

## Analysis of Growth and Yield of *Echinacea Purpurea* with the Addition of Biochar and Plant Growth Regulator

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**Abstract**—*Echinacea purpurea* is a North American herbal plant (*Asteraceae*) that is well-known worldwide as an alternative option to prevent the outbreak of COVID-19. *E.purpurea* contains antioxidants, is anti-inflammatory, and is immunomodulatory. The various potentials and benefits included in *E.purpurea*, can be developed by adding biochar and plant growth regulators. This study aimed to determine the optimal potential of secondary metabolite content in treating biochar type and growth regulator concentration. *E.purpurea* were prepared from the Experimental Field at Universitas Sebelas Maret, Jumantono, Karanganyar and were grown using the Split-plot research design treated with type biochar (control, husk charcoal, wood charcoal) and concentration growth regulator (0 ml/l, 2 ml/l, 4 ml/l, 6 ml/l). The data obtained were analyzed by variance analysis followed by Duncan's test at a 5% level and described. The results showed that husk charcoal biochar gave the highest yield but was not significantly different from the wood charcoal biochar or control: plant height (38.8 cm) and the number of flowers (8.22). The growth regulator at a concentration of 6 ml/L gave the highest yield but was not significantly different from the concentration of 2-4 ml/L: plant height (39.8 cm), flowering time (11 WAP), number of flowers (9), and root length (21.9 cm). The highest C-organic plant tissue content yield was 35.44% (with husk charcoal) and 35.55% (with wood charcoal). It is shown that the provision of biochar and growth regulators can develop *E. purpurea* cultivation, especially on growth and yield components.

**Keywords**—Biochemical compounds; herbs; immunomodulatory.

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### I. INTRODUCTION

Plant species have always been a source for the discovery of medicinal needs. Dependence on medicinal plants may have increased worldwide because it could be an alternative option to prevent the COVID-19 pandemic. On the other hand, the World Health Organization (WHO) explained that medicinal plants can support and improve health and the immune system. *E. purpurea*, a plant from North America, is the most popular and is used worldwide to prevent outbreaks of common cold disease and other upper respiratory infections. *E. purpurea* has phenolic compounds, namely caffeic acid, chicory acid, and echinacoside [1]. Caffeic acid can act as an antioxidant and anti-inflammatory molecule [2]. Chicoric acid stimulates T-cell activation, accelerates wound healing, and reduces inflammation [3]. Chicoric acid increases the production of interferons, immunoglobulins, and other chemicals essential for the immune system [4].

Echinacoside has shown potential for use in the treatment of nerve conditions in nerve cells [5].

Cultivation of *E. purpurea* in Germany can produce 2-6 t/ha of dry roots, but in New Zealand, only 1.3-2.6 t/ha. Meanwhile, in America, the production of dry roots of *E. purpurea* reached 45.4 tonnes/ha. Cultivation of *E. purpurea* in Indonesia in the Pacet-Cipanas area at 3.5 BST produces an average of 0.78 tons/ha of dry roots. Ungaran (altitude 600 masl) only averages 0.32 tons/ha of dry roots. The total accumulation rate of *E.purpurea* biomass cultivated in Ungaran (altitude 600 masl) was relatively lower, namely 35.4 g/plant, compared to that grown in Pacet-Cipanas (altitude 1100 masl), which could reach 75.5 g/plant [6]. *E. purpurea* can survive under various soil conditions and stresses, such as saline, drought, and cold, owing to its highly developed fibrous root system, vigorous growth, and significant biomass [7]. The difference in climate between the subtropics and the tropics, especially in Indonesia, will affect

the growth and yield of *E.purpurea*. *E.purpurea* is challenging to develop in Indonesia with the addition of biochar and growth regulators. Growth regulators trigger biochemical reactions because they contain nucleotide (uridine diphosphate N-acetyl-d-glucosamine (UDP-GlcNAc), which acts to activate signals to form enzymes. The enzymes produced are Phenylalanine Ammonia Lyase (FAL) and Tyrosine Ammonia Lyase (TAL), which play a role in increasing the activity of phenolic compounds [8]. Adding biochar to soil increases yields in the presence of significant cations and the availability of phosphorus, total N, and soil cation exchange capacity (CEC) [9].

## II. MATERIAL AND METHOD

### A. Materials

Samples of *E.purpurea* came from the Experimental Field of Sebelas Maret University, Surakarta, Jumantono, Karanganyar ( $\pm 300$  meters above mean sea level with  $7^{\circ}37'829''S$ ,  $110^{\circ}56'901''W$ ). Samples were grown using the Split-plot Design, which was treated with 3 types of biochar (control, husk charcoal, and wood charcoal) and 4 levels of growth regulator (with a trademark "Hormax") concentration (0ml/L, 2ml/L, 4ml/L, and 6ml/L) in 3 repetitions. Hormax contains IAA/Auxin (108.56 ppm), Cytokinin (Kinetin 98.34 ppm and Zeatin 107.81 ppm), ABA (89.35 ppm), IBA (83.72 ppm) Gibberellin/GA3 (118.40 ppm), Ethylene (168 ppm), Traumalin Acid (212 ppm) and Humic Acid (354 ppm).

The samples were all parts of the *E.purpurea* plant, including stems, leaves, roots, and flowers. Samples were taken when entering the generative or flowering phase and then dried until the water content was less than 10%. Data analysis was conducted using the Duncan Multiple Range Test (DMRT) SPSS 24.0 and Excel, presented in tables and diagrams.

The materials used are *E.purpurea* seeds from B2P2TOOT Karanganyar (Indonesia), a type of biochar (husk charcoal and wood charcoal), fertilizer (leaf compost fertilizer), and plant growth regulators (with a trademark "Hormax"). The materials used in the laboratory analysis were *E.purpurea* extract,  $H_2SO_4$ ,  $K_2Cr_2O_7$  2 N, and 5.000 ppm C solution. The tools used are 65% net, sprayer, and meter. Laboratory analysis equipment used is an analytical balance, water bath, micropipette, test tube rack, centrifuge, incubator, and visible spectrophotometer.

### B. Procedure

1) *Biomass (gram)*: All plant parts were weighed on an analytical balance as wet weight. All plant parts were oven to  $60^{\circ}C$  for 24 hours and then weighed as a dry weight. The harvest index is carried out after harvesting at the time when the plant has been dried until its moisture content is reduced from 10% [10]. Measurements of root weight and weight of herbaceous dry (upper part) are carried out to determine the harvest index [11]. The percentage was calculated with the following formulation:

$$\text{Harvest Index} = \frac{\text{Upper dry weighth (herb)}}{\text{Total dry weighth}} \quad (1)$$

2) *Plant Tissue C-organic Content(%) (Walkley and Black)*: The Walkley – Black (WB) method is a laboratory

analysis method commonly used to calculate organic carbon [12]. The sample was ground to a fine powder. The sample that has become powder is weighed as much as 0.05-0.1 grams and then put into a bottle, 5 ml of  $K_2Cr_2O_7$  2 N and 7 ml  $H_2SO_4$ , then mixed until homogeneous for 30 minutes. Preparation of 250 ppm C standard solution was added with 5 ml of 5000 ppm C standard solution, 5 ml of  $H_2SO_4$ , and 7 ml of 2 N  $K_2Cr_2O_7$  solution, mixed and shaken until homogeneous. The standard solution that has been made is left overnight. After being stored for 24 hours, the samples were measured using a spectrophotometer at 651 nm. The recommended absorbance value for laboratory analysis is 635.0 nm for a spectrophotometer used at visible wavelengths. Photometric measurements aim to validate the stability and linear level of photometric value measurements [13].

$$C\text{-organic Content} = \frac{\text{ppm curve} \times 100}{\text{sample (mg)} \times f_k} \quad (2)$$

where  $f_k$  is the water content correction factor.

## III. RESULT AND DISCUSSION

Biomass plants are related to accumulating photosynthetic products and water content in plant organs. Accumulation of biomass during vegetative growth and part of harvested biomass determines the yield of biomass [14]. Some studies conducted by [15] explained that growth reflects the increase in protoplasm, size, and the total number of plant cells. This biomass production results in weight gain which can also be followed by an increase in plant size so that crop yields also increase.

TABLE I  
EFFECT OF BIOCHAR TYPE AND GROWTH REGULATOR CONCENTRATION ON AVERAGE BIOMASS *E.PURPUREA*

| Treatment | Weight    |         |               |
|-----------|-----------|---------|---------------|
|           | Wet       | Dry     | Harvest Index |
| B1H1      | 121.62a   | 27.74a  | 0.864a        |
| B1H2      | 151.66ab  | 38.70a  | 0.875a        |
| B1H3      | 212.04bc  | 47.49ab | 0.882a        |
| B1H4      | 189.10abc | 46.06ab | 0.872a        |
| B2H1      | 142.29ab  | 35.94a  | 0.859a        |
| B2H2      | 212.34bc  | 52.78ab | 0.892a        |
| B2H3      | 206.02bc  | 52.80ab | 0.898a        |
| B2H4      | 232.20c   | 60.93b  | 0.898a        |
| B3H1      | 187.21abc | 39.45a  | 0.852a        |
| B3H2      | 205.43bc  | 41.87a  | 0.861a        |
| B3H3      | 182.66abc | 52.80bc | 0.904a        |
| B3H4      | 210.89bc  | 52.92ab | 0.897a        |

\*\*Note: Means followed by different letters in the same column are significant in DMRT at the 5% level. BIH1: control + growth regulator 0ml/L, BIH2: control + growth regulator 2ml/L, BIH3: control + growth regulator 4ml/L, BIH4: control + growth regulator 6ml/L, B2H1: husk charcoal + growth regulator 0ml/L, B2H2: husk charcoal + growth regulator 2ml/L, B2H3: husk charcoal + growth regulator 4ml/L, B2H4: husk charcoal + growth regulator 6ml/L, B3H1: wood charcoal + growth regulator 0ml/L, B3H2: wood charcoal + growth regulator 2ml/L, B3H3: wood charcoal + growth regulator 4ml/L, B3H4: wood charcoal + growth regulator 6ml/L

Table 1 explains that the combination treatment of husk charcoal biochar with a concentration of 6ml /L growing regulating agents produces the highest biomass parameters such as wet weight, dry weight, and harvest index compared to other treatment combinations. This is related to each other because the higher the yield of wet and dry weights will increase the index of crop yields from plants. Wet weight also

reflects the nutritional status of plants because the wet weight depends on the total number of cells, size of cells, or quality of the plant constituent cells. This depends on the availability of hormones and the amount of accumulation of organic compounds from photosynthetic results, is a reflection of the ability of plants to absorb hormones from the growth regulator content [16]. Growth regulators can optimize plant yields by modifying growth development to stressful conditions in the plant life cycle [17]. In addition, the plant growth regulator growing regulatory substance will be absorbed by the plant to accelerate the protoplasmic flow of cells and activate metabolism. The results of the studies [18] explained that the provision of growing regulatory substances can increase the permeability of the cell wall, which will increase the absorption of nutrients and enhance photosynthesis. Increased photosynthesis will increase crop yields.

Adding husk charcoal biochar results in optimal treatment in increasing *E.purpurea* biomass. An increase in yield corresponds to the amount of charcoal added to the soil since charcoal increases porosity, which is beneficial for the development of root openings and helps plants to grow well [19]. Biochar not only increases crop yields but works as a soil enhancer [20]. The situation around the *E.purpurea* plant that becomes more porous greatly facilitates the absorption of available nutrients and inorganic nutrients that affect the process of crop harvesting. Biochar can replace peat moss media and increase 80 % of the growth and yield of ornamental, aromatic, and medicinal plants [21].

TABLE II  
EFFECT OF BIOCHAR TYPE AND GROWTH REGULATOR CONCENTRATION ON  
AVERAGE BIOMASS *E.PURPUREA*

| Biochar       | Plant Height (cm) | Flowering Time (WAP) | Number of Flowers | Root Length (cm) |
|---------------|-------------------|----------------------|-------------------|------------------|
| No Biochar    | 35.54a            | 12.72b               | 7.28a             | 19.02a           |
| Husk Charcoal | 38.80b            | 11.81a               | 8.22a             | 19.56a           |
| Wood Charcoal | 37.18ab           | 11.67a               | 8.00a             | 20.81a           |

| Growth Regulator | Plant Height (cm) | Flowering Time (WAP) | Number of Flowers | Root Length (cm) |
|------------------|-------------------|----------------------|-------------------|------------------|
| 0 ml/L           | 32.49a            | 12.89b               | 5.69a             | 19.30a           |
| 2 ml/L           | 39.25b            | 11.92ab              | 8.29b             | 18.30a           |
| 4 ml/L           | 37.18b            | 12.11ab              | 8.11b             | 19.70a           |
| 6 ml/L           | 39.77b            | 11.33a               | 9.15b             | 21.88b           |

\*\*Note: Numbers with different notations represent significant differences at 5% DMRT

Table 2 explains that the treatment that showed the significant value and the highest average yield on plant height for the husk charcoal biochar treatment produced significantly different values from the treatment without biochar of 38.80 cm. Some studies by [22] explained that phosphorus elements will increase when entering the flowering process because energy is needed in large quantities and functions as a constituent of enzymes and ATP for energy translocation. The growth regulator concentration of 6 ml/L was 39.77 cm, which was not significantly different from the 2-4 ml/L concentration. This is by research from [23] that explained that the division and elongation of apical meristem cells driven by auxin will promote plant height growth. Auxin can affect plant growth and development through the cell

membrane osmosis process, which allows water and organic and inorganic molecules to enter the cells [25].

Plant growth regulators can increase stress tolerance through properties such as phytohormones and hydrogen cyanide synthesis, cell wall degrading enzymes, antagonist activity, deaminase enzymes [24]. Biochar increases the potential influence of bacteria that produce phytohormones, siderophores, hydrogen cyanide, and ACC deaminase and can dissolve phosphate through stimulation of the root system. Biochar and plant growth regulators can increase soil acid phosphomonoesters activity. Exogenous use of auxins and cytokinin in appropriate concentrations increases the dry matter yield of plants (increases potassium and calcium content but does not change the concentration of phosphorus in plants) and increases their stability by stimulating rhizosphere regeneration processes. Auxin and cytokinin can reduce the occurrence of disease by decreasing the vitamin C content and increasing the phenolic compound content [25].

*E.purpurea* plants are included in short-day plants with a critical period that requires less than 12 hours per day for generative growth and more than 14 hours for vegetative growth [26]. Table 2 explained that wood charcoal gave the fastest flowering time but was not significantly different from husk charcoal which was 11.67 WAP. Growth regulator administration with a concentration of 6 ml/L produced high values and significantly differed from the concentration level of 2-4 ml/L of 11.33 WAP.

The treatment that showed the highest average number of flowers in the husk charcoal biochar treatment was 8.22 fruit, similar to the wood charcoal treatment and the control. Growth regulator administration with a concentration of 6 ml/L amounted to 9.15 flowers, identical to 2-4 ml/L concentrations. Protein is a building block for plants and increases the percentage of flower formation [27]. Wood charcoal gave the highest root length but was not significantly different from husk charcoal without biochar, which was 20.81 cm. Growth regulator administration with a concentration of 6 ml/L produced the highest root length value and significantly differed from the concentration level of 0-4 ml/L of 21.88 cm. Cells in the roots can convert IBA into IAA because IBA is the precursor of IAA.

The accumulation of IBA as a source of auxin can trigger adventitious root production and root formation [28]. The treatment that showed the highest average root volume was the husk charcoal biochar treatment of 22.36 ml, which was similar to the wood charcoal treatment and the control. Auxin and cytokines can regulate the formation of pro cambium, stem cell homeostasis, and phloem development [29] Growth regulator administration at a concentration of 6 ml/L was 23.70 ml, which was not significantly different from a concentration of 2-4 ml/L. [30] stated that physiological processes driven by plant auxin assimilation will provide good chrysanthemum root growth.



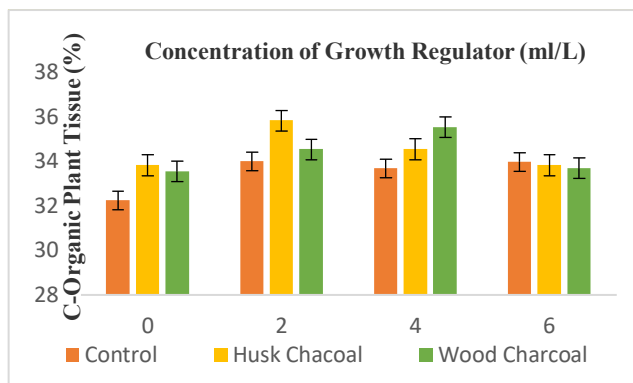


Fig. 1 Percentage Plant Tissue C-Organic Content (%) of *E. purpurea*

Carbon is an essential organic substance as a constituent of plant dry matter [31]. Figure 1 indicated that the husk biochar treatment had the greatest value, with a 2 ml/L ZPT concentration of 35.84%, followed by the wood charcoal biochar treatment, with a 4 ml/L ZPT concentration of 35.55%. The lowest results were found without biochar with a ZPT concentration of 0 ml/L of 32.56%. The increase in C-Organic in plant tissue can be due to the carbon content stored in biochar. The contribution of organic carbon in biochar is caused by a decomposition process that releases carbon (C), which increases organic-C levels in the soil. The ash content composition of rice husk charcoal was 22.88%, and wood charcoal was 6.91%. The bound carbon content and ash content describe the content of biochar, which consists of chemical elements of carbohydrate salts, sulfates, phosphates, silicates, potassium, calcium, and magnesium [32]. The content of these extractive substances will affect the carbon content in biochar and is a determinant of the quality of biochar as a soil improvement agent [33].

#### IV. CONCLUSION

This study concludes that adding biochar and the concentration of growth regulators can affect the response of plants, such as the result of growth and physiological processes. The husk charcoal biochar gave the highest yield. Still, it was not significantly different in the wood charcoal biochar treatment or without biochar (control) in the observed variables, namely plant height (38.8 cm) and the number of flowers (8.22). The growth regulator at a concentration of 6 ml/L gave the highest yield but was not significantly different from the concentration of 2-4 ml/L on the observed variables, namely plant height (39.8 cm), flowering time (11 WAP), number of flowers (9) and root length (21.9 cm). The highest C-organic plant tissue content yield was 35.44% (with husk charcoal) and 35.55% (with wood charcoal). Meanwhile, the addition of biochar in both husk charcoal and wood can increase the response of the final production and the content of secondary metabolites (flavonoids) from *E. purpurea*. This indicates that the treatment is optimal in supporting the final productivity and flavonoid content in the growth of *E. purpurea* in lowland land.

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