experimental design used was a Completely Randomized Design (CRD) with six treatments, consisting of control (no inorganic fertilizer and no biofertilizer biofilm), A (100% inorganic fertilizer), B (75% inorganic fertilizer and 25% biofertilizer biofilm), C (50% inorganic fertilizer and 50% biofertilizer biofilm), D (25% inorganic fertilizer and 75% biofertilizer biofilm), and E (100% biofertilizer biofilm) with four repetitions.

2.4 Procedure

The fungal inoculum was propagated on PDB (Potato Dextrose Broth) media. The bacterial inoculum was reproduced using several media, namely YEMA (Yeast Extract Mannitol Agar) for symbiotic BPN, Pikovskaya for BPF, and Jansen for non-symbiotic BPN using the slant media method. 6 ml of each, then taken and grown on NB (Nutrient Broth) media. Monocultures are stored in a shaker for three days at 33-37°C. After three days, a micropipette transferred the fungal inoculum to a new PDB. At the same time, each bacterial inoculum was inoculated into a PDB medium containing fungal inoculum and allowed to form a biofilm. Bacterial cell density was measured using a hemacytometer. Biofilm formation was observed qualitatively using the staining method [14]. The bacterial dose given was 10^6 cells.g^-1 of soil. In its application, the inoculum density is 10^{10} sel.mL^-1. The inorganic fertilizers used are 300 kg/ha of Urea fertilizer, 200 kg/ha of SP-36 fertilizer, and 100 kg/ha of KCl fertilizer [15]. The dose of fertilizer is then adjusted to the needs of the Pak choi plant. Based on soil weight, the amount of Urea fertilizer for Pak choi plants was 0.45 g/pot (100%) and adjusted to each amount of 75%, 50%, and 25%, respectively. The amount of SP-36 fertilizer for Pak choi is 0.3 g/pot (100%) and adjusted to each amount of 75%, 50%, and 25%, respectively. The amount of KCl fertilizer for Pak choi is 0.15 g/pot (100%) and adjusted to each amount of 75%, 50%, and 25%, respectively. Inorganic fertilizer incubation was carried out one day before transplanting. The planting medium used is Andisol soil. The soil is put into heat-resistant plastic and weighed 3 kg. The soil is then sterilized using steam (steamed for 2 hours/day for three consecutive days) [16]. The application of biofertilizer biofilm was carried out 7 HST around the roots. The amount of inoculum given was 25%, 50%, 75%, and 100%, respectively. Seeding is carried out for one week in the tray or until the seedlings have an average number of leaves of 3-4. After that, the Pak choi seedlings were transferred to pots. One pot contains 1 Pak choi seed. The application of inorganic fertilizer was carried out one day before transplanting, while the application of biofilm biofertilizer was carried out at 7 HST. Maintenance carried out is watering and spraying organic pesticides. Harvesting is around 30-45 days after transplanting. The observation parameters were plant height, leaf width, number of leaves (direct observation and
measurement in the field using a ruler), wet weight, and dry weight (direct weighing including leaves, roots, and stems). Observations are made every week. Data were analyzed statistically using Analysis of Variance (ANOVA) (95% significance level) followed by Duncan’s Multiple Range Test (DMRT) (95% significance level). A correlation test was conducted to see the observed parameters' relationship.

3 Result and discussion

3.1 Plant growth

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Height (cm)</th>
<th>Number of Leaves (strand)</th>
<th>Leaf Width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.100a</td>
<td>5.500a</td>
<td>2.750a</td>
</tr>
<tr>
<td>A</td>
<td>21.175d</td>
<td>17.250e</td>
<td>7.125e</td>
</tr>
<tr>
<td>B</td>
<td>19.800d</td>
<td>16.000de</td>
<td>7.200e</td>
</tr>
<tr>
<td>C</td>
<td>17.775c</td>
<td>14.000cd</td>
<td>6.050c</td>
</tr>
<tr>
<td>D</td>
<td>19.600d</td>
<td>13.750c</td>
<td>6.300c</td>
</tr>
<tr>
<td>E</td>
<td>14.775b</td>
<td>10.250b</td>
<td>3.850b</td>
</tr>
</tbody>
</table>

Notes: Control (no inorganic fertilizer and no biofertilizer biofilm), A (100% inorganic fertilizer), B (75% inorganic fertilizer and 25% biofilm biofertilizer), C (50% inorganic fertilizer and 50% biofilm biofertilizer), D (25% fertilizer inorganic and 75% biofilm biofertilizer), and E (100% biofilm biofertilizer).

3.1.1 Plant height

Based on the ANOVA results, treatment significantly affected plant height (Table 1). The highest average increase in plant height was in treatment A (100% inorganic fertilizer), namely 21.175 cm or 75.91% higher than the control. Treatment A had no significant difference from treatments B and D. The control treatment had the lowest mean with a value of 5.1 cm. Nutrients N, P, and K are essential nutrients for plants. Biological fertilizers act as inoculants made from living organisms' active ingredients, which fix certain nutrients or facilitate the availability of nutrients in the soil for plants. Biological fertilizer can be effective if organic or inorganic fertilizer is added to the application as a substrate to reproduce [17]. Providing input in the form of adding inorganic fertilizers can help metabolic processes in plants run well. Inorganic fertilizers are needed to stimulate the formation of roots, which will support the establishment of plants and the building of plant height [18].

3.1.2 Number of leaves

Based on the ANOVA results, treatment significantly affected the number of leaves (Table 1). The highest average increase in leaf number was in treatment A (100% inorganic fertilizer), namely 17.25 pieces or 68.11% higher than the control. Treatment A had no significant difference from treatment B. The control treatment had the lowest mean with a value of 5.5 strands. Inorganic fertilizers have an advantage compared to biological fertilizers, which dissolve quickly in water so that nutrients are readily available to plants. The addition of inorganic fertilizer will enable plants to grow their vegetative organs optimally from the start of their development because inorganic fertilizer is fast-release (quickly available) [18]. In addition, the number of leaves will be affected by the height of
the plant. The higher the plant, the more leaves will also increase because the leaves are located on the stem segments [19].

### 3.1.3 Leaf width

Based on the ANOVA results, treatment significantly affected leaf width (Table 1). The highest mean increase in leaf width was found in treatment B (75% inorganic fertilizer + 25% biofilm biofertilizer), which was 7.2 cm or 61.80% higher than the control. Treatment B had no significant difference from treatment A. The control treatment had the lowest average, with a value of 2.75 cm. Combining inorganic fertilizers and biofilm biofertilizers can increase leaf width because the amount of nutrients contained is sufficient to supply the nutrient needs of the Pak choi plant. An adequate supply of nutrients will help plants to form protein so that with enough macro and micronutrient requirements for plants, the amount of protein included will increase and will increase the amount of protoplasm in plant cells and will eventually increase the width of leaves that are rich in chlorophyll. The nutrient element that most influences the growth and development of leaves is nitrogen [20].

### 3.2 Plant weight

Plant weight is differentiated between wet and dry weight. The following is data on the wet weight of Pak choi (Figure 1).

#### 3.2.1 Wet weight

![Wet Weight Chart](https://doi.org/10.1051/e3sconf/202346701027)

Notes: Control (no inorganic fertilizer and no biofertilizer biofilm), A (100% inorganic fertilizer), B (75% inorganic fertilizer and 25% biofertilizer biofilm), C (50% inorganic fertilizer and 50% biofertilizer biofilm), D (25% fertilizer inorganic and 75% biofilm biofertilizer), and E (100% biofilm biofertilizer).

**Figure 1.** Wet weight of Pak choi plants.

Based on Figure 1, biofilm can increase plant wet weight. The highest increase was in treatment B (75% inorganic fertilizer + 25% biofilm biofertilizer), which was 28.083 g or 97.29% higher than the control. The difference between treatment B and other treatments
was also significant. The control treatment had the lowest result, with a value of 0.761 g. The wet weight content of plants is influenced by the water content in plant tissues or organs, nutrients, and organic matter contained in a plant. Microorganisms included in the biofertilizer biofilm play a role in decomposing organic matter. The more decomposition processes by decomposer microorganisms, the availability of nutrients in the planting medium will increase, affecting crop production. The availability of nutrients in sufficient conditions will allow the photosynthesis process to run smoothly so that assimilation can be translocated to all plant parts. In the end, there will be an increase in plant wet weight [21]. Next is the dry weight data for Pak choi plants (Figure 2).

### 3.2.2 Dry Weight

![Dry weight graph]

Notes: Control (no inorganic fertilizer and no biofertilizer biofilm), A (100% inorganic fertilizer), B (75% inorganic fertilizer and 25% biofertilizer biofilm), C (50% inorganic fertilizer and 50% biofertilizer biofilm), D (25% fertilizer inorganic and 75% biofilm biofertilizer), and E (100% biofilm biofertilizer)

**Figure 2.** Dry weight of Pak choi plants.

Based on Figure 2, biofilm can increase plant dry weight. The highest increase was in treatment B (75% inorganic fertilizer + 25% biofilm biofertilizer), which was 1.869 g or 95.88% higher than the control. Treatment B was not significantly different from treatment A. The control treatment had the lowest results, with a value of 0.077 g. Plant dry weight reflects the nutritional status of a plant. It is also an indicator that determines whether or not a plant's growth and development are closely related to nutrient availability. The higher the dry weight value of the plant produced, the better the plant's growth and the more nutrients it will absorb. By adding microbial inoculant, the presence of nutrients in the soil can increase so that it can stimulate plant growth [22]. The results of the correlation analysis between each variable can be seen in Table 2 below:
Table 2. Correlation between observed variables in Pak choi plants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Plant height</th>
<th>Number of leaves</th>
<th>Leaf width</th>
<th>Wet weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>1</td>
<td>0.902**</td>
<td>0.896**</td>
<td>0.770**</td>
<td>0.782**</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>0.902**</td>
<td>1</td>
<td>0.925**</td>
<td>0.826**</td>
<td>0.850**</td>
</tr>
<tr>
<td>Leaf width</td>
<td>0.896**</td>
<td>0.925**</td>
<td>1</td>
<td>0.856**</td>
<td>0.853**</td>
</tr>
<tr>
<td>Wet weight</td>
<td>0.770**</td>
<td>0.826**</td>
<td>0.856**</td>
<td>1</td>
<td>0.979**</td>
</tr>
<tr>
<td>Dry weight</td>
<td>0.782**</td>
<td>0.850**</td>
<td>0.853**</td>
<td>0.979**</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes:**= Very Significant Correlation (Level 0.01); *= Significant Correlation (0.05 Level).

Based on Table 2, it is known that all plant growth variables have a close correlation and are positive. Harvest results show optimal photosynthesis processes. Optimal photosynthesis is supported by leaf color, which has a high level of greenness; leaf green is influenced by the availability of N in the soil or growing media [23]. So that, in the end, it supports all components of growth and optimal harvest results, too. The results of this correlation indicate that the combination of inorganic fertilizers and biofilm biofertilizers can be used as complete fertilizers because these two types of fertilizers can complement each other's nutrient needs in Pak choi plants.

4 Conclusion

The treatment of 25% biofilm biofertilizer with 75% inorganic fertilizer was able to increase plant height by 21.175 cm or 74.24% higher than control, number of leaves by 17.25 stand or 65.62% higher than control, leaf width by 7.125 cm or 61.80% higher than control, wet weight by 28.083 g or 97.29% higher than control, and dry weight by 1.869 g 95.88% higher than control so that this treatment can be an alternative to reduce the use of inorganic fertilizer.

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