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Response of Growth and Yield of Mint (*Mentha spicata*) Cuttings to Auxin and Composition of Planting Media

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ABSTRACT

Consumption of herbal medicines by Indonesian people is increasing because of the price and ease of obtaining them. One of the main ingredients of herbal medicine is mint leaves because they contain tannin and flavonoid secondary metabolites. However, the generative mint plant is very slow and needs vegetative cuttings propagation. This study aims to examine the effect of auxin-type growth regulators and the composition of the growing media on the growth and yield of mint plant cuttings. This study used a complete randomized block design with two factors. The first factor was the auxin growth regulator with four levels: without growth regulator, IBA 500 ppm, IAA 500 ppm, NAA 500 ppm. The second factor was the composition of the planting medium with three levels, namely soil, husk charcoal, and goat manure 3:3:1; 3:2:2, and 3:1:3. Three repetitions were carried out. Auxin affects the number and area of leaves. Applying 500 ppm NAA encouraged the highest leaf growth, with the number of leaves at 163.38. Growth regulator NAA 500 ppm increased the number of leaves and leaf area of mint plants, i.e., by 0.01% and 1.16% compared to the control. The growing media composition affected the crown fresh weight, root biomass, and crown root ratio. The media composition of 3:3:1 produced a crown fresh weight of 41.07 g, and the media composition of 3:3:3 produced the highest biomass of 5.97 g. The media composition 3:1:3 showed the highest root crown ratio, 2.69%. Auxin and the composition of the growing media synergistically promote the growth and yield of mint cuttings.

Keywords: Benzyl Amino Purine; Crown root ratio; Indole Acetic Acid; Naphthalene Acetic Acid

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INTRODUCTION

COVID-19 is a health disaster that has hit Indonesia and the world (Anyanwu et al. 2022; Caren et al. 2022). This health disaster increased the awareness of the Indonesian people about health (Fauk et al. 2022), thus increasing interest in herbal medicine (Liu et al. 2023). Indonesia has millions of medicinal plants that can potentially be developed to add value to the herbal medicine industry (Silva et al. 2023). One of the plant ingredients that can develop as herbal medicine is mint. Mint plant leaves contain tannins and flavonoids, which have the potential to facilitate digestion (Gorjian et al. 2022). Mint leaves also contain antioxidants such as flavonoids, phenolic acids, triterpenes, vitamin C and provitamin A, phosphorus minerals, calcium, iron, and potassium (Brahmi et al. 2022). In addition, this plant contains menthol, methionine, beta-carotene, beta-sitosterol, borneol, calcium, ethanol, eugenol, geraniol, niacin, thiamin, and carvone as the main active ingredients of spearmint oil (Mokhtarikhah et al. 2022).

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The content of mint plants encourages the need for mint plants to increase. However, all this time, mint plants have been propagated vegetatively by cuttings. Plants resulting from stem cuttings have the same characteristics as their parents, and the results of cuttings reach the maturity period faster, so they can produce generative organs more quickly (Moosavi-Nezhad et al. 2022). The success of plants in propagation by cuttings is significantly influenced by nutrition (Li et al. 2023).

One of the intensification efforts that can be made to increase the growth and yield of mint plant cuttings is by administering growth regulators and the composition of the planting medium. The growth of cuttings can be stimulated by providing nutrients through the soil or directly to the plants. Growth regulators are used to encourage the formation of roots on cuttings such as auxin (Deswiniyanti et al. 2020). There are various auxin growth regulators, namely IAA, IBA, and NAA (Shekhawat et al. 2021). The application of auxin hormone can be applied singly or in combination. Indole Acetic Acid (IAA) is a natural auxin in plants synthesized from tryptophan in primordial, young leaves and developing seeds. IAA plays a role in stimulating longitudinal growth, cell division, and expansion (de

Jesús Romo-Paz et al. 2021). IAA is used to encourage cell elongation and increase the ability of cells to absorb water so that it can increase the water potential of the tissue, as a result of which cells will experience elongation (Rehman et al. 2022). Indole Butyric Acid (IBA) is an auxin compound that stimulates cuttings' rooting (Sosnowski et al. 2019). IBA is more effective than natural auxin IAA or other synthetic auxins. However, the higher the concentration of IBA, the lower the ability to produce roots (Yu et al. 2019). In addition, NAA (Naphthalene Acetic Acid) has a role in stimulating root growth. NAA is stable to light, so the NAA component is more effective over a more extended period than the Indole component (Zhang et al. 2022). Auxin can play a role in cell development, thereby increasing protein synthesis (Pasternak et al. 2023). With an increase in protein synthesis, it can be used as a source of energy for growth (Perico et al. 2022).

Fulfillment of nutrients other than growth regulators, namely with the composition of the planting media, because plants propagated by cuttings do not yet have strong roots, so a media composition that has high porosity is needed. The porosity of the planting medium greatly affects the success of the cuttings. The planting medium must provide sufficient water and nutrients for plant growth (Luo et al. 2022). The planting medium affects the growth of plant stem cuttings (Boeckx et al. 2020). The best planting medium is sand + husk charcoal + manure with a ratio of 1:1:1. Manure in a combination of growing media can further enhance the growth of cuttings. Goat manure and husk charcoal planting media are suitable for planting media because they contain nitrogen (N), potassium (K), phosphorus (P), magnesium (Mg), and calcium (Ca) needed by plants and soil fertility (Sodiq et al. 2019). This study examines the role of auxin-type growth regulators and the composition of the growing media on the growth and yield of mint plant cuttings.

MATERIALS AND METHODS

The research was conducted in a screen house located in Puntukrejo Village, Ngargoyoso District, Karanganyar Regency, with an altitude of 900 meters above sea level and a geographical location of 07° 37′ 15″ South Latitude and 111° 06′ 31″ East Longitude with average rainfall in Ngargoyoso District of 407 mm per month. The temperature at the research location, in the morning, the highest temperature is 29.5˚C, and the lowest temperature is 24˚C; during the day, the highest temperature is 32.5˚C, and the lowest temperature is 25˚C, and in the afternoon, the highest temperature is 26˚C, and the lowest temperature is 25˚C. The study used a Complete Randomized Block Design with two factors. The first factor was the growth regulator type with four levels: without growth regulator, IBA 500 ppm, IAA 500 ppm, NAA 500 ppm. The second factor consisted of the composition of the soil planting medium, husk charcoal, and goat manure at three levels with the ratios: 3:3:1, 3:2:2, and 3:1:3 so that 12 combinations of treatments were obtained, which were repeated three times. The data obtained were analyzed by analysis of variance. If there is a significant difference, proceed with the 5% Multiple Range Test (Duncan's Multiple Range Test). The materials used in this study were mint plant seeds, growth regulators (IBA, IAA, and NAA), 1N NaOH and distilled water as materials for dissolving ZPT, planting media (soil, husk charcoal, and goat manure), and water. The tools used in this study were polybags measuring 20 cm x 20 cm, measuring cups or measuring pipettes, ruler or tape measure, digital scales, LAM (Leaf Area Meter), thermohygrometer, lux meter, safe envelope, knife, label paper, gloves, tub or bucket, knife, pen or pencil, and documentation tools.

Planting was carried out using stem cuttings of mint plants that were 7 HST old, and roots had appeared and were transplanted into polybags filled with prepared planting media. It was harvested when the mint plants were 10 WAP. The observed variables included the number of shoots, number of leaves, leaf area, shoot fresh weight, root biomass, and crown root ratio. Observation of leaf area was carried out by calculating the leaf area of each leaf using the LAM (Leaf Area Meter) at 10 WAP. Canopy fresh weight was weighed during harvest using a digital scale with an accuracy of up to 0.01 grams. Root biomass was carried out after the roots were baked in the oven for 1 x 24 hours at a temperature of 65oC, which was then weighed with a digital scale with an accuracy of up to 0.01 gram. Research data were analyzed using analysis of variance with a level of 5%. If it is significant, then it is continued with a significant difference test, namely the Duncan Multiple Range Test, at a level of 5%.

RESULTS AND DISCUSSION

The results showed that the auxin treatment and the composition of the planting medium did not affect the number of shoots (Table 1). The emergence of shoots is an important indicator to determine the growth and development of plants resulting from cuttings. The emergence of new nodes on the shoots or stem segments of plant cuttings marks the emergence of branch shoots on cuttings. Branches are where leaves grow on plants. Leaf production is essential in mint plant propagation, which is also influenced by the regeneration rate of explants forming side shoots and branches. One of the roles of auxin is to accelerate cell elongation (Damodaran and Strader 2019; Nardi et al. 2021). Giving exogenous auxin will increase the activity of endogenous auxins already present in plants, thereby encouraging cell division and causing shoots to appear earlier (Ahmad et al. 2023).

There was no interaction between auxin and the composition of the growing media. However, auxin application affected the number of leaves (Table 2). The 500 ppm NAA application showed the highest number of leaves, 163.38. The number of leaves in the 500 ppm NAA application significantly differed from those without plant regulators, IAA 500 ppm, and IBA 500 ppm. This is supported by the ability of NAA to be stable to light so that the NAA component is more effective over a more extended period than other indole components (Gomes and Scortecci 2021; Wang et al. 2022). The lowest number of leaves was in the application of 500 ppm IBA because the higher the concentration of IBA, the lower the ability to produce roots, which affects the growth of the number of leaves. Leaf growth has a positive correlation with cutting root growth (Suharjo and Nadja 2021).

Table 1. Effect of growth regulator on the number of shoots of mint

No Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	69.56	61.56	67.56	66.22a
IBA 500 ppm	66.44	70.22	67.33	68.00a
IAA 500 ppm	61.67	65.44	73.11	66.74a
NAA 500 ppm	67.89	66.89	72.89	69.22a
Average	66.39a	66.03a	70.22a	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (*α*=0.05), and the sign (-) indicates no interaction

Table 2. Effect of growth regulator on the number of leaves of mint

Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	170.33	146.00	165.11	160.48c
IBA 500 ppm	134.33	133.89	149.56	139.26b
IAA 500 ppm	121.11	128.11	144.67	131.30a
NAA 500 ppm	159.22	166.26	164.67	163.38d
Average	146.25a	143.57a	156.00a	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (α=0.05), and the sign (-) indicates no interaction

The results showed that there was no interaction between auxin and the composition of the growing media. However, auxin application affected the leaf area (Table 3). Treating NAA 500 ppm resulted in a leaf area of 4950.96 mm2. The leaf area in the 500 ppm NAA treatment significantly differed from the auxin-free treatment, 500 ppm IBA, and 500 ppm IAA. This is supported by the role of NAA in increasing cell division, cell elongation, and membrane permeability permeability (Wang et al. 2021; Jha et al. 2022). In addition, the application of NAA can increase photosynthetic pigments such as chlorophyll fluorescence (51.80%), total chlorophyll content (30.92%), and carotenoids (47.53%) (Rehman et al. 2022). The leaf area is closely related to photosynthetic pigments and light received by plants and leaf chlorophyll content (Xing et al. 2020). A decrease in photosynthetic pigments can reduce the rate of photosynthesis so that it can produce a smaller leaf area (Tan et al. 2020).

The combination of auxin treatment and the composition of the growing media did not affect shoot fresh weight. However, the planting medium's composition affected the shoot's fresh weight (Table 4). The soil composition, rice husk charcoal, and manure (3:3:1) showed the highest shoot fresh weight of 41.07g and was significantly different from the other treatments. The composition of soil planting medium, husk charcoal, and goat manure (3:1:3) showed the lowest crown fresh weight, namely 30.44g. Growing media is fundamental to providing plants with water, nutrients, and support. These results indicate that the higher the husk charcoal ratio encourages the growth of the mint plant canopy. Chaff charcoal acts as a soil enhancer that can affect N mineralization during decomposition, reducing nitrogen-N's leaching from the soil (Uchida et al. 2019). The planting medium's composition directly affects plant performance (Sardar et al. 2022). The ideal plant-growing medium can sustain plant life, requires little input, and is not easily damaged (Graceson et al. 2013). Chaff charcoal is light in weight but has less water-holding capacity (Singh et al. 2022). In contrast, clay, sand, and topsoil have good physical attributes for plant growth but are heavy, especially in water-saturated conditions, and are prone to waterlogging (Sodiq et al. 2019).

The results showed no interaction between auxin and the composition of the growing media on root biomass. However, the composition of the planting medium affects root biomass (Table 5). The soil composition, rice husk charcoal, and manure 3:2:2 showed the highest root biomass, 5.97 g. While the composition of the media 3:1:3 showed the lowest root biomass, namely 3.43 g. These results indicate that a balanced ratio between husk charcoal and manure can encourage optimal root growth. Root biomass reflects nutritional status because plant dry weight depends on photosynthesis and respiration (Kapczyńska et al. 2020). Media is an essential substrate for plant cultivation because it provides physical support, plant development, moisture, and sufficient plant aeration (Small and Degenhardt 2018). Manure is a rich nutrient source, resulting in increased plant growth (Hussain et al. 2022). Adding components such as manure and husk charcoal can increase the water-holding capacity (Vives-Peris et al. 2020).

The crown-root ratio is the percentage ratio between the crown's dry weight and the roots' dry weight. Comparison of crown root ratio to measure root development of seedlings treated with planting media compared to fresh weight and dry weight from the base of the root to the lowest source. According to Budiastuti et al. (2021), the crown root ratio is a characteristic that can be used as an indication of a lack or excess of water in plants. Excess water inhibits root growth more than shoot growth (Harahap et al. 2020). The results showed no interaction between auxin and the composition of the planting medium on the ratio of crown roots. However, the planting medium's composition affects the root crown ratio (Table 6). The soil composition, rice husk charcoal, and manure 3:1:3 showed the highest root crown ratio, 2.02%. These results indicate that higher goat manure can support the development of plant roots because it can increase microbial activity (Ren et al. 2022). In addition, goat manure contains high potassium for root growth (Adewoyin and Arimoro 2023). Nutrients absorbed by the roots are needed in the process of photosynthesis, the results of which can be used to stimulate plant growth, especially plant height (Iftikhar et al. 2020; Ma et al. 2021).

Table 3. Effect of growth regulator on the leaf Area (mm^2)

Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	4117.89	3776.13	3862.08	3918.70a
IBA 500 ppm	5132.56	4654.30	4758.48	4848.45c
IAA 500 ppm	4096.12	4319.23	4427.70	4281.02b
NAA 500 ppm	5348.11	4689.47	4815.31	4950.96d
Average	4673.67a	4359.78a	4465.89a	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (α=0.05), and the sign (-) indicates no interaction

Table 4. Effect of growth regulator on the shoot fresh weight (g)

Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	37.69	34.48	23.51	31.89a
IBA 500 ppm	40.52	38.48	30.32	36.44a
IAA 500 ppm	44.62	32.11	33.44	36.72a
NAA 500 ppm	41.46	34.22	34.48	36.72a
Average	41.07c	34.82b	30.44a	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (*α*=0.05), and the sign (-) indicates no interaction

Table 5. Effect of growth regulator on the root biomass (g)

Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	6.92	5.58	2.58	5.03a
IBA 500 ppm	4.92	6.66	3.68	5.09a
IAA 500 ppm	5.02	6.16	3.54	4.91a
NAA 500 ppm	3.59	5.46	3.89	4.31a
Average	5.12b	5.97b	3.42a	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (*α*=0.05), and the sign (-) indicates no interaction

Table 6. Effect of growth regulator on the crown root ratio (%)

Growth Regulator	Planting Media Composition			Average
	3:3:1	3:2:2	3:1:3	
No Growth Regulator	1.97	1.67	3.45	2.37a
IBA 500 ppm	1.82	1.43	2.45	1.90a
IAA 500 ppm	2.11	1.29	2.05	1.82a
NAA 500 ppm	2.19	1.52	2.81	2.17a
Average	2.02b	1.48a	2.69c	(-)

Note: Numbers followed by the same letters in the same column and row are not significantly different based on the results of the DMRT Test (α=0.05), and the sign (-) indicates no interaction

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Growth regulator NAA 500 ppm increased the number of leaves and leaf area of mint plants ie, 0.01% and 1.16% compared to the control. The growing media composition affected the crown fresh weight, root biomass, and crown root ratio. The media composition of 3:3:1 produced a crown fresh weight of 41.07 g, and the media composition of 3:3:3 produced the highest biomass of 5.97 g. The media composition of 3:1:3 showed the highest root crown ratio, 2.69. Auxin and the growing medium's composition synergistically promote mint plants' growth and yield.

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