Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS
Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2024.290.303

Research Article
Growth and Essential Oil Production of Vetiver Plants Under Salinity Condition with Salicylic Acid and Mycorrhiza Application

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Abstract
Background and Objective: Salinity is a real challenge in agricultural crop production in many regions. Several strategies should be applied to reduce the adverse effects of salinity and increase crop tolerance. Therefore, more than one application can support the mitigation of salinity stress in vetiver plants through the application of salicylic acid and mycorrhiza. This study aims to evaluate the growth and production of vetiver under salinity conditions with the application of salicylic acid and mycorrhiza. **Materials and Methods:** This study used a factorial completely randomized design with 3 factors. The first factor is salinity consisting of 4-5 dS/m salinity and 8-9 dS/m salinity, the second factor is salicylic acid consisting of no salicylic acid, 0.5 mM salicylic acid and 1 mM salicylic acid and the third factor is mycorrhiza consisting of no mycorrhiza, *Glomus* sp. and *Gigaspora* sp., where each treatment is made 3 replications. The recorded parameters are plant height, number of leaves, leaf area, number of tillers, root length, root volume, root fresh weight and production of essential oil. All the recorded data were analyzed by using Analysis of Variance (ANOVA). **Results:** The application of salicylic acid and mycorrhiza had a significant effect on increasing all parameters of vetiver growth and production observed in this study. The results showed that the best of vetiver oil production was obtained with the application of 0.5 mM salicylic acid and *Gigaspora* sp., mycorrhiza. **Conclusion:** The use of mycorrhiza species *Gigaspora* sp., can increase the growth and production of essential oil under salinity conditions. The best essential oil production under salinity condition was obtained with the application of 0.5 mM salicylic acid and *Gigaspora* sp.

Key words: Mycorrhizae, salicylic acid, vetiver, essential oil, *Gigaspora* sp., salinity

Citation: Novita, A., L.A.M. Siregar, Rosmayati and N. Rahmawati, 2024. Growth and essential oil production of vetiver plants under salinity condition with salicylic acid and mycorrhiza application. Asian J. Plant Sci., 23: 290-303.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The vetiver plant is one of the Gramineae family plants originating from India. Vetiver is one of the plants that produce essential oil. The main content of essential oil in vetiver is β-vetivenene, khusimol, vetiselinenol, isovalencenol, vetivenicacid, α-vetivone and β-vetivone[1]. These essential oils are products of plant secondary metabolites, where secondary metabolites are produced by plants in certain amounts as a defense in stressful conditions, one of which is salinity.

Salinity is one of the factors limiting plant growth and productivity. Most cultivated plants are sensitive to salinity due to high saline content. Increased salinity in the soil causes osmotic stress, nutrient imbalance, oxidative stress, ion toxicity in the soil in addition to reducing the ability of plants to absorb water and photosynthesize so that metabolic processes are disrupted. Plant growth that decreases is caused by osmotic stress which makes it difficult for plants to absorb water and the influence of excessive Na^+ and Cl^- ions causes cell division and enlargement to be inhibited[2].

Salinity stress is known to suppress plant growth by affecting vegetative development and net assimilation rate which results in a decrease in yield productivity[3,4]. The results of research by Novita *et al.*[5] reported that there was a decrease in vetiver growth at a salinity level of 8 dS/m, where vetiver plants showed decreasing vegetative growth seen from a decrease in plant height, number of leaves specific leaf area and leaf dry weight[6]. Although vetiver grass is widespread in saline-affected areas, few studies have reported that vetiver is moderately saline tolerant[7] and the mechanisms underlying vetiver's saline tolerance remain unclear.

Some strategies that can be done to reduce the adverse effects of salinity include nutrient application[8], organic matter application[9], breeding tolerant cultivars[10] and the use of rhizobacteria[11]. However, some of the previous applications have limited effects when applied singly. Therefore, more than one application is needed to be able to support abiotic stress mitigation in salinity-sensitive crops[12]. In this study, the strategy applied was the application of nutrients in the form of salicylic acid combined with the use of mycorrhiza species.

Salicylic acid has been recognized as an important molecule to contribute to abiotic stress responses[13]. Salicylic acid plays a role in plant tolerance to various abiotic stresses including metals/metalloids[14] and salinity[15]. Salicylic acid has the effect of protecting the development of anti-stress programs and accelerating the growth normalization process after removing stress factors[16]. When biotic or abiotic stress occurs in cells, tissues, or organs, the salicylic acid signaling process is initiated. To trigger defense signals, SA must connect to several different receptors. The main SA receptors, SA Methyl Transferase 1 (SAMT1) and SA binding protein 2 (SABP2), regulate the ratio of SA to MeSA17[17].

Besides, arbuscular mycorrhiza fungi (AMF) are regarded as another effective method for bioameliorating salt stress. The AMF uses several physiological and biochemical processes that work together to provide the host plant a higher resistance to salinity. Enhanced nutrient uptake and preservation of ionic homeostasis, superior water use efficiency and osmoprotection, enhanced photosynthetic efficiency, preservation of cell ultrastructure and intensified antioxidant metabolism are a few of the well-known mechanisms[18]. Several studies have highlighted that FMA imparts salinity tolerance to host plants based on higher biomass compared to plants without mycorrhiza[19]. The higher biomass further leads to dilution of Na^+ and Cl^- and manifests as better crop yields[20].

Based on the description of the problems above, it is necessary to conduct research aimed at evaluating the growth and production of essential oil from vetiver plants grown in salinity conditions with the application of salicylic acid and mycorrhiza.

MATERIALS AND METHODS

Materials: The materials used in this study were Padang ecotype vetiver seedlings obtained from the results of phase I research by Novita *et al.*[21] as plant material, saline soil, sea water, salicylic acid, mycorrhiza *Glomus* sp., mycorrhiza *Gigaspora* sp. and other materials that support this research.

Research sites: This research was conducted in the Greenhouse, Agricultural Development Polytechnic Medan, North Sumatra, Indonesia from May, 2022 to August, 2022.

Research methods: This study used a factorial Completely Randomized Design (CRD) with 3 factors. The first factor is the best ecotype and salinity consisting of Padang ecotype at 4-5 dS/m salinity and Padang ecotype at 8-9 dS/m salinity, the second factor is salicylic acid consisting of no salicylic acid, 0.5 mM salicylic acid and 1 mM salicylic acid and the third factor is mycorrhiza consisting of no mycorrhiza, *Glomus* sp. and *Gigaspora* sp., where each treatment is made 3 replicates.

Salicylic acid application: Salicylic acid was sprayed using a spraying system in accordance with the concentration of each treatment (none, 0.5 mM and 1 mM) at one and two weeks after planting (WAP).

Mycorrhiza application: Arbuscular mycorrhiza fungi (AMF) were applied once at planting stage II of the research and placed under the roots in the rhizosphere (root zone) according to each treatment type.

Data analysis: The data were recorded on the traits i.e., plant height, number of leaves, leaf area, number of tillers, root length, root volume, root fresh weight and production of essential oil, all these parameters are observed at 8 weeks after planting (WAP).

All the recorded data were analyzed by using Analysis of Variance (ANOVA) and the means were further compared and separated with Duncan's Multiple Range Test (DMRT) at α 5%[22].

RESULTS AND DISCUSSION

Plant height: Observation data and analysis of variance showed that salinity treatment, salicylic acid application and mycorrhiza had a significant effect on plant height. The interaction between salinity treatment and salicylic acid, salinity and mycorrhiza and the interaction between salinity treatment, salicylic acid and mycorrhiza had a significant effect on plant height. The height of vetiver plants under salinity conditions with the application of salicylic acid and mycorrhiza was presented in Table 1.

Table 1 presented data on vetiver plant height, where salicylic acid treatment on vetiver plant height was highest at 1 mM salicylic acid application. The application of salicylic acid under salinity has an effect on increasing plant height, where the observation results showed that the height of vetiver plants increases as the concentration of salicylic acid given increases and the highest is obtained at a concentration of 1 mM. This means that the 1mM concentration is the optimal concentration of salicylic acid application. These results were in line with the research of Farouk and Omar[23] which stated that *Ocimum basilicum* L. plants with the application of salicylic acid concentrations of 0.75 mM and 1 mM are able to maintain growth from stress and maintain the highest growth in plant height. In line with Abdi *et al.*[24] which states that the application of salicylic acid affects the physiological and biochemical processes of plants, increases defense enzymes in counteracting free radicals, prevents membrane peroxidation so that it affects cell elongation and plant height growth.

The use of mycorrhizal type *Glomus* sp., produced the highest vetiver plants. The increase in plant height with the application of mycorrhiza under salinity stress is because mycorrhiza provides several benefits to plants, including increased absorption of nutrients and water, as well as protection against soil pathogens. According to Ilangumaran and Smith[25], under salinity stress, plants require additional energy to reduce the toxic effects of Na^+ ions and also face nutrient deficiencies, this has a negative impact on plant growth. Afrinda and Islami[26] stated that the application of mycorrhiza can increase P uptake in plants which serves to increase yield, besides being able to produce the highest plant height so as to provide the highest yield. Furthermore, Adetya *et al.*[27] stated that the use of mycorrhiza can increase the availability of nutrients, bind toxic substances and make plants resistant to stress.

The interaction of 1 mM salicylic acid treatment and *Glomus* sp., mycorrhiza species produced the highest plants at 4-5 dS/m salinity, which was significantly different from other treatment interactions. The interaction between salinity with the application of salicylic acid and mycorrhiza had an effect on increasing the growth of vetiver plants, this was seen in the increase in plant height. This finding demonstrated salicylic acid's capacity to lessen soil stress situations. Similar findings were also noted by Ali and Mahmoud[28], who found that applying salicylic acid can lessen the detrimental effects of salt stress on plant productivity, growth and physiological and morphological traits. Moreover, El-Taher *et al.*[29] claimed that salicylic acid is a plant hormone that controls a variety of physiological and biochemical processes in plants, particularly when they are stressed. According to Per *et al.*[30], salicylic acid affects various important physiological functions in plants, such as increasing nutrient absorption and decreasing Na^+ concentration under salinity stress. In addition, Dastogeer *et al.*[31] reported that mycorrhizal application increased plant height and its effect on growth was better under salinity stress conditions than under normal conditions. Shamshiri and Fattahi[32] also reported that mycorrhiza application has been shown to increase plant growth under salinity stress conditions. Furthermore, Khalloufi *et al.*[33] stated that the application of mycorrhiza increases nutrient uptake, water uptake, maintains osmotic balance and stimulates antioxidant activity to reduce the harmful effects of salinity on plant growth.

Number of leaves: Observation data and analysis of variance showed that salinity treatment, salicylic acid application and mycorrhiza significantly affected the number of leaves. The interaction between salinity treatment and salicylic acid and the interaction between salinity treatment, salicylic acid and mycorrhiza had a significant effect on the number of leaves. The interaction between salinity treatment and mycorrhiza application had a significant effect on the number of leaves. The number of vetiver leaves under salinity conditions with salicylic acid and mycorrhiza application was presented in Table 2.

Table 1: Vetiver plant height under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (cm)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp (C_1)	*Gigaspora* sp (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	150.33^{e}	153.58^{d}	155.58^{c}	144.11^{c}
8-9 dS/m (A_2)		131.42^{j}	135.33^{i}	135.92^{i}	
4-5 dS/m (A_1)	Salicylic acid 0.5 mM (B_1)	154.67^{cd}	158.75^{b}	159.33^{b}	149.10^{b}
8-9 dS/m (A_2)		137.83^{h}	144.08^{f}	139.92^{g}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	159.25^{b}	162.00^{a}	158.00^{b}	152.36^{a}
8-9 dS/m (A_2)		140.17^{g}	150.75^{e}	144.00^{f}	
Mean		145.61^{c}	150.85^{a}	149.11^{b}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 2: Number of vetiver leaves under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (helai)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	12.92^{i}	18.50^{e}	15.67^{g}	13.93^{c}
8-9 dS/m (A_2)		10.83^{j}	13.08^{i}	12.58^{i}	
4-5 dS/m (A_1)	Salicylic acid 0.5 mM (B_1)	17.33^{f}	22.50^{b}	20.67^{c}	18.75^{b}
8-9 dS/m (A_2)		14.50^{h}	19.50^{d}	18.00^{ef}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	18.58^{e}	24.67^{a}	22.83^{b}	19.82^{a}
8-9 dS/m (A_2)		15.92^{g}	18.67^{e}	18.25^{e}	
Mean		15.01^{c}	19.49^{a}	18.00^{b}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 2 presented data on the number of vetiver leaves, where salicylic acid treatment on the highest number of vetiver leaves was obtained at 1 mM salicylic acid application. The number of vetiver leaves increased with the addition of salicylic acid concentration given. This result shows a positive effect that the application of salicylic acid can increase plant growth. According to Khan *et al.*[14], leaves are an important organ in plants that function as a transpiration and photosynthesis process. The results of photosynthesis will be distributed to all plant organs for growth and development. The more the number of leaves produced, the more photosynthetic assimilates produced. So that plants will grow and develop faster. According to Leiwakabessy *et al.*[34] salicylic acid given to plants can increase the process of photosynthesis in leaves, the assimilation of carbon dioxide (CO_2) and nutrient uptake by plants.

Growth in the number of leaves is also influenced by the application of mycorrhiza where the highest number of leaves is found in *Glomus* sp. This is evidenced by the results of the study that the number of leaves of vetiver plants with mycorrhiza application is higher than without mycorrhiza. In line with Husna[35], mycorrhiza application is significant to plant growth, namely the number of leaves. According to Farida and Chozin[36], mycorrhiza application affects the number of leaves because mycorrhiza helps plants in the absorption of water and nutrients that increase biomass production, especially in the stems and leaves. Furthermore, Hartanti *et al.*[37] also explained that mycorrhiza in the soil will be symbiotic with plant roots so that it can increase the uptake of phosphorus (P) and other nutrients such as nitrogen (N) and potassium (K).

The interaction effect of salinity treatment and salicylic acid application on the number of leaves also increased in this study. The interaction of 1 mM salicylic acid treatment and mycorrhiza type *Glomus* sp. (B2C1) produced the highest number of leaves at 4-5 dS/m salinity, which was significantly different from other treatment interactions. Previous research by Bin-Jumah *et al.*[38] showed that the application of salicylic acid can reduce plant damage due to salinity stress. Salicylic acid is a hormone that regulates plant development and reduces the negative impact of salinity. According to Hasan *et al.*[39], salicylic acid application can control stomatal opening under stress, reduce transpiration and water loss, maintain turgor and control plant growth under stress conditions. In addition, there is a significant increase in plant growth in soil media previously treated with mycorrhiza, where the higher the level of mycorrhiza, the higher the increase in the number of leaves[40]. In order to improve soil fertility over time, mycorrhiza contains a variety of propagules, such as spores, hyphae and rhizomorphs, which are structures that may last in saltwater settings. It has been demonstrated that using mycorrhiza can improve nutrient absorption and preserve plant conditions under stress. Mycorrhiza is a type of fungus that grows on plant roots and aids in nutrient absorption[41].

Table 3: Number of vetiver tillers under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (anakan)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	3.75^{bc}	3.50^{c}	3.67^{bc}	3.54^{b}
8-9 dS/m (A_2)		3.00^{d}	3.83^{bc}	3.50^{c}	
4-5 dS/m (A_1)	Salicylic acid 0.5 mM (B_1)	3.67^{bc}	4.00^{b}	3.67^{bc}	3.61^{b}
8-9 dS/m (A_2)		3.42^{c}	3.50^{c}	3.42^{c}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	3.83^{bc}	5.00^{a}	4.67^{a}	4.13^{a}
8-9 dS/m (A_2)		3.50^{c}	4.00^{b}	3.75^{bc}	
Mean		3.53^{c}	3.97^{a}	3.78^{b}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 4: Leaf area of vetiver under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (cm^2)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	5199.88^{g}	5603.85^{e}	5445.70^{ef}	5118.89^{c}
8-9 dS/m (A_2)		4680.29^{i}	4999.38^{h}	4784.22^{i}	
4-5 dS/m (A_1)	Salicylic acid 0,5 mM (B_1)	5611.66^{e}	6113.83^{c}	5852.05^{d}	5571.32^{b}
8-9 dS/m (A_2)		5323.04^{fg}	5391.87^{f}	5135.47^{gh}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	6300.93^{b}	7371.47^{a}	7277.05^{a}	6306.52^{a}
8-9 dS/m (A_2)		5260.16^{fg}	6020.48^{cd}	5609.04^{e}	
Mean		5395.99^{c}	5916.81^{a}	5683.92^{b}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Number of tillers: Observation data and analysis of variance showed that salinity treatment, salicylic acid application and mycorrhiza singly significantly affected the number of tillers. Almost all treatment interactions significantly affected the number of tillers, except for the interaction between salinity and mycorrhiza. The number of vetiver tillers under salinity conditions with salicylic acid and mycorrhiza application was presented in Table 3.

Table 3 presented data on the number of vetiver tillers, where the salicylic acid treatment on the number of vetiver tillers was highest at 1 mM salicylic acid application. The application of salicylic acid has an effect on increasing the number of vetiver tillers, where the number of tillers increases with the higher the addition of salicylic acid concentration. This was in line with El-Shafey *et al.*[42] which states that the application of salicylic acid through the leaves significantly increases the number of tillers. The results of Usharani *et al.*[43] also reported that spraying salicylic acid had a significant effect on the number of tillers.

The number of tillers of vetiver plants also increased with the application of *Gigaspora* sp., mycorrhiza, this is because mycorrhiza in plants acts as a helper for nutrient absorption so as to increase plant growth. According to Akbar *et al.*[44] the application of mycorrhiza to plants through leaves increases the number of tillers per plant. Mycorrhiza applications also stimulate higher germination, root system growth and increase the number of tillers and plant weight. In some plants, the effect of mycorrhizal application increases the number of tillers and plant weight[45].

The interaction of 1 mM salicylic acid treatment and mycorrhiza *Glomus* sp., produced the highest number of tillers, namely 5.00 tillers at 4-5 dS/m salinity, which was not significantly different from the interaction of 1 mM salicylic acid treatment and mycorrhiza type *Gigaspora* sp. (B2C2), namely 4.67 tillers at 4-5 dS/m salinity and significantly different from other treatment interactions. The application of salicylic acid and mycorrhiza under salinity stress has a significant effect on increasing the number of vetiver tillers. This was in line with Pirasteh-Anosheh *et al.*[46], which stated that salinity treatment increases the rapid growth phase in the number of tillers and the application of salicylic acid at different concentrations can improve some of the negative impacts on plant growth under salinity stress. Furthermore, Emami *et al.*[47] stated that the number of tillers is considered the most sensitive trait to salinity stress, this will be related to the percentage of root dry weight. The results of research by Zhang *et al.*[48], also reported that there was an increase in the number of tillers in plants treated with mycorrhiza under saline stress. Furthermore, Ansari *et al.*[49] stated that the increase in the number of tillers has an impact on production because mycorrhiza is able to increase the uptake of macro nutrients such as N, especially in saline conditions.

Leaf area: The results of observations of vetiver leaf area and analysis of variance showed that salinity treatment, salicylic acid application, mycorrhiza application both singly and the interaction between treatments had a significant effect on leaf area. Vetiver leaf area under salinity conditions with salicylic acid and mycorrhiza application was presented in Table 4.

Based on Table 4, it can be seen that the salicylic acid treatment on the widest vetiver leaf area was obtained at 1 mM salicylic acid application. Leaf area increases with the addition of salicylic acid concentration. According to Li *et al.*[50] that the difference in concentration in the application of salicylic acid shows that this compound has an important role in plant growth. Furthermore, Sahar *et al.*[51] explained that the application of salicylic acid is able to increase the content of proteins and sugars that can maintain osmotic pressure in the cell so as to increase tolerance to dehydration in the leaves. Increased tolerance in leaves will trigger leaf growth such as leaf area.

The increase in leaf area of vetiver plants is also influenced by the application of mycorrhiza, this result can be seen that vetiver leaves are wider in the application of *Glomus* sp., mycorrhiza than without mycorrhiza application. According to Roth and Paszkowski[52], mycorrhiza acts as a source of metabolism so that it affects photosynthesis. In addition, Qi *et al.*[53] stated that mycorrhiza can affect growth by increasing leaf area in accordance with plant morphology. These results are in line with some previous research results that mycorrhiza significantly increases leaf area in some plants[54-56].

The interaction of 1 mM salicylic acid treatment and mycorrhiza *Glomus* sp. (B2C1) produced the largest vetiver leaf area of 7371.47 cm^2 at 4-5 dS/m salinity, which was not significantly different from the interaction of 1 mM salicylic acid treatment and mycorrhiza type *Gigaspora* sp. (B2C2) which was 7277.05 cm^2 at 4-5 dS/m salinity, but significantly different from other treatment interactions. The effect of the interaction of salinity with the application of salicylic acid and mycorrhiza on the leaf area of vetiver plants has increased, these results indicate that the application of salicylic acid can increase growth even under salinity stress conditions. Salicylic acid plays an important role in maintaining plants against biotic and abiotic stress conditions, where salicylic acid application affects various plant processes including stomatal closure and photosynthesis rate[30,57]. In addition, salicylic acid has increased antioxidant enzyme activity and proline content so as to reduce the harmful effects of saline stress and promote growth[58,59]. The results of research by Abdelaal *et al.*[60], reported that the application of salicylic acid to leaves under salinity conditions can reduce the adverse effects of salt stress on plant growth and improve proline accumulation. On the other hand, the detrimental effect caused by salinity on the area of one leaf per plant can also be reduced by the application of mycorrhiza. According to Çekiç *et al.*[61], salinity-affected mycorrhiza will form root symbiosis in most plant species, this allows plants to explore a larger volume of soil to absorb more nutrient uptake and water transport, increase the absorption of mineral elements such as phosphorus and improve water use efficiency. Therefore, mycorrhizal application increases leaf area, delays senescence and improves salinity resistance in plants[62].

Root length: The results of observations of vetiver root length and analysis of variance showed that salinity treatment, salicylic acid application, mycorrhiza application and interaction between treatments had a significant effect on root length, only the treatment interaction between salinity treatment and salicylic acid application had no significant effect on root length. The root length of vetiver under salinity conditions with salicylic acid and mycorrhiza application was presented in Table 5.

Based on Table 5, it can be seen that the longest vetiver was obtained at 0.5 mM salicylic acid application. This shows that the application of salicylic acid on vetiver plants affects the length of the roots. Different concentrations of salicylic acid give different results, where the best results are obtained at a concentration of 0.5 mM, which is the lowest concentration in this study so that it can stimulate root growth, otherwise at higher concentrations it is thought to inhibit root growth. In some other plants also the application of salicylic acid with high concentration differences shows inhibition on root length such as in legumes[63]. In line with Khan *et al.*[14], who stated that high salicylic acid application can inhibit root growth, this is due to part of the mechanism of plant response to biotic and abiotic stress, so that under these conditions, plants will prioritize the allocation of resources for other functions, such as defense mechanisms rather than root growth. Mahajan and Pal[64] said that when plants experience stress, salicylic acid levels often increase, triggering defense responses such as the production of antimicrobial and antioxidant compounds. These responses affect several aspects of plant growth including root length.

Mycorrhiza application also affects the increase in root length, where vetiver plants treated with *Gigaspora* sp., mycorrhiza give better results than without mycorrhiza. In order to maximize the roots and enable plants treated with mycorrhiza to absorb more water and nutrients, the mycorrhiza application works by deeply enlarging the nutrient absorption zone[65]. In a symbiotic relationship with plants, mycorrhiza and roots work together to enhance the nourishment of the host plant. This type of symbiosis also serves as a plant growth-promoting hormone, a bioprotectant against root pathogen infections and an increase in plant resilience to drought stress[66].

Table 5: Root length of vetiver under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (cm)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	83.98^{gh}	86.07^{f}	92.30^{cd}	82.32^{c}
8-9 dS/m (A_2)		71.37^{m}	79.14^{k}	81.03^{ij}	
4-5 dS/m (A_1)	Salicylic acid 0.5 mM (B_1)	92.93^{cd}	99.09^{b}	104.60^{a}	92.96^{a}
8-9 dS/m (A_2)		80.41^{jk}	89.08^{e}	91.65^{d}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	88.43^{e}	92.74^{cd}	93.63^{c}	86.38^{b}
8-9 dS/m (A_2)		76.28^{l}	82.50^{hi}	84.70^{fg}	
Mean		82.23^{c}	88.10^{b}	91.32^{a}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 6: Root volume of vetiver under salinity conditions with salicylic acid and mycorrhiza application

		Mycorrhiza (cm^3)			
Salinity	Salicylic acid	No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	Mean
4-5 dS/m (A_1)	No salicylic acid (B_0)	212.58^{g}	220.17^{f}	247.92^{d}	199.40^{c}
8-9 dS/m (A_2)		164.83^{m}	172.08^{kl}	178.83^{jk}	
4-5 dS/m (A_1)	Salicylic acid 0.5 mM (B_1)	241.58^{de}	269.25^{c}	292.50^{a}	237.19^{a}
8-9 dS/m (A_2)		177.50^{jkl}	201.25^{h}	241.08^{de}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	235.92^{e}	247.92^{d}	280.58^{b}	217.58^{b}
8-9 dS/m (A_2)		170.67^{lm}	189.08^{i}	181.33^{j}	
Mean		200.51^{c}	216.63^{b}	237.04^{a}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

The interaction of 0.5 mM salicylic acid treatment and *Gigaspora* sp., mycorrhiza (B1C2) produced the longest vetiver roots of 104.60 cm at 4-5 dS/m salinity and was significantly different from other treatment interactions. Root length increased with the interaction of salinity treatment with the application of salicylic acid and mycorrhiza, this indicates that the application of salicylic acid and mycorrhiza can prevent the negative effects of salinity stress. This result was in line with Jini and Joseph[67] who reported that there was an increase in root length due to salicylic acid application under salinity stress. Salicylic acid application protects various plant species against biotic and abiotic stresses including salinity[68,69]. Nguyen *et al.*[70] explained that salinity stress reduces the availability of H_2O in various parts of the plant including the roots, but the application of salicylic acid can improve the availability of water under stress. In addition, in salinity stressed soil, osmotic pressure and imbalance in nutrient availability for plants are factors that are detrimental to plant growth.

According to Atamimi and Sugiyarto[71], the application of mycorrhiza to plants contributes to expanding the field of root absorption in taking water and nutrients needed by plants through its hyphae, because it can penetrate small soil pores that cannot be reached by roots so that the area of root absorption of water and soil minerals increases.

Root volume: The observation of vetiver root volume and analysis of variance showed that salinity treatment, salicylic acid application, mycorrhiza application both singly and the interaction between treatments had a significant effect on root volume. The root volume of vetiver under salinity conditions with salicylic acid and mycorrhiza application is presented in Table 6.

Based on Table 6, it can be seen that the highest vetiver root volume was obtained at 0.5 mM salicylic acid application. Salicylic acid application affects the volume of vetiver roots, where the application of salicylic acid with a concentration of 1 mM has a lower root volume compared to the concentration of 0.5 mM, but higher than without application. According to Motte *et al.*[72], salicylic acid can affect root shape including branching and root density, changes in root shape can affect root volume by redistributing root biomass in the soil for example, increased branching of lateral roots can lead to a more extensive root system and consequently higher root volume, while decreased branching can result in reduced root volume.

The application of *Gigaspora* sp., mycorrhiza also proved to have a significant effect on increasing the volume of vetiver roots. According to Wahab *et al.*[73], one of the main benefits of mycorrhiza is the increase in the surface area of plant roots, mycorrhiza forms specialized structures such as hyphae and arbuscules around plant roots. These hyphae expand the root surface area, thereby increasing the plant's ability to absorb water and nutrients from the soil. In addition, mycorrhiza can also stimulate the growth of plant roots, thus producing certain growth hormones that stimulate the formation of roots and root branches, this can result in an overall increase in the number as well as volume of roots[74]. The results of this study are in line with Ilangamudali and Senarathne[75], who reported that the application of mycorrhiza can increase plant root growth in the form of root volume.

The interaction of 0.5 mM salicylic acid treatment and *Gigaspora* sp., mycorrhiza (B1C2) produced the highest vetiver roots of 292.50 cm^3 at 4-5 dS/m salinity and was significantly different from other treatment interactions. The effect of the interaction on root volume increased, this is in line with root growth because the application of salicylic acid and mycorrhiza shows a defense response to salinity stress conditions. According to Bernstein[76], the root is the first organ to come into contact with salt ions, so the toxic effect mainly occurs in this part. Furthermore, Ahmad *et al.*[77] stated that salicylic acid has an impact on root metabolism so that it significantly increases the growth rate of plants under saline conditions. This is in line with Ardebili *et al.*[78], who stated that the role of salicylic acid in the mechanism of plant growth and reduce plant damage due to salinity. In addition, Aroca *et al.*[79] reported that mycorrhiza changes plant hormones and affects plant physiology to better suit salinity conditions. According to Porcel *et al.*[80], the application of mycorrhiza in saline conditions can increase the mineral content of plants, so that plants treated with mycorrhiza have increased root volume than plants without mycorrhiza in saline conditions[81].

Root fresh weight: The observation of root fresh weight and analysis of variance showed that salinity treatment, salicylic acid application, mycorrhiza application, interaction between salicylic acid and mycorrhiza application and interaction between the three treatments had a significant effect on root fresh weight, while the interaction between salinity treatment and salicylic acid application and interaction between salinity treatment and mycorrhiza application had no significant effect on root fresh weight. The fresh weight of vetiver roots under salinity conditions with salicylic acid and mycorrhiza application is presented in Table 7.

Based on Table 7, it can be seen that the highest fresh weight of vetiver roots was obtained at 0.5 mM salicylic acid application. Salicylic acid affects the fresh weight of the roots, where applications with various concentrations of salicylic acid show the best results on vetiver plants. In line with the results of research by Lolaei *et al.*[82], that salicylic acid increases root fresh weight. Pasternak *et al.*[83] reported that salicylic acid controls root growth by regulating the activity of the root apical meristem and the application of salicylic acid at a concentration of 30 mM reduces the number of cells expressed in genes in root meristem cells[84].

The fresh weight of plant roots increased with the application of *Gigaspora* sp., mycorrhiza compared to without mycorrhiza, this indicates that the application of mycorrhiza has a significant effect on the growth of plant root fresh weight. Increased mycorrhizal growth and development compared to non-mycorrhiza plants has been reported in many plant species[54,85,86]. According to Chandrasekaran[87], mycorrhizal inoculation increased root fresh weight significantly. Furthermore, Giovannini *et al.*[88] explained that mycorrhiza can affect root allocation and translocation so that the growth rate and root fresh weight increase due to higher nutrient absorption and translocation. This may be due to an increase in root absorption surface[89].

The interaction of 0.5 mM salicylic acid treatment and *Gigaspora* sp., mycorrhiza (B1C2) produced the highest vetiver roots of 1287.92 g at 4-5 dS/m salinity and was significantly different from other treatment interactions. The application of salicylic acid and mycorrhiza under salinity stress proved to increase the fresh weight of vetiver roots compared to the control treatment. This result was in line with Hundare *et al.*[90], that the application of salicylic acid can reduce the negative impact of salinity and the maximum increase is seen in the fresh weight of the roots. According to Ma *et al.*[91] and Mohammadi *et al.*[92] salicylic acid plays a role in activating the defense system which includes osmoregulation and ion homeostasis which can increase plant growth and defense system from saline conditions. Furthermore, Khoso *et al.*[93] explained that the root system of a plant has many functions, including providing stability by facilitating the absorption of water and ions, storing important nutrients and supporting vegetative growth. Furthermore, Jerbi *et al.*[94] and Klinsukon *et al.*[95] reported that mycorrhiza application significantly increased plant biomass at various salinity levels, where mycorrhiza affected root fresh weight at all levels of salt stress compared to uninoculated plants. Mycorrhizae increase the number of extraradical hyphae and increase the surface area of mycelial tissue, contributing to increased root biomass[96].

Production of essential oil: The results of observations of essential oil production from vetiver plants and analysis of variance showed that salinity treatment, salicylic acid application, mycorrhiza application both singly and the interaction between treatments had a significant effect on essential oil production. The production of vetiver essential oil under salinity conditions with the application of salicylic acid and mycorrhiza is presented in Table 8.

Table 7: Fresh weight of vetiver roots under salinity conditions with salicylic acid and mycorrhiza application

Salinity	Salicylic acid	Mycorrhiza (cm^3)			Mean
		No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	
4-5 dS/m (A_1)	No salicylic acid (B_0)	944.83^{hi}	1001.00^{fgh}	1085.58^{cde}	948.58^{b}
8-9 dS/m (A_2)		805.67^{j}	902.42^{i}	952.00^{ghi}	
4-5 dS/m (A_1)	Salicylic acid 0,5 mM (B_1)	1021.58^{efg}	1166.33^{b}	1287.92^{a}	1092.00^{a}
8-9 dS/m (A_2)		968.83^{ghi}	1022.50^{efg}	1084.83^{cde}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	1113.67^{bcd}	1146.67^{bc}	1134.92^{bc}	1062.70^{a}
8-9 dS/m (A_2)		909.83^{i}	1024.42^{efg}	1046.67^{def}	
Mean		960.74^{c}	1043.89^{b}	1098.65^{a}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 8: Production of vetiver essential oil under salinity conditions with salicylic acid and mycorrhiza application

Salinity	Salicylic acid	Mycorrhiza (cm^3)			Mean
		No mycorrhiza (C_0)	*Glomus* sp. (C_1)	*Gigaspora* sp. (C_2)	
4-5 dS/m (A_1)	No salicylic acid (B_0)	6.12^{hi}	6.33^{gh}	6.95^{e}	5.99^{c}
8-9 dS/m (A_2)		4.92^{k}	5.42^{j}	6.19^{ghi}	
4-5 dS/m (A_1)	Salicylic acid 0,5 mM (B_1)	7.31^{cd}	8.50^{b}	8.92^{a}	7.50^{a}
8-9 dS/m (A_2)		6.44^{fg}	6.66^{f}	7.18^{de}	
4-5 dS/m (A_1)	Salicylic acid 1 mM (B_2)	6.33^{gh}	7.45^{c}	8.36^{b}	6.78^{b}
8-9 dS/m (A_2)		6.00^{i}	6.35^{gh}	6.18^{ghi}	
Mean		6.19^{c}	6.79^{b}	7.30^{a}	

Numbers followed by different letters indicate significantly different according to Duncan's Multiple Range Test at the 5% level

Table 8 presents data on vetiver essential oil production, salicylic acid treatment on the production of vetiver essential oil is mostly obtained at 0.5 mM salicylic acid application. The application of salicylic acid affects the production of vetiver essential oil under salinity, this is because salicylic acid can stimulate enzyme activity and certain metabolic pathways in plants so as to increase the production of compounds such as essential oils. According to Rebey *et al.*[97], environmental stress can lead to the accumulation of essential oils, as antioxidant compounds, to reduce oxidative stress caused by salinity. Furthermore, Akula and Ravishankar[98] explained that the increased content of each constituent in essential oils under salinity could be attributed to the effects of this stress on the synthesis of several secondary metabolites and the function of these metabolites as self-defense components against stressful conditions. In addition, salinity may alter essential oil biosynthesis through apparent changes in biochemical and physiological processes[99]. Salicylic acid has triggered roles in secondary metabolites such as glucosinolates[100], alkaloids[101] and anthraquinones[102].

The application of mycorrhiza under salinity stress significantly increased vetiver essential oil production compared to without mycorrhiza where the highest production was obtained with the application of *Gigaspora* sp. This is in accordance with the statement stating that mycorrhizal plants usually grow better than non-mycorrhizal ones. One reason is that mycorrhiza can effectively increase the absorption of macro nutrients and some micro nutrients[103]. In addition to helping plant rooting work, mycorrhiza is also able to increase plant tolerance to unfavorable environmental conditions such as drought and salinity[104].

Salinity stress is a major abiotic stress that inhibits crop and yield production. The interaction of salicylic acid and mycorrhiza plays an important role in eliminating the adverse effects of salinity stress. This is evidenced by the interaction of 0.5 mM salicylic acid treatment and *Gigaspora* sp., mycorrhiza (B1C2) which produced the most essential oil, namely 8.92% at 4-5 dS/m salinity. According to Hao *et al.*[105], the symbiosis of salicylic acid and mycorrhiza can reduce the adverse effects of salinity stress by modulating ion homeostasis and carbohydrate metabolism in plants. Sugars synthesized through carbohydrate metabolism act as osmotic regulators and signal molecular inactivation of various defense responses to salinity stress[106]. Thus, salicylic acid application further enhances sugar export to plant roots thereby favoring mycorrhizal symbiosis[107]. According to Talaat and Shawky[108], mycorrhiza can overcome salt stress in plants with effective symbiosis due to its ability to tolerate high salinity conditions. Mycorrhiza species *Gigaspora* sp. and *Glomus* sp., showed viability and more stable under saline stress conditions than other types of mycorrhiza[109]. The combination of mycorrhiza with high salinity concentrations, especially *Gigaspora* sp., mycorrhiza, increased growth compared to low salinity concentrations[110]. Abdel-Rahman *et al.*[111] stated that stomatal opening is affected by *Gigaspora* sp., colonization in high salinity stress, but in low salinity *Glomus* sp., is better in affecting stomatal opening.

CONCLUSION

The best growth and essential oil production under salinity conditions were obtained with 0.5 mM salicylic acid application. The use of mycorrhiza species *Gigaspora* sp., can increase the growth and production of essential oil under salinity conditions. The best essential oil production under salinity conditions was obtained with the application of 0.5 mM salicylic acid and *Gigaspora* sp. It is hoped that the results of this research can contribute to increasing the potential of saline land.

SIGNIFICANCE STATEMENT

The application of salicylic acid and mycorrhiza simultaneously aims to mitigate salinity stress in vetiver plants. The results showed that there was an increase in growth and essential oil production of vetiver plants under salinity stress with the application of salicylic acid and mycorrhiza compared to without application. The best growth and essential oil production under salinity conditions were obtained with application of 0.5 mM salicylic acid and *Gigaspora* sp. Furthermore, a deeper analysis is needed regarding the mechanism of plants interacting with salicylic acid and mycorrhiza in dealing with salinity stress.

ACKNOWLEDGMENT

The authors gratefully thank the Ministry of Education, Culture, Research and Technology, Directorate General of Higher Education, Research and Technology for supporting the research through the research budget with contract number: 17/UN5.2.3.1/PPM/KP-DRTPM/L/2023.

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