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Effect of Water Hyacinth Root Extract and Liquid Smoke on the Growth of Soybean Plants in Saline Soil

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Abstract
Soybean production can be increased by optimizing salinity marginal land and using Plant Growth Regulators (PGR) gibberellins. Phosphate solubilizing bacteria, specifically *Pseudomonas fluorescens*, were applied to help mitigate the elevated salt content in saline soil. The study aimed to describe the effect of water hyacinth root extract, liquid smoke, and their interaction on soybean plant growth. The study used a two-factor Randomized Group Design with the concentration of water hyacinth root extract (0 ppm, 500 ppm, 1000 ppm) and liquid smoke (0%, 1%, 2%). Data analysis used two-way ANOVA and continued with Duncan's test. The results showed that the interaction between the concentration of water hyacinth root extract and the concentracion of liquid smoke significantly affected the number of leaves, plant wet biomass, number of pods, and pod biomass. The concentration of 1000 ppm water hyacinth root extract and 1% the concentration of liquid smoke is the best combination for the number of leaves of 14.67 strands, plant wet biomass of 15.33 grams, the number of pods 13.00 pods, and soybean plant pod biomass of 8.00 grams.

Keywords: Acetic acid; exogenous hormone; gibberellin; sodium; plant growth.
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INTRODUCTION

Indonesia is one of the countries with the highest population, which shows that food needs are increasing. Still, on the other hand, land use is also growing, so agricultural land is becoming narrower. One of the food needs of the Indonesian people that is widely consumed is soybean crops. Based on data from the Badan Pusat Statistik (2014), soybean production increased by 14.44% or 112.61 thousand tons compared to 2013. This study shows an increase in consumption directly proportional to the demand for soybean crops. One of the efforts that can be made is to expand the area of agricultural land towards marginal coastal land and turn it into productive land. Approximately ±27.4 million hectares of marginal coastal land (saline soil) have the potential to be developed as productive agricultural land (Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian, 2012). Increased levels of salt dissolved in the soil are the main obstacles to the utilization of saline soils, and these obstacles disrupt the absorption process of water and nutrients, potentially inhibiting plant growth (Kurnia and Suprihati, 2016). Saline soil inhibits plant growth because high sodium in the soil causes Fe^{+}, Mg^{2+}, and Al^{+} ions not to be absorbed by plants and these ions are high in the soil, which causes nutrients such as P to be absorbed and unavailable by plants (Luthfi, 2012). In addition, improper soil management can cause the endogenous hormone content in plants to decrease (Wulandari, 2014). Based on these limitations, efforts can be made to improve nutrients in the soil by applying phosphate-solubilizing bacteria and utilizing exogenous plant regulators to enhance the quality of soybean plants.

Scientists define phosphate-solubilizing bacteria as microorganisms capable of dissolving phosphate from insoluble phosphate bonds through the secretion of organic acids. One phosphate-solubilizing bacteria is *Pseudomonas fluorescens*. *P. fluorescens* has many favorable properties that can affect plant health and soil fertility, including helping to increase plant growth (Dorjey et al., 2017). This is happens because *P. fluorescens,* a phosphate-solubilizing microorganism, can affect plants by increasing phosphate availability and producing growth regulators (Asril, 2019). The research of Abulfaraj and Jalal (2021) shows that the application of *P. fluorescens*can increases the growth of stem length and fresh weight of soybean plants under salinity stress of 400 mM NaCl salt. Based on this study shows that *P. fluorescens* bacteria can dissolve phosphate for soybean plants in saline soil.

Growth regulators are plant organic compounds in low concentrations that affect physiological processes, especially plant differentiation and development (Salisbury and Ross, 1995). Plant growth regulators are natural or synthetic organic hormones that can trigger, inhibit, or alter plant growth and development (Varalakshmi and Malliga, 2012). Gibberellin is a hormone that contributes to cell division, seed dormancy breaking, and flower and fruit development. Commonly used exogenous gibberellins that are available in the market are GA_3 (gibberellin-3). The role of GA_3 can increase growth in saline conditions (Iqbal et al., 2011). Some plants that can serve as a source of gibberellins are water hyacinth roots and liquid smoke from coconut shells.

Water hyacinth *(Eichhornia crassipes)* is an aquatic plant that is considered a weed in the aquatic environment because of its uncontrolled spread and can cause many losses, including reducing the productivity of water land and competing nutrient uptake with fish communities (Wardini, 2008). However, water hyacinth roots are known to have a reasonably high protein content, which is between 12-18%, and a relatively complete amino acid content that can be utilized as a substitute for the hormone gibberellin (Bayyinatul et al., 2012). The roots of water hyacinth are known to contain the hormone gibberellin (Musbakri, 1999). According to Permatasari (2016), gibberellin hormone in water hyacinth root extract can accelerate the process of flower formation and increase the amount of fruit production in plants. Ummah and Rahayu (2019) reported that there was a natural effect of gibberellin concentration of water hyacinth root extract as exogenous gibberellin, which at the concentration of 500 ppm gives the best effect on the percentage of germination. This research shows that water hyacinth root extract affects plant growth as a source of gibberellin.

In addition to water hyacinth root extract, coconut shell liquid smoke can allegedly function as gibberellin. According to Jayanudin and Suhendi (2012), liquid smoke contains 46.56% acetic acid. Acetic acid then forms acetyl Co-A. Acetyl Co-A is a precursor to gibberellin biosynthesis (Taiz and Zeiger, 2002). Liquid smoke from coconut shells results from condensation of coconut shell smoke in pyrolysis at temperatures around 400°C (Budijanto et al., 2008). The results of research by Putri et al. (2023) reported that coconut shell liquid smoke at a concentration level of 1% significantly affected plant height, leaf width, and wet weight of lettuce. Based on the research of Aisyah et al. (2018), they reported that the application of 0.5% coconut shell liquid smoke can spur vegetative growth and increase the activity of banana plant resistance enzymes. Therefore, the function of liquid smoke and the main composition of coconut shells have the potential to be developed as a raw material for making liquid smoke, which will produce acetic acid as a precursor to the biosynthesis of gibberellin formation in plants.

Based on the background and previous studies, this research will examine the role of gibberellins in affecting the growth of soybean plants (*Glycine max* L.) in saline soil enriched with *P. fluorescens* bacteria.

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MATERIALS AND METHODS

This experimental research was carried out in January - March 2024 in the greenhouse of the Biology study program at Surabaya State University. This study used a Randomized Group Design (RGD) with two treatment factors, namely the concentration of water hyacinth root extract (0, 500, and 1000 ppm) and the concentration of liquid smoke (0%, 1%, and 2%). The study consisted of 9 treatments with three repetitions, so 27 sample units were obtained. The tools used in this study consisted of polybags, shovels, a refractometer, a soil pH meter, a blender, a rotary evaporator, scales, stationery, and documentation tools. The materials used included Argomulyo soybean seeds, coconut shell liquid smoke, water hyacinth roots, methanol, base fertilizer, *and P. fluorescens* bacteria.

The research procedure consists of the first stage, namely the preparation of water hyacinth root extract, the second stage of preparation of saline soil planting media, and the third stage of planting and maintaining soybean plants by giving treatment. The first stage begins with collecting as much as 10 kg of water hyacinth roots and then washing them thoroughly. Water hyacinth roots were dried in an oven for three days at 60°C. Next, they were blended into 300 grams of simplisia powder. Then, it was re-measured with 60% methanol in a ratio of 1:4 with a composition of 300 grams of simplisia: 1200 ml methanol. Evaporation was carried out using a rotary evaporator, which resulted in 6 grams of water hyacinth root extract in methanol. After that, dilution with $NaHCO_3$ and ethyl acetate was 50 ml, and then extraction was done with HCl 1 M and ethyl acetate 75 ml. The concentration of water hyacinth root extract produces the final extract.

The second stage is to collect regosol soil and sand in a ratio of 1:1 or 2.5 kg:2.5 kg. Next, 0.25 grams of urea and 0.50 grams of KCl were applied as essential fertilizer. After that, the planting media was watered with a seawater solution of 0.004 M concentration. Before being treated, the soil was applied with the first phosphate-solubilizing bacteria as much as 100 ml, then watered again at 14 Days After Planting (DAP), 28 DAP, and 42 DAP as much as 50 ml. The last stage began with sowing soybean seeds for 20-21 Days After Sowing (DAS). After that, transplanting was done to 35 cm x 35 cm polybags, each filled with two seeds. After 7 DAP, the liquid smoke was applied six times at 7, 15, 21, 29, 35, and 43 DAP, while water hyacinth root extract was applied six times as well as 9 DAP, 17 DAP, 24 DAP, 31 DAP 38 DAP, and 45 DAP. Watering maintenance is carried out using seawater to see the condition of the soil that has dried up, and this watering is done in the morning or evening. In addition to watering, weeding is carried out so plants have enough nutrients. Moreover, the last step is harvesting. Harvesting of soybean plants was done 45 DAP (maximum vegetative period) by uprooting soybean plants and taking data.

The parameters in this study consisted of plant height, number of leaves, root length, plant wet biomass, P nutrient content, flowering time, number of pods, and pod wet biomass. Observations of plant height parameters and number of leaves were observed at 15, 30, and 45 DAP, while root length, wet biomass, number of pods, and pod biomass were carried out at 45 DAP. Flowering time was observed when the first flower appeared, and P nutrient levels were tested on plant leaves by analyzing them at the BSIP Aneka Kacang testing laboratory. Furthermore, data analysis was carried out using the SPSS 23.0 software Two-Way ANOVA test. If the data showed the effect of the concentration of water hyacinth root extract and the concentration of liquid smoke on soybean plant growth, then Duncan's test was continued at the significance level (α) 0.05 to see the real difference between the concentration of water hyacinth root extract, the concentration of liquid smoke, and the interaction between the concentration of water hyacinth root extract and the concentration of liquid smoke.

RESULTS

The results obtained showed a significant effect of the concentration of water hyacinth root extract, the concentration of liquid smoke, and the interaction between the concentration of water hyacinth root extract and the concentration of liquid smoke on the growth of soybean plants in saline soil. The results of parameter measurements can be seen in the soybean growth chart (Figure 1 and Figure 2). Before being treated with water hyacinth root extract and liquid smoke, saline soil was measured with several parameters such as soil pH, soil moisture, and nutrient content of Na, N, P, and K. Based on Table 1 shows the measurement results of pH and soil moisture of 7.5 and 65%. The content of available sodium nutrients in the soil was 1.20 $cmol^+/kg$ (very high), available potassium was 0.37 $cmol^+/kg$ (low), total nitrogen was 0.30% (medium), and phosphate was 36 ppm (very high). The measurement of soil conditions and content shows that the soil is based on the needs of the study, namely the soil under salinity stress.

Table 4.1 Results of pH, soil moisture, N, P, K, and Na content tests in saline soil

Parameters	Measurement Results	Criteria*
pH	7.5	Bases
Soil Moisture (%)	65	Moist
Na ($cmol^+/kg$)	1.20	Very High
K ($cmol^+/kg$)	0.37	Low
N (%)	0.30	Medium
P_2O_5 (ppm)	36	Very High

Notes: *) Based on the criteria of Sulaeman et al. (2005)

The study results of the concentration of water hyacinth root extract and the concentration of liquid smoke showed that the interaction between the concentration of water hyacinth root extract and the concentration of liquid smoke significantly affected the parameters of the number of leaves and wet biomass of plants. However, in the parameters of plant height, root length, and leaf P nutrient content, there was no significant effect of the interaction between the concentration of water hyacinth root extract and the concentration of liquid smoke. The interaction treatment of 1000 ppm the concentration of water hyacinth root extract and 1% the concentration of liquid smoke showed the best results on the number of leaves and plant wet biomass parameters. Still, it was not significantly different from the interaction treatment of 1000 ppm the concentration of water hyacinth root extract and 2% the concentration of liquid smoke.

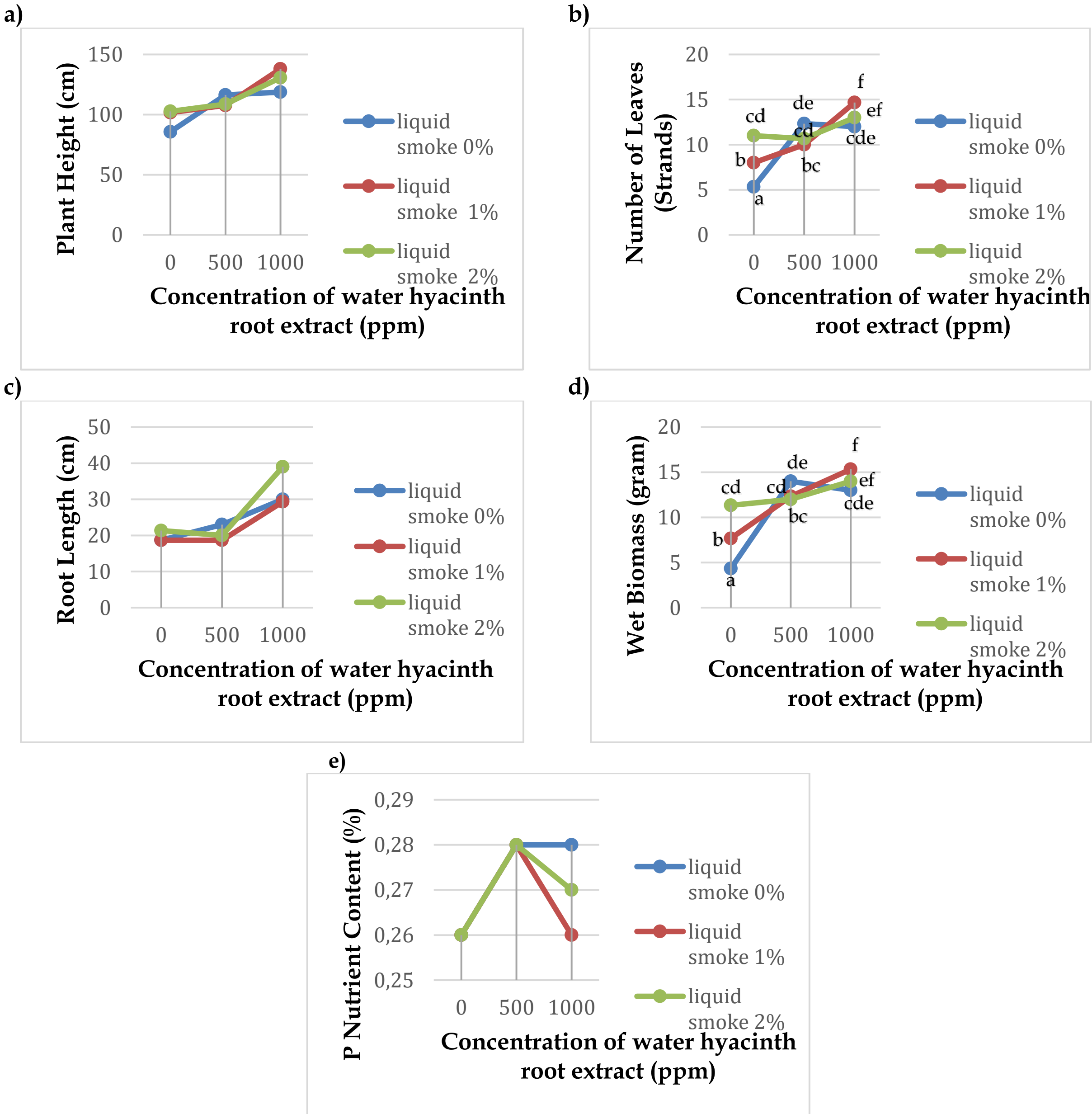

Figure 1. The effect of the combination of water hyacinth root extract and liquid smoke on a) plant height; b) number of leaves; c) root length; d) wet biomass; and e) P nutrient content (Different letters on the graph indicate a significant effect based on Duncan's test at the 0.05 level).

The vegetative period of soybean plants is 45 DAP (Noviani and Rahayu 2019), but in observations, it has been found that flowers appear before the maximum vegetative period ends. Therefore, observations of plant growth also observe the timing of flowers, the number of pods, and the wet biomass of pods. The results of applying the concentration of water hyacinth root extract and the concentration of liquid smoke showed a significant effect on the number of pods and pod biomass (Figure 2). There was no interaction between the concentration of liquid smoke and the concentration of water hyacinth root extract on the flowering time parameter of soybean plants.

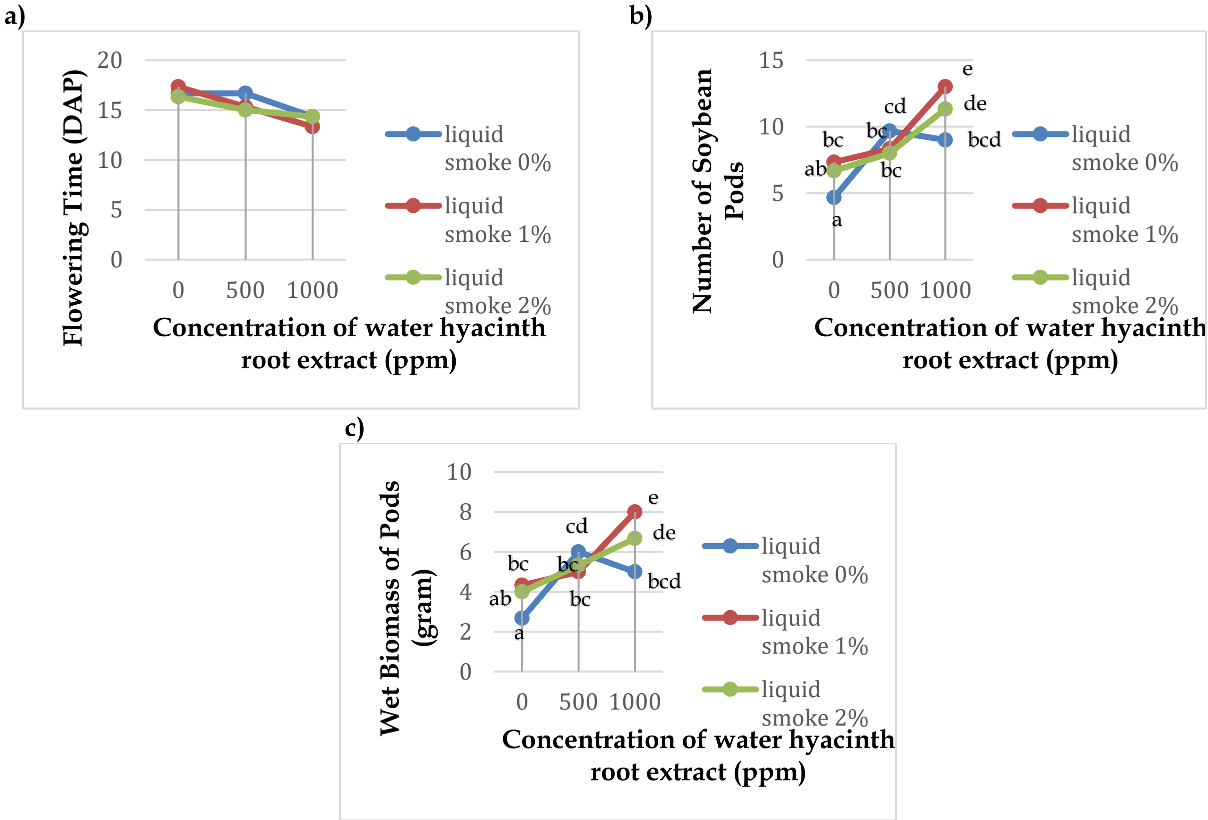

Figure 2. The effect of the combination of water hyacinth root extract and liquid smoke on a) flowering time; b) number of soybean pods; and c) wet biomass of pods (The difference in letters on the graph indicates a significant effect based on Duncan's test at the 0.05 level).

DISCUSSION

Based on the results of soil tests (Table 1), the sodium nutrient content of the soil was found to be 1.20 cmol$^+$/kg. The value of sodium content is classified as high salinity, according to Sulaeman et al. (2005), sodium levels in the soil are said to be saline if it exceed 0.6 cmol+/kg. In addition to sodium nutrients, there is a total nitrogen nutrient content of 0.30% with low criteria and a potassium nutrient content of 0.37 cmol$^+$/kg with moderate criteria. Sutarto and Suharsono (2013) stated that the optimal requirement of nitrogen (N) in the soil for soybean plants ranges from 1.5 - 2.5% N, and the optimal requirement of potassium (K) ranges from 0.4 - 0.6 cmol$^+$/kg, so that the nutrients N and K in this saline soil have not met the nutrient requirements.

In the soil test results (Table 1), the nutrient phosphate (P_2O_5) in the soil has a value of 36 ppm. According to Sulaeman et al. (2005), phosphate nutrients are very high if they exceed 15 ppm, but plants may not necessarily use the presence of P nutrients in the soil if the P is unavailable. The availability of P for plants depends on the influence of cations, anions and the pH of the soil (Havlin et al., 1999). On the other hand, the saline soil in this study had a very high Na^+ content of 1.20 cmol$^+$/kg, which reduced plant uptake of Fe^{2+}, Mg^{2+}, and K^{2+} cations, preventing them from being absorbed. As a result, these cations become more concentrated in the soil and bind phosphate nutrients into new bonds. Therefore, cations that bind phosphate nutrients in the soil caused P nutrients to be trapped and unavailable to plants (Lutfi, 2012). This study aimed to balance the phosphate nutrients available for soybean plants with the addition of phosphate-solubilizing bacteria *Pseudomonas fluorescens*. Research by Abulfaraj and Jalal (2021) showed that the use of *P. fluorescens* increased the fresh weight growth of soybean plants affected by 400 mM salinity stress. The above research proves that *P. fluorescens* in saline soil could play a role in balancing the growth of plants experiencing salinity stress.

Based on the research results, it is known that the application of water hyacinth root extract and liquid smoke affects the growth of soybean plants. Growth parameters in vegetative period research consisted of plant height, number of leaves, root length, plant wet biomass, and leaf P nutrient content. Moreover, the generative period parameters consisted of flower time, number of pods, and wet biomass of pods. The results of the growth data of soybean plants during the vegetative period (Figure 1) show that plant height, root length, and P nutrient content have no significant effect on the interaction treatment between the concentration of water hyacinth root extract and the concentration of liquid smoke. In the parameters of the number of leaves and wet plant biomass, there is a significant effect on the interaction treatment between the concentration of water hyacinth root extract and the concentration of liquid smoke. The combination of 1000 ppm water hyacinth root extract and 1% concentration of liquid smoke produces the best data, the number of leaves of 14.67 strands, and wet plant biomass of 15.33 grams.

Furthermore, the growth data of soybean plants during the generative period (Figure 2) shows that the flower time parameter has no significant effect on the interaction treatment between the concentration of water hyacinth root extract and the concentration of liquid smoke. In the parameters of the number of pods and wet biomass of pods, there is a significant effect on the interaction treatment between the concentration of water hyacinth root extract and the concentration of liquid smoke. The combination of 1000 ppm water hyacinth root extract and 1% concentration of liquid smoke produced the best data, a number of 13.00 pods and wet plant biomass of 8.00 grams.

This happens because water hyacinth root extract contains 12-18% protein, which can be helpful as a body regulator of the hormone gibberellin (GA_3) (Bayyinatul et al., 2012). Water hyacinth roots contain gibberellin phytohormones that can help plant growth. Liquid smoke has 46.56% acetic acid content (Jayanudin and Suhendi, 2012). Liquid smoke has acetic acid that can induce hormone substances (Mu et al., 2004). Acetic acid binds to coenzyme A to form acetyl Co-A for the precursor of gibberellin biosynthesis. The two ingredients prove this research is a source of gibberellin hormone for soybean plants. Gibberellin is a hormone that can stimulate plant growth in stems and leaves and stimulate flower and fruit development. The results of this research are in line with this theory, where in the growth parameters of the number of leaves, wet plant biomass, number of pods, and pod biomass, there is a significant effect of giving water hyacinth root extract and liquid smoke which acts as a source of gibberellin.

Gibberellin hormone from water hyacinth root extract belongs to the category of exogenous hormones; the research of Ummah and Rahayu (2019) stated that the content of gibberellin hormone in water hyacinth root extract was 2995.50 ppm; the study used water hyacinth root extract as an exogenous hormone to help synergize the work of endogenous hormones that already exist in plants, thus causing plants to be stimulated faster and the growth time is relatively shorter. Naturally, plants already have growth hormones in their meristem tissues called endogenous hormones. However, environmental stress factors or improper cultivation patterns result in low endogenous hormone content in plants, so plant growth becomes slower. Adding exogenous hormones such as gibberellins can solve the issue of plant growth constraints due to environmental factors (Wulandari et al., 2014).

Liquid smoke contains acetic acid. Acetic acid binds to coenzyme-A to form acetyl-CoA (Taiz & Zeiger, 2002). Furthermore, acetyl-CoA, through the acetic mevalonic acid pathway, produces terpenoid compounds in the secondary metabolite pathway, which become precursors of GA compounds. GA compounds will undergo biosynthesis to become gibberellin compounds active for plants. From this process, liquid smoke plays a role in contributing acetic acid, which is used in the formation of synthesized compounds through various processes that require a long time and journey until the gibberellin hormone is formed, so from the results of this study, liquid smoke treatment tends not to have a significant effect on parameters. There is no significant difference between each treatment concentration.

At a concentration of 1000 ppm water hyacinth root extract and 1% the concentration of liquid smoke, the best results are close to the role of gibberellin, which increases cell division and enlargement, controlling the active growth of plants. The effect of gibberellin increases the process of cell division by activating cell division enzymes when entering into the process of xylem and phloem cell differentiation (Widyastuti, 2015). Gibberellin activates cell division enzymes such as protease enzymes so that cell division runs well in developing xylem and phloem tissues. Therefore, gibberellin helps increase both tissues' ability to carry out water and nutrient transportation to run smoothly. As a result, if cell division increases, growth results will also increase from the increase in plant height, the number of leaves accumulating into plant wet biomass.

The role of gibberellins is not only in the vegetative growth of plants, gibberellins influence the generative period. Gibberellin is a hormone that can stimulate flowering and fruit development (Taiz and Zeiger, 2002). Applying the gibberellin hormone in pod formation begins with inducing flowers, and gibberellin stimulates the expression of flower-forming meristem genes by producing proteins that can induce the expression of flower organ formation genes (Husnul, 2013). Gibberellin hormones increase mitotic activity in pod formation, so pods are stimulated to elongate cells and develop with greater seed capacity. Soybean plants usually appear as flowers at 45 DAP and maximum generative until ready to harvest at 90 DAP (Noviani and Rahayu, 2022). However, in this study, it was found that flowers appeared before the maximum vegetative period ended. Namely, 13 DAP and pods had formed before 45 DAP. Based on the results of this study, the increase in the number of pods and pod biomass is likely due to good growth during the vegetative process. Better growth causes the first flowers to appear faster, and many flowers do not fall, so the number of pods formed increases.

Furthermore, based on the research results (Figure 1 and Figure 2), plant height, root length, and flowering time showed no effect. The plant height and length are growth parameters that have no effect because the plant quickly reaches the maximum vegetative period and begins to enter the generative period. The maximum vegetative period is during the highest growth and cell division. The plant is in its most active period of growth in this phase. This phase is characterized by the highest growth in each part of the stem, leaves, and roots so that the plant can increase fruit development due to optimal growth time. Plants that have entered the generative period will decrease vegetative growth (Aji and Supijatno, 2015).

The flowering time parameter does not have a significant effect because the varieties of flowering time are relatively the same and not much different between each treatment. In this research, the role of gibberellin after inducing flowering for soybean plants is that it helps pod formation in cell division, where gibberellin stimulates cell division and differentiation in flower tissue, which makes pods quickly formed. Gibberellin hormone increases mitotic activity by increasing cell division enzymes such as proteases in pod formation so that pods are stimulated to elongate cells and develop with greater seed capacity. Based on this process, the role of gibberellin is diverted towards pod formation, and the effect of flowers on soybean plants is absent. This result is in line with the research of Arnanto et al. (2024), which states that the gibberellin hormone does not significantly affect flowering age.

Parameters of P nutrient content of soybean leaves showed no significant effect when treated with the concentration of water hyacinth root extract and the concentration of liquid smoke as a source of gibberellin in saline soil enriched with *P. fluorescens*. The results show that the value of P nutrient levels is similar. These happen when plants absorb nutrients from the soil. Plants absorb nutrients to carry out photosynthesis, and photosynthesis is carried out in the leaves of plants. In this research, the high value of the number of leaves of soybean plants is not only due to the influence of gibberellins but also because there are phosphate-solubilizing bacteria in the planting media that increase the absorption of nutrients from soil affected by salinity stress. The application of phosphate-solubilizing bacteria is made for all treatments with the same dose of 50 ml. With saline soils that often retains P nutrients, the role of P nutrients in soybean plants is reduced, and nutrient deficiencies can occur, which causes plants to experience slow growth. The role of phosphate solubilizing bacteria in saline soils is to release the P nutrients that bind to cations that make nutrients available to plants.

Therefore, by giving phosphate solubilizing bacteria, plants can absorb phosphorus so that the production process of secondary metabolites for plants is not inhibited and plants have sufficient phosphorus nutrient levels. According to Adisarwanto (2013), phosphorus nutrient levels of 0.28% are included in phosphorus nutrient levels sufficient for soybean plant growth. The results of phosphorus levels in this study are in line with these statements, where the value of nutrient levels that have a relative value is not much different from the others, which is around 0.26-0.28% so that it is included in the P nutrient levels that are sufficient for the growth of soybean plants.

Based on the study's results, the treatment of the concentration of water hyacinth root extract and the concentration of liquid smoke increased the growth of soybean plants in saline soil enriched with *P. fluorescens.* The concentration of 1000 ppm water hyacinth root extract and 1% the concentration of liquid smoke gave the highest results on the number of leaves, plant wet biomass, number of pods, and pod wet biomass. However, it cannot be denied that the concentration of 1000 ppm water hyacinth root extract and 2% the concentration of liquid smoke were also able to provide the best growth because, according to the results of data analysis, the two treatments did not show significant differences.

CONCLUSION

Based on the research, it can be concluded that the treatment of the concentration of water hyacinth root extract significantly affects the number of leaves, root length, plant wet biomass, P nutrient content, flowering time, number of pods, and pod wet biomass. The concentration of liquid smoke affects the number of leaves, plant wet biomass, number of pods and pod biomass. The interaction between the concentration of water hyacinth root extract and the concentration of liquid smoke has a significant effect on the parameters of the number of leaves by 14.67 strands, plant wet biomass 15.33 grams, the number of pods by 13.00 grams and pod biomass by 8.00 grams. The treatment that showed the best results was the concentration of water hyacinth root extract 1000 ppm and the concentration of liquid smoke 1%. However, the concentration of water hyacinth root extract 1000 and the concentration of liquid smoke 2% are also recommended because the results were not significantly different from the previous best results.

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CONFLICT OF INTEREST

There is no conflict of interest.

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