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Growth Responses of Peppermint Plant (*Mentha arvensis* L.) to Several Sources of Natural Plant Growth Regulators

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ABSTRACT

Indonesia, endowed with diverse medicinal plants, is exploring the commercial cultivation of peppermint plants (*Mentha arvensis* L.) to meet increasing demand for peppermint oil. This study investigated the effects of various natural plant growth regulators (PGRs) on peppermint plant growth and yield. Conducted in Bengkulu City from December 2020 to March 2021, the study used a Completely Randomized Design with 9 treatments of natural plant growth regulators and 3 replications. Results indicated that natural PGRs, including extracts from mung bean sprout filtrate, corn, shallots, and young coconut water, influenced leaf and tiller growth but showed no significant effects on plant height, number of branch shoots, stem segments, stolon, roots, leaf size, or essential oil aroma concentration. The combination of mung bean sprout filtrate and shallot filtrate yielded the greatest number of leaves. The findings suggest that while natural PGRs can enhance specific growth parameters, their overall impact on peppermint plant development may be limited by optimal soil conditions and nutrient availability.

INTRODUCTION

Indonesia boasts rich natural resources, including diverse medicinal plants with valuable active compounds utilized in scientific research, health, medicine, and culinary practices. Among these, the peppermint plant, originating from subtropical regions and commonly found in countries like Japan, Brazil, China, and Argentina, stands out. Peppermint plant yields essential oil, known as peppermint oil, widely employed in pharmaceuticals, food, and beverages (Zhao et al., 2022). This essential oil serves various purposes such as aroma enhancement, flavouring, and medicinal applications for coughs, colds, and headaches (Pangestu and Tyasmoro, 2019).

Peppermint oil imports to Indonesia during 2023 was 623.7 tons, increasing 47% from those during 2022, highlighting the growing demand (WITS, 2023). To meet this demand and potentially bolster the economy through exports, there's a pressing need for widespread cultivation of peppermint plants. However, commercial cultivation remains limited in Indonesia, necessitating the development of optimal cultivation methods to enhance both the quantity and quality of peppermint plant yields.

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Plants (2019), it was emphasized that Indonesia's medicinal plants, including peppermint plant, hold promising opportunities in the export market, indicating their potential to contribute significantly to foreign exchange earnings and job creation. Hence, intensive and sustainable development of peppermint plant cultivation is crucial to capitalize on expanding market opportunities.

Peppermint plant propagation predominantly occurs vegetatively due to its prolonged flowering and seed-setting process in Indonesia (Hadipoentyanti et al., 2009). Utilizing cuttings offers advantages such as rapid seed production and uniform harvests when essential oil production peaks. Various approaches have been explored to accelerate growth and enhance peppermint plant quantity, including the application of growth regulators (PGRs) derived from synthetic or natural sources (Kusuma et al., 2016).

Natural sources are gaining their popularity for use as natural plant growth regulators as they are available in large amount and hence being feasible for use (Adam et al. 2024; Namratha et al., 2024). Corn seed extract and mung bean sprout extract; contain hormones like auxin, gibberellin, and cytokinin, which have been shown to stimulate growth in different plant species (Latunra et al., 2016; Ulfa, 2014). Similarly, young coconut water, rich in cytokinin and auxin hormones, has demonstrated significant effects on plant height and growth acceleration in various plants (Bey et al., 2006; Fardha, 2024; Tiwery, 2010). These natural growth regulators offer promising avenues for enhancing peppermint plant cultivation in Indonesia, potentially leading to increased yields and economic benefits. The objective of this research was determining the optimum concentration of a natural growth regulator solutions for the growth of peppermint plants.

MATERIALS AND METHODS

This research was conducted in a plastic house from December 2020 to March 2021 at Muara Bangkahulu District, Bengkulu City at an altitude of ±19 meters above sea level. The average daily sunlight intensity ranged from 49.16% to 68.68%, with average temperatures between 26.33°C and 27.05°C, and humidity levels ranging from 82.18% to 85.13%. The land's topography was flat, open, and close to water source.

The materials used in this research includes: peppermint shoot cuttings 5-10 cm long (5 internodes, 4 nodes) from the mother plant propagated at The Indonesian Spices and Medicinal Crops Research Institute (Balittro), carbofuran, polybags 12 cm x 10 cm and 30 cm x 30 cm, 50% shading paranett, polyethylene plastic cover, soil, cow manure, and inorganic fertilizers (Urea, SP-36, and KCl). Natural plant growth regulator (PGRs) included shallots, young corn, mung bean sprouts and young coconut water.

This research used a completely randomized design (CRD) with one factor and three replications, consisting of 9 treatments, namely distillate water (DW, control), mung bean sprout filtrate (MBSF), corn filtrate (CF), shallot filtrate (SF), young coconut water (YCW), MBSF+CF, MBSF+SF, CF+SF, and MBSF+ CF+SF. Each treatment was repeated 3 times, so there were 27 experimental units. Each experimental unit consisted of 4 plant samples.

The research field was cleaned and freed from weeds. The polyethylene plastic roof was installed to protect plants from direct rainwater and to prevent volatilization of PGR during application.

Initial soil analysis was carried out by taking soil samples at a depth of 20 cm from 5 points to represent all soil conditions. At each point, 0.5 kg of soil was sampled, mixed in a bucket and stirred evenly. Subsequently, the soil mix was analysed in Soil Science Laboratory, Faculty of Agriculture, Bengkulu University. The results of the initial soil analysis were C-organic content 2.92% (medium), N 0.31% (medium), P 4.55 ppm (medium), K 0.29 me/ 100 (mow) and pH 4.30 (very acidic).

The planting material used was stem cuttings. Healthy and young peppermint stems were cut for ± 10 cm long (5 internodes or 4 nodes) at the angle of 45%. Leaves were removed from the stem to reduce evaporation, leaving two young top leaves. The propagation media consisted of a mixture of top soil and manure with a ratio of 2:1 (v/v), sieved using 0.5 cm x 0.5cm mesh siever. This mixture was filled into polybags (size 12 cm x 10 cm) to ¾ parts, then left for 4-5 days. Carbofuran at a dose of 0.12 $g.polybag^{-1}$ was added into the planting hole to prevent pest attacks on the roots. The cuttings were planted to a depth of 2 -3 cm in an upright position. The polybags were put under area shaded with a paranet with a density of 50%. The polybags with cuttings were then covered with polyethylene plastic tunnel measuring 1 m wide, 0.5 m high, to maintain humidity. Watering was done once a day in the morning, and the plastic tunnel was opened after one week (Hadipoentyanti, 2012).

Planting medium was in the form of top-soil or top layer of soil at a depth of 10 – 30 cm then dry the soil for 3 – 4 days. The soil was then smoothed using a sieve with a 0.5 cm sieve and put into 30 cm x 30 cm polybags which then arranged in a prepared place and incubated for 4 - 5 days.

Cuttings of 4 weeks old were planted in polybags containing planting media in the evening to reduce evaporation. The media were watered until they were saturated so that the planting medium was moist. A hole with diameter of 10 - 15 cm was made in the media. The peppermint cuttings were planted along with the soil by tearing the nursery polybag so that it did not disturb the plant roots.

Watering the plants was done to the planting medium very day in the afternoon. Fertilization was given as basic fertilizer in the form of organic fertilizer of composted cow dung 125.6 $g.polybag^{-1}$ and NPK fertilizer 6.28 $g.polybag^{-1}$. Pest was controlled by regular maintenance. To mitigate pest attacks by grasshoppers and leaf-sucking insects, chemical control with Deltamethrin 25 effectively used for managing these pests.

Various natural growth regulator sources were added into distilled water at the same weights and crushed with a mixer for 2 minutes. Their liquid was separated from the mixture by using a Whatman paper overnight. The liquid extracts were combined according to the treatment, diluted with distilled water with a concentration of 25% v/v, with a volume of 100 ml per treatment and applied by pouring it onto the root area of the plant. The treatment solutions were applied at weekly interval until harvest.

Harvesting was carried out when 50-75% of the plants are flowering (late vegetative), because during this period the oil and peppermint levels reach their peak. Terna were harvested by cutting shoot parts of the plant using cutting scissors ± 20 cm from the ground surface (Hadipoentyanti, 2012).

Plant growth were measured in term of plant height, branch shoot length, number of branch shoots, number of tillers, number of leaves, leaf length, leaf width, number of stem segments, leaf age, number of roots, and number of stolons. In addition, essential oil content; level of aroma, and concentration of essential oils were also assessed.

The data obtained were analysed using ANOVA at $\alpha = 5\%$. Main comparisons were performed by DMRT at $\alpha = 5\%$ level.

RESULTS AND DISCUSSION

Significant effects of natural plant growth regulators were highlighted on leaf and seedling growth at specific stages (Table 1), indicating the potential of some sources for enhancing peppermint plant growth and yield.

The combination of MBSF and SF resulted in the highest number of leaves at 46 days after planting (DAP), with 36.75 leaves on peppermint plants (Table 1). This result was statistically comparable to treatments involving DW, SF, YCW, and their combination with CF. Conversely, the lowest leaf count was observed in plants treated solely with MBSF, recording 25.5 leaves at 46 DAP. Mung bean sprouts contain cytokinin, while shallot contain auxin, both of which stimulate cell division and differentiation pathways, influencing leaf growth. Additionally, adequate levels of nutrients such as nitrogen (N), phosphorus (P), and potassium (K) in the soil are crucial for leaf formation, aiding in cell division and enlargement. The initial soil analysis indicated medium levels of N (0.31%) and P (4.55 ppm) contributing to optimal leaf development.

Table 1. Effect of natural plant growth regulators on the number of leaves of peppermint and number of stolon

Treatment	Number of leaves at 46 DAP	Number of stolon at 84 DAP
DW	31.67 ab	6.00 a
MBSF	25.50 c	5.67 a
CF	27.67 bc	3.17 b
SF	30.58 ab	6.83 a
YCW	35.17 ab	7.17 a
MBSF + CF	29.92 bc	7.00 a
MBSF + SF	36.75 a	6.67 a
CF + SF	26.33 c	5.42 a
MBSF + CF + SF	32.42 ab	5.58 a

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water. Numbers followed by the same letter in the same column are not significant difference according to DMRT at α=5%.

The application of YCW resulted in the highest number of branches of peppermint plants as compared to those of other treatments (Table 1). This outcome was statistically similar to treatments involving DW, MBSF, SF, and various combinations thereof, but significantly differed from CF. Conversely, the lowest number of stolon was observed in plants treated with CF, with a mean value of 3.17, significantly different from all other treatments. Young coconut water contains cytokinins, which stimulate shoot growth and enhance cell activity, aligning with findings that suggest cytokinins accelerate germination, promote shoot growth, and increase leaf number. Research also indicates the efficacy of coconut water in enhancing shoot proliferation in dendrobium orchids (Irmayanti et al., 2025).

Observations of plant height conducted weekly (Table 2) revealed that the application of natural PGR had no significant effect. Plant height growth was influenced by the presence of compounds such as auxins, gibberellins, and cytokinins, which actively participate in apical meristem formation and stem elongation. However, the lack of significant effect in this study may be attributed to the consistent content of these compounds across all plant samples.

The initial soil analysis indicated an Organic C content was 2.9%, which was within the optimal range of 2% to 3% for peppermint plant growth. This abundance of organic matter serves as a crucial carbon source, supporting microbial activity and nutrient mineralization in the soil. As a result, the application of natural PGR may not exhibit a significant effect, as the soil's organic content already meets the plant's requirements. Additionally, the provision of basic fertilizers such as cow manure, SP-36, KCl, and Urea at recommended dosages ensures the peppermint plants receive sufficient nutrients for optimal growth, further diminishing the discernible impact of natural application.

Weekly observations of the increase in the number of branch shoots (Table 3) revealed no significant effect from the application of natural PGR. This lack of impact may be due to the interaction between cytokinin and auxin compounds, where auxin content predominates, and influencing shoot growth. Cytokinins, discovered for their role in stimulating plant cell division alongside auxin, often face antagonistic interactions when auxin levels exceed cytokinin levels in plants, potentially

Table 2. Effect of PGR on plant height of peppermint at 1 to 6 weeks after planting

Treatment	Plant height (cm) at different weeks after planting					
	1	2	3	4	5	6
DW	2.29	6.05	8.18	10.34	11.74	13.29
MBSF	2.90	6.99	9.64	11.29	13.12	14.39
CF	2.74	6.47	6.74	11.22	13.27	14.84
SF	2.45	6.29	8.97	11.63	13.93	15.57
YCW	2.64	6.62	8.70	10.68	12.29	13.42
MBSF + CF	2.09	5.48	7.88	9.64	11.68	12.77
MBSF + SF	2.12	5.87	8.29	10.57	12.23	13.40
CF + SF	2.23	6.52	9.16	11.27	13.32	14.20
MBSF + CF + SF	2.08	6.19	8.48	10.68	12.43	13.39

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

Table 3. Effect of PGR on weekly increased number of peppermint's branch shoot from 1 to 5 weeks after planting

Treatment	Week				
	1	2	3	4	5
DW	5.6	3.3	4.2	25.4	5.6
MBSF	5.8	3.2	4.4	27.7	6.0
CF	5.8	3.4	4.2	23.8	5.4
SF	4.6	3.5	4.1	23.9	5.4
YCW	4.7	3.5	4.2	25.1	5.5
MBSF+CF	4.9	2.9	4.0	23.3	5.6
MBSF+SF	5.2	3.0	4.4	30.1	6.4
CF+SF	5.1	3.4	4.3	24.8	5.7
MBSF+CF+SF	4.9	3.3	2.7	20.9	5.1

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

affecting shoot development (Taiz and Zeiger, 2010; Makmur, 2019). Furthermore, the sufficient C-Organic content in the soil (2.9%), within the optimal range for peppermint plants, and the provision of recommended basic fertilizers like cow manure and SP-36, might contribute to the absence of a significant effect. These factors support microbial activity and nutrient mineralization in the soil, ensuring the availability of essential elements for plant growth (Pirngadi, 2009). Therefore, the influence of natural PGR on peppermint plant growth may not be clearly discernible due to the suitable soil conditions and nutrient supply.

Based on observations of shoot branch length every week (Table 4), the provision of natural PGR did not show a significant effect. The gibberellin content in plants is an important factor in the elongation of branch shoots. According to Taiz and Zeiger (2010), one of the most striking effects of biologically active gibberellin is its ability to promote cell elongation. The absence of an effect may be due to the equality of gibberellin content in each plant sample.

The application of natural PGR did not have a significant effect on leaf length and leaf width (Table 5). The content of auxin and cytokinin compounds in plants is a factor that plays an active role in leaf growth. Auxin functions to increase osmotic pressure, cell permeability, develop cell walls, and stimulate cell elongation and enlargement. Meanwhile, cytokinins function in cell division, and the combination of auxin and cytokinin will stimulate cell division and influence the differentiation pathway (Taiz and Zeiger, 2010). According to Hurtado and Cajacuri (2024), the elements N, P, and K in the planting medium also help the process of cell division and enlargement, which makes young leaves reach their perfect shape more quickly. The lack of effect may be due to the equal content of auxin and cytokinin in each plant sample, as well as the inadequate need for N, P and K nutrients for growth in length and width of leaves.

Organic matter is an important factor as a carbon source that supports the activity of various types of microbes in the soil, so that the process of mineralizing nutrients into elements available to plants can run optimally (Pirngadi, 2009). Initial soil analysis data shows an organic C content of 2.9%, which is included in the criteria for being very suitable for the growth of peppermint plants. The availability of very suitable organic C, coupled with the provision of basic fertilizer in the form of cow dung manure, SP-36, KCl, and

Table 4. Effect of PGR on the increasing shoot branch length at 1 to 6 weeks after planting.

Treatment	Increase shoot branch length (cm)					
	1	2	3	4	5	6
DW	0.93	1.46	1.98	2.19	2.40	2.58
MBSF	0.82	1.64	2.13	2.38	2.52	2.65
CF	0.83	1.67	2.11	2.39	2.62	2.77
SF	0.97	2.15	2.19	2.36	2.48	2.63
YCW	0.96	1.53	2.18	2.36	2.72	2.84
MBSF + CF	0.74	1.47	2.29	2.45	2.25	2.40
MBSF + SF	0.92	1.98	2.13	2.25	2.27	2.43
CF + SF	0.87	1.57	2.01	2.30	2.47	2.62
MBSF + CF + SF	0.92	1.48	2.19	2.45	2.50	2.65

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

Table 5. Effect of natural PGR on increase in leaf length and leaf width of peppermint plant at 2 to 6 weeks after planting.

Treatment	Increase in leaf length and width					
	Leaf length (cm)			Leaf width (cm)		
	2	4	6	2	4	6
DW	-0.73	-0.78	-1.20	0.60	0.60	-0.32
MBSF	-0.85	-0.89	-1.32	0.60	0.60	-0.34
CF	-0.68	-0.70	-1.17	0.59	0.62	-0.32
SF	-0.64	-0.68	-1.29	0.60	0.64	-0.27
YCW	-0.65	-0.64	-1.10	0.60	0.64	-0.28
MBSF+ CF	-0.69	-0.73	-1.06	0.62	0.61	-0.29
MBSF + SF	-0.64	-0.65	-1.27	0.58	0.58	-0.37
CF + SF	-0.74	-0.74	-1.16	0.62	0.62	-0.28
MBSF + CF+ SF	-0.80	-0.87	-1.26	0.61	0.62	-0.27

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

Urea according to the recommended dosage, has sufficient nutrient requirements for optimal growth of peppermint plants. Therefore, with sufficient nutrient requirements for optimal growth of peppermint plants, the effect of providing natural PGRs is not really visible on the growth of peppermint plants.

Observation of the number of stem segments (Table 6) showed that natural PGR application does not have a significant effect. The gibberellin content in plants is an important factor in the growth of stem segments. According to Taiz and Zeiger (2010), one of the most striking effects of biologically active gibberellin is its ability to promote cell elongation, especially in the induction of internode elongation. This finding indicated that the equal content of gibberellin compounds in each plant sample causes no influence on the number of plant internodes.

Observation of the number of stolons (Table 6) also showed that giving natural PGRs did not have a significant effect. The auxin and cytokinin content in plants plays an active role in stolon formation. The interaction between cytokinin and auxin compounds is a factor that can influence stolon growth. Apical dominance is an antagonistic interaction between auxin and cytokinin that occurs due to excessive auxin activity at the top of the stem or branch shoots, so that the side shoots remain in a dormant condition (Makmur, 2019). The lack of effect is thought to be caused by this antagonistic interaction, where the auxin content is greater than the cytokinin content in each plant, thus affecting stolon growth.

Organic matter is an important factor as a carbon source that supports the activity of various types of microbes in the soil, so that the process of mineralizing nutrients into elements available to plants can run optimally (Pirngadi, 2009). Initial soil analysis data shows an organic C content of 2.9%, which is included in the criteria for being very suitable for the growth of peppermint plants. The availability of carbon, coupled with the provision of basic fertilizer in the form of cow dung manure, SP-36, KCl, and Urea according to the recommended dosage, has sufficient nutrient requirements for optimal growth of peppermint plants. Therefore, with sufficient nutrient requirements for optimal growth of peppermint plants, the effect of providing natural PGRs was not shown on the growth of peppermint plants.

Table 6. Effect of natural Plant Growth Regulator on number of stem segments, number of stolon, and number of roots of peppermint

Treatment	Number of stem internodes	Number of stolon	Number of roots
DW	180.25	3.42	77.00
MBSF	209.50	3.83	82.83
CF	190.50	3.58	72.50
SF	187.08	4.42	85.50
YCW	178.58	4.08	79.00
MBSF + CF	185.42	4.83	82.17
MBSF+ SF	229.17	4.08	79.67
CF + SF	193.33	4.00	78.17
MBSF+CF +SF	171.67	4.33	77.17

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

This research also revealed that natural PGR application did not have a significant effect on leaf age . The cytokinin content in plants is one of the factors playing a role in the leaf aging process. Cytokinins have been shown to influence many physiological and developmental processes, including leaf senescence (Taiz and Zeiger (2010). Therefore, it is suspected that the equal content of cytokinin compounds in each plant sample causes no influence on the lifespan of plant leaves.

In this experiment, the use of various natural plant growth regulators did not significantly affect plant growth of peppermint. This result seems not in line with those of other reports on the use of the same natural filtrates. Several reason can be proposed for these result differences. Firstly, pH of the growing medium was very low (4.3). This medium acidity is not suitable for optimal growth of peppermint plants which was reported to be not adjusted to those optimal for between 6.0 and 7.5 (Shukla et al. 1997). Although the plant can tolerate a slightly wider range, from 5 to 7, but growth may be affected outside of the preferred pH range of 6.0 to 7.5.

The Presence of Peppermint Essential Oil

Based on organoleptic test on 10 respondents, it was found that MBSF + SF gave the best assessment results with an average score of 2.7 which was included in the fragrant criteria (Table 7). This indicated that MBSF + SF treatment produced the best essential oil aroma. The essential oil aroma with the lowest score was obtained from DW treatment with an average score of 1.1 which is in the criteria of a slightly fragrant aroma. Treatments MBSF, CF, SF, YCW, MBSF + CF, CF + SF, and MBSF + CF + SF produced aromas with sufficient fragrant criteria.

Based on the results of the tester test, it can be assumed that all treatments with natural PGRs have an effect on the level of aroma concentration of the essential oil of the peppermint plant. Natural PGRs can improve the aroma quality of essential oils (fragrant criteria), with some treatments providing better results than others. The treatment of MBSF + SF produced the best aroma with an average score of 2.7 (fragrant). Distillate water has the lowest score with an average of 1.1 (slightly fragrant). Other treatments produced aromas that were quite fragrant. The use of natural PGR has a positive effect on the intensity of the aroma of the essential oil of the peppermint plant. Thus, it can be concluded that the application of natural PGRs affected the aroma quality of essential oils, and treatment with green bean sprout filtrate and SF were the most effective in improving the aroma of peppermint plant essential oils.

CONCLUSIONS

Liquid of mung bean sprout filtrate + shallot filtrate produced the greatest number of plant leaves and gave the highest assessment score on the aroma concentration of peppermint essential oil. Young coconut water provided the greatest number of stolon. Further researches should focus on evaluating several concentration of the promising natural plant growth regulators combinations.

Table 7. Assessment of the aroma of peppermint essential oil

Treatment	Respondent number										Average	Note
	1	2	3	4	5	6	7	8	9	10		
DW	2	3	1	1	1	1	0	0	1	1	1.1	Slightly fragrant
MBSF	3	2	4	3	4	3	0	0	1	3	2.3	Quite fragrant
CF	3	4	1	1	1	3	0	1	2	2	1.8	Quite fragrant
SF	2	4	2	3	1	1	1	1	1	2	1.8	Quite fragrant
YCW	1	3	2	2	2	1	2	2	2	2	1.9	Quite fragrant
MBF + CF	1	3	0	2	1	2	3	0	3	3	1.8	Quite fragrant
MBF + SF	2	4	3	3	2	2	3	3	3	2	2.7	Fragrant
Corn + SF	4	4	2	2	2	2	1	1	1	3	2.2	Quite fragrant
MBF + CF + SF	4	3	4	2	1	2	1	1	3	2	2.3	Quite fragrant

Note: DW= distillate water, MBSF= mung bean sprout filtrate, CF= corn filtrate, SF= shallot filtrate, YCW= young coconut water.

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