JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(7), 286–294
https://doi.org/10.12911/22998993/188880
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.05.06
Accepted: 2024.05.22
Published: 2024.06.01

Organic Fertilizer Alleviates Salt Stress in Shallot by Modulating Plant Physiological Responses

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ABSTRACT

Salinity is a major constraint for crop productivity as it reduces agricultural land area. This problem can be ameliorated by the application of organic materials such as manure, which plays an important role in supporting plant growth and reducing soil toxicity by binding toxic compounds. The purpose of this study is to analyse the effect of manure in overcoming the impact of salt stress on shallots. Here randomised block design (RBD) consisting of 2 factors and 3 replications was used. The first factor is salinity levels (0, 50, 100, and 150 mM), and the second is manure doses (0, 10 and 20 t·ha^{-1}). This study finds that the application of 20 t·ha^{-1} of manure decreases the shallot's leaf tissue thickness, but the 50 mM of salinity significantly increases it. Then, the application of 20 t·ha^{-1} of manure increases the shallot's number of tillers and bulbs, while the 100 mM of salinity significantly decreases its number of tillers. The application of 10 t·ha^{-1} of manure decreases the proline and flavonoids content of the plant's leaves. In addition, plants treated and not treated with manure under 50 mM of salinity have higher proline and flavonoids levels in their leaf. Therefore, shallots can grow under salinity conditions if manure is applied.

Keywords: allium, flavonoids, manure, proline, salinity.

INTRODUCTION

Shallot (*Allium ascalonicum*) is a horticultural crop that is widely cultivated and used for food and medicine throughout the world (Solouki et al., 2023). According to Statistics Indonesia (2023), the shallot harvest area in Indonesia in 2022 was 184,386 ha, decreasing from 194,575 ha in the previous year. Shallot production can be increased by expanding the planting area as Indonesia has about 440,330 ha of saline land (Usnawiyah et al., 2021), which has not well utilised. However, some special treatments are required. They involve correct plant species selection, soil property improvement through water management, mechanical soil management, chemical improvement with the addition of gypsum and sulphur, and the use of mulch and organic materials, not to mention improvements in farmer awareness (Karolinoerita and Annisa, 2020).

High salinity adversely affect the morphology, physiology and yield of shallot plants (Shoaib et al., 2018) as it prolongs shoot emergence, slows leaf growth, reduces height, and alters the bulb shape and decrease its size and weight (Alam et al., 2023). Salinity determines the plant's ability to grow as it damages cells. It inhibits plant growth as it creates imbalances in nutrient ion balance and causes ion toxicity in addition to diminishes water availability, respiration rate, mineral distribution, membrane stability, turgor pressure, growth rate, and yield rate (Golldack et al., 2014; Makhloufi et al., 2014). High salinity affects water uptake by the plants due to the presence of salts around plant roots, which also causes oxidative stress. The impact of salinity on the physiological condition of shallots includes a decrease in proline levels, phenolic compounds, and pyruvic acid precursors (Hanci et al., 2016). Highly saline water inhibits plant's membrane instability, relative water content, total chlorophyll content, and carotenoids content (Venâncio et al., 2022). The impact of soil salinity on plant physiology can be reduced by the addition of organic matter such as manure as it can improve soil conditions to certain levels where plants can grow. Organic matter added to saline environment can improve osmotic regulation between plant root cells and soil nutrient solutions so as to reduce the effects of saline stress (Adil Aydin, 2012; Morrissey et al., 2014). Organic amendments are known to reduce the effects of salinity with the help of soil microorganisms and positively influence microbial activity and nutrient cycling (Wichern et al., 2020). Improved soil nutrition with the addition of manure results from microbial activity that breaks down organic matter into nutrients needed by plants. The use of compost, especially K, P, Zn, and N, can increase soil nutrient content so that the number and weight of tubers can increase (Yan et al., 2018; Showler, 2022).

The availability of nutrients in saline soils can be increased by adding organic matter which greatly affects the growth of plants, especially shallots. The response of shallots to highly saline conditions is related to the activity of aquaporin genes, especially PIP2, which is related to Zn uptake (Solouki et al., 2023). Another study on shallot resistance to such conditions was conducted by Sanwal et al. (2022), who found that the effects of higher antioxidant enzyme activity and lower H_2O_2 production and lipid peroxidation. Shallot's tolerance to salinity can be increased by adding Si fertiliser, as found by Venâncio et al. (2022) that Si application increases the yield, bulb freshness, and bulb size of shallots (≥ 50 mm) as well as decreases salinity stress to 2.8 dS m^{-1}. Solouki et al. (2023) found that a significant decrease in shallot growth only occurred when treated with salt concentrations of 50 mM. The utilisation of saline land for shallot cultivation can be done by adding organic matter in the form of manure, which improves osmotic regulation.

MATERIALS AND METHODS

This research was conducted from August to November of 2023 in Poncokusumo, Malang Regency, at an altitude of 500–600 MASL. The materials were shallot var. tajuk, NaCl, goat manure, basic fertiliser (N, P, and K fertiliser), and polybags with the diameter of 20 cm. This study uses randomised blok design (RBD) consisting of 2 factors and 3 replications. The first factor was salinity levels (0, 50, 100, and 150 mM), and the second was manure doses (0, 10, and 20 t·ha^{-1}).

The soil media were given NaCl that had been dissolved according to the salinity treatment levels of 50, 100, and 150 mM; they were then mixed with manure. Planting media that have been mixed evenly were put into polybags with a diameter of 20 cm weighing ± 5 kg and given a bucket base. Here healthy, fresh, dense, not wrinkled, and brightly coloured shallot seedlings were used to accelerate sprouting and ensure uniform growth. They were cut at the 1/3 end. The planting was done one week after filling the planting medium containing one shallot bulb. The plant care included watering, N, P, K fertilising (started 7 days after planting, 6 times with the span of 7 days), weeding, and pest management (started after right after the planting).

The observations were conducted on leaf epidermal tissue thickness, proline content, leaf flavonoid content, tiller number per clump, and tuber number per clump. The thickness of leaf epidermal tissue was measured using the paraffin method by Nakamura (1995) with semi-permanent preparations. The proline levels were measured using the method proposed by Bates et al. (1973). The leaf's flavonoid levels were measured using the spectrophotometric (quercetin) method used by (Lindawati and Ma'ruf, 2020). The number of tillers and tubers per clump was calculated using the destructive method. The data obtained from the study were analysed using variance analysis (F-test) at the 5% test level in order to determine the effect of the treatment and whether a real effect was obtained. Tt was continued with the honestly significant difference (HSD) at the 5% level.

RESULTS AND DISCUSSION

Leaf epidermal thickness

Manure doses and salinity levels significantly affect the thickness of shallot leaf epidermis. The addition of 20 t·ha^{-1} of manure was significantly influential and was able to reduce the thickness of shallot leaf tissue (Table 1 and Figure 1). This is caused by increased plant growth from higher nutrient content in manure (Agegnehu et al., 2017), as the formed cells tend to be larger but less dense (Nath et al., 2010). Thick leaf epidermal tissue

Table 1. Average leaf epidermal thickness tissue due to manure dose

Treatment	Leaf epidermal thickness (μm)
Manure	
0 t·ha^{-1}	351.13 b
10 t·ha^{-1}	325.94 b
20 t·ha^{-1}	284.06 a
HSD 5%	39.30
0 mM	215.20 a
50 mM	321.93 b
100 mM	351.30 bc
150 mM	393.08 c
HSD 5%	50.16

Note: numbers accompanied by the same letter in each row are not significantly different based on the HSD test at the 5% level.

affects the plants' transpiration rate, which results in a decrease in growth rate (Giuliani et al., 2013; Maylani et al., 2020). The use of manure and N s kg/ha significantly increased plant growth and yield (Ncayiyana et al., 2018).

Salinity levels are directly proportional to the increase in shallot's leaf epidermal thickness. The concentrations of 50, 100, and 150 mM significantly increase the thickness of the leaf's epidermis, compared to that of plants that do not experience salinity. The leaf epidermal thickness of shallots under 50 mM of salinity is not significantly different from those under 100 mM but is significantly different from those under 150 mM. However, such thickness in the plants under 100 and 150 mM of salinity was not significantly different. Salt-tolerant plants can initiate protective mechanisms that

Figure 1. Leaf epidermal thickness of shallot leaves by manure dose and salinity level

allow them to grow in saline environments (Zandalinas et al., 2017). Epidermal tissue can thicken as a form of structural resistance adaptation to the environment, including saline conditions (Ozturk et al., 2021). Thick leaf epidermal tissue can help reduce water losses and increase the production of anti-stress compounds that can protect plants from damage caused by salinity (Bhattacharya, 2021).

Leaf proline level

Manure doses and salinity levels have a significant interaction effect on the average proline content in shallot leaves (Table 2). The average proline content of in the leaves decreased along with the addition of manure. The addition of 10 t·ha^{-1} of manure can reduced the leaf's proline content, but the depletion rate is not significantly different from the addition of 20 t·ha^{-1} in the treatment without salinity. As for the treatment with 50, 100, and 150 mM of salinity, the addition of 10 t·ha^{-1} of manure was also significantly able to reduce leaf proline levels compared to the same addition on plants without manure. The addition of 20 t·ha^{-1} of manure resulted in the lowest leaf proline levels compared to the addition of 10 t·ha^{-1} of manure. These results are in line with the findings of Sanwal et al. (2022) that one of the factors affecting proline levels is stress in salinity conditions since the levels of soluble salts and sodium which decrease along with the addition of manure and gypsum (Foronda and Colinet, 2022). The use of organic matters can maintain good soil structure, increase cation exchange capacity, serve as a soil nitrogen reservoir, increase water retention, and enhance mineralisation (Havlin and Heiniger, 2020).

In contrast to the effect of manure, increasing salinity improves the average proline content of shallot leaves. The salinity of 50, 100, and 150 mM can significantly increase proline levels in leaves compared to such content in plants that do not experience salinity. The salinity of 150 mM produced higher proline levels compared to the salinity of 50 mM and 100 mM, with or without manure. This shows that salinity can increase the amount of proline in shallot leaves. In addition to salinity, the proline amino acid levels in *Allium cepa* L. shoots increased under alkaline soil conditions (Sivasamy et al., 2022). The impact of salinity on the physiological conditions of Allium ascalinicum includes increased proline levels and decreased phenolic compounds and pyruvic acid precursors (Mohamed and Aly, 2008; Hanci et al., 2016). The increase in proline levels in Allium cepa shoots is caused by plant regulation in maintaining cellular osmotic balance and surviving oxidative damage caused by salinity stress (Solouki et al., 2023). Sanwal et al. (2022) also revealed that one of the factors affecting proline levels is salinity stress.

Leaf flavonoids level

Higher manure doses and salinity levels have an interaction effect on the flavonoid levels in shallot leaves (Table 3). The leaf's flavonoid levels decreased along with the addition of manure. The addition of 10 and 20 t·ha^{-1} of manure on plants that do not experience salinity and plants that experience salinity of 50 and 100 mM can significantly reduce leaf flavonoid levels. As for plants experiencing 150 mM of salinity, the

Table 2. Interaction between manure and salinity on average proline content in shallot leaves

Treatment	Prolin (μM/g fresh weight)			
	0 mM	50 mM	100 mM	150 mM
0 t·ha^{-1}	0.0441 a	0.0539 b	0.0770 c	0.0924 d
	B	C	C	C
10 t·ha^{-1}	0.0329 a	0.0497 b	0.0637 c	0.0805 d
	A	B	B	B
20 t·ha^{-1}	0.0322 a	0.0483 b	0.0618 c	0.0756 d
	A	A	A	A
HSD 5% Salinity	0.00091			
HSD 5% Manure	0.00072			

Note: numbers accompanied by the same lowercase letter in the same row are not significantly different based on the HSD test at the 5% level, numbers accompanied by the same capital letter in the same column are not significantly different based on the HSD test at the 5% level.

addition of 10 t·ha^{-1} of manure did reduce the flavonoid levels, but the addition of 20 t·ha^{-1} of manure significantly decreased them (Table 3). Flavonoid levels in shallot leaves can be influenced by various other factors such as plant species, environmental conditions, and interactions with microorganisms (Anh et al., 2023).

The type of soil or place of growth also affects the content of substances formed in plants (Hawari et al., 2022). The addition of manure can affect the flavonoid production in plants. Those containing flavonoids will produce orange, pink, and red spots (Riyana et al., 2018). Environmental stress caused by biotic and abiotic factors affects the production of secondary metabolites and generally increases the production of secondary metabolites (Mazid et al., 2011). The formation of secondary metabolites is plants' protective response to environmental stress (Ramakrishna and Ravishankar, 2011).

In contrast to the effect of manure, salinity increased flavonoid levels. Plants that did not get manure addition and receive 10 t·ha^{-1} of manure addition, should they be under 50 mM of salinity treatment, were able to produce more flavonoid in their leaves compared to plants that did not experience salinity stress. Plants that were under 100 mM of salinity treatment but did not receive manure addition produced more flavonoid; the level was significantly different from plants under 50 mM of salinity condition but not significantly different from plants under 150 mM of salinity treatment. Plants under 50 mM of salinity condition that were added with 20 t·ha^{-1} of manure were able to increase their flavonoid levels compared to those that did not experience salinity treatment. Meanwhile, plants added with 10 to 20 t·ha^{-1} of manure under 150 mM of salinity had the highest flavonoid levels. Abdelrahman et al. (2020) found that shallots reprogramme their metabolism towards high accumulation of amino acids and flavonoids as an adaptive response. Flavonoids not only provide protection against harmful abiotic factors but also facilitate interactions with other plants and microorganisms due to their physical and biochemical properties (Khalid et al., 2019). A systematic review of the therapeutic uses of shallots highlight their high flavonoid content and antioxidant activities (Moldovan et al., 2022). Flavonoids have a role in frost hardiness and drought resistance and play a functional role in heat acclimatisation of plants. Flavonoids in plants act as antioxidants, antimicrobials, photoreceptors, visual attractants, food repellents, and light filters (Panche et al., 2016).

Number of tillers

Manure doses and salinity levels have a significant effect on the number of tillers in shallots. The average number of shallot tillers per clump with manure application increased at all plant ages (Table 4). The addition of 20 t·ha^{-1} of manure was significantly influential and increased the number of tillers. Onion bulbs increase along with the increasing application of manure (Díaz-Pérez et al., 2018). Manure added to soil has a positive effect on shallot growth (Ikrarwati et al., 2021; Bijay-Singh and Sapkota, 2022). Manure application can also increase soil biological activity, which facilitates nutrient cycling and particle aggregation, resulting in better soil health and

Table 3. Interaction between manure and salinity on average flavonoid content in shallot leaves

Treatment	Flavonoid (mg QE/g of sample)			
	0 mM	50 mM	100 mM	150 mM
0 t·ha^{-1}	169.94 a	193.97 b	283.87 c	300.91 c
	C	C	C	B
10 t·ha^{-1}	125.79 a	156.00 b	224.19 c	296.74 d
	B	B	B	B
20 t·ha^{-1}	107.35 a	126.59 b	139.76 b	187.74 c
	A	A	A	A
HSD 5% Salinity	17.95			
HSD 5% Manure	14.07			

Note: numbers accompanied by the same lowercase letter in the same row are not significantly different based on the HSD test at the 5% level,.numbers accompanied by the same capital letter in the same column are not significantly different based on the HSD test at the 5% level.

plant growth (Hoffland et al., 2020). Increasing salinity causes a decrease in the number of shallot tillers per clump at 4 to 6 weeks after planting (Table 4). The number of tillers of shallots treated with 100 mM of salinity is lower than that of plants not experiencing salinity as it inhibits plant growth. Inhibited growth is an adaptive mechanism for survival, which allows plants to resist salt stress (Munns, 2002). A decrease in the number of tillers can lead to a decrease in the number of tubers per plant, thus negatively affecting yield (Venâncio et al., 2022). When Allium tuberosum plants are stressed with NaCl, their growth will be inhibited, and their yield will decrease (Liu et al., 2022). Na^+ is particularly detrimental at high concentrations in the cytosol of leaf cells as it interferes with metabolic processes such as photosynthesis (Schmöckel and Jarvis, 2016). High salinity can reduce crop production and further growth as well as cause physiological abnormalities which ultimately threaten global food security (Balasubramaniam et al., 2023).

Table 4. Average number of shallot tillers per clump due to manure dose and salinity level

Treatment	Average number of tillers each clump at age (WAP)		
	4	6	8
Manure:			
0 t ha^{-1}	3.62 a	4.71 a	4.54 a
10 t ha^{-1}	4.17 b	5.62 b	5.37 b
20 t ha^{-1}	4.33 b	7.29 c	5.54 b
HSD 5%	0.51	0.58	0.72
Salinity:			
0 mM	4.72 c	6.72 c	5.50
50 mM	4.28 bc	6.11 bc	5.39
100 mM	3.89 ab	5.89 b	4.94
150 mM	3.28 a	4.78 a	4.78
HSD 5%	0.66	0.74	ns

Note: numbers accompanied by the same letter in the same column and treatment are not significantly different based on the HSD test at the 5% level; WAP – week after planting; ns: not significant.

Bulb weight per clump

Manure doses and salinity levels have a significant interaction effect on the weight of shallot bulbs per clump. The average weight of shallot bulbs per clump increased along with the addition of manure (Table 5). Shallot plants that did not experience salinity and that were treated with salinity levels of 50, 100, and 150 mM, that were added with 20 t·ha^{-1} of manure had weight of the bulbs compared to those that did not receive manure addition. Köninger et al. (2021) found that manure significantly increases soil carbon reserves and stabilises organic matter content, which can indirectly affect the growth of Allium plants. In addition, applying manure can increase the cation exchange capacity of the soil, which can help overcome plant nutrient deficiencies (Lumbanraja and Harahap, 2015), thus increasing the weight of shallot bulbs (Setyaningrum and Arbiwati, 2021). Manure contains nutrients needed by plants for optimal growth. It also increases soil fertility so that water and air can be absorbed well by plant roots. This is due to the fact that nutrient contents in manure can improve soil properties and provide nutrients

Table 5. Interaction between manure and salinity on the average weight of shallot.bulbs per clump

Treatment	Average weight of shallot bulbs (g) per clump			
	0 mM	50 mM	100 mM	150 mM
0 t·ha^{-1}	19.85 d	12.31 c	6.11 b	1.76 a
	A	A	A	A
10 t·ha^{-1}	23.71 d	21.05 c	16.30 b	11.17 a
	B	B	B	B
20 t·ha^{-1}	25.77 c	24.60 c	16.41 b	13.70 a
	C	C	B	C
HSD 5% Salinity	1.96			
HSD 5% Manure	1.54			

Note: numbers accompanied by the same lowercase letter in the same row are not significantly different based on the HSD test at the 5% level, numbers accompanied by the same capital letter in the same column are not significantly different based on the HSD test at the 5% level.

needed by plants for growth and tuber formation (Köninger et al., 2021). In addition, manure can also increase the photosynthetic capacity of plants, which contributes to the formation of larger tubers (Lasmini et al., 2022). Increased salinity causes a decrease in shallot bulb weight per clump. Shallot plants, both those receiving manure addition and receiving 10 t ha^{-1} of manure addition, under 50 mM of salinity had lower bulb weight than those that did not experience salinity stress. Plants under 100 mM of salinity that received 20 t·ha^{-1} of manure have lower bulb weight than those that did not experience salinity stress. The addition of organic matter to saline soils can increase the availability of nutrients in the soil, which greatly affects the growth of plant, especially shallots (Alam et al., 2023). Increased salinity of irrigation water has a negative impact on the physiology of ‘Rio das Antas’ shallot plants, namely decreased bulb fresh weight, bulb production, bulb yield, and water use efficiency (Venâncio et al., 2022). Salinity can damage plant roots, resulting in a reduced water and nutrient absorption ability (Zhou et al., 2023). It also causes dehydration so the plants become weak and unable to produce large tubers. Salinity can affect plant growth, which is characterised by a decrease in plant dry weight (Suharjo et al., 2021). A significant decrease in shallot growth occurs when the plant is treated with salt at a concentration of 50 mM (Solouki et al., 2023) the condition can inhibit plant growth and yield, including bulb diameter. Salinity negatively affects the growth and yield of shallot plants, including their per-clump weight (Venâncio et al., 2022), which impacts the growth of shallot plants (Solouki et al., 2023).

CONCLUSIONS

Shallots can grow well under saline conditions if they are treated with manure addition. Plants under the salinity level of 50 mM that were treated with 20 t·ha^{-1} of manure had lower proline and flavonoid levels than those under the 100 mM and 150 mM of salinity. Then, plants under 150 mM of salinity produced higher proline and flavonoid levels and produced smaller tubers. Based on these findings, further research on the dose of manure and levels of salinity needs to be done.

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