Effects of Liquid Organic Fertilizer on Growth and Volatile Components of Arugula under Salinity

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The effects of liquid organic fertilizer (LOF) made from anaerobic digestion on some physiological factors and volatile components of arugula plants that were grown in salty conditions were investigated. The experiment was conducted in three different stages. In the first two stages, seeds were grown in petri dishes and pots for seven days. The third stage involved growing seedlings in pots for 60 days. Salinity inhibited the germination of 7-day arugula seedlings in petri dishes and their emergence in pots. In these stages, LOF pretreatment failed to eliminate stress-induced inhibition. Some physiological parameters were analyzed in 60-day seedlings in the third stage. Salt-induced inhibition showed significant negative effects on all parameters. In contrast to previous stages, LOF (1% and 5%) and NPK had positive effects in all groups at the third stage, eliminating salt stress in all parameters except water content. Contrary to expectations, volatile components showed no significant change but had fluctuating values due to salt stress or fertilizer treatments.

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INTRODUCTION

Climate change is a complex phenomenon with causes ranging from greenhouse gas emissions to human-induced environmental changes. Its effects encompass economic, environmental, health, and social dimensions, impacting biodiversity, human health, livelihoods, and vulnerable populations. Understanding and addressing the causes and effects of climate change are crucial for developing effective mitigation and adaptation strategies to combat this global challenge. It has significant effects on biodiversity, impacting ecosystems and species at various levels. Research has demonstrated that climate change can result in shifts in species distribution, range adjustments, modifications in interspecific relations, changes in species richness and abundance, and increased rates of extinction. These impacts are exacerbated by factors such as habitat fragmentation, nutrient deposition, and alterations in ecosystem services (He *et al.* 2019). The effects of climate change on biodiversity are projected to influence all levels of biodiversity, from genes to biomes, underscoring the broad implications of this global challenge (Sintayehu 2018). It is essential to acknowledge the interactive effects of land use and climate change on biodiversity, as these interactions can have nonadditive impacts on ecosystems and species (Oliver and Morecroft 2014). Climate change has been identified as a significant driver of soil salinity, with various factors contributing to its increase. Studies have shown that salt-water intrusion, shallow water tables, degraded water reuse, and anthropogenic

activities including irrigation and dryland management can lead to a rise in soil salinity levels (Corwin 2020). The impact of climate change on soil salinity is projected to intensify over time, with models indicating substantial increases in salinity levels by 2050 (Dasgupta *et al.* 2015). Additionally, the inter-season variability of rainfall and temperature has been identified as a key driver of changes in soil salinity (Payo *et al.* 2017). The consequences of soil salinity induced by climate change are far-reaching, affecting agricultural productivity and food security. Research has highlighted that soil salinity, exacerbated by climate change, poses a significant threat to global food production, particularly in arid regions with limited water resources (Aiad *et al.* 2021). Furthermore, the negative impact of soil salinization on plant growth and living organisms is severe, regardless of whether the salinity is of primary or secondary origin (Tedeschi 2020).

Liquid organic fertilizers (LOF) are essential for sustainable agriculture as they provide a valuable source of nutrients for crops and aid in recycling organic waste. Anaerobic digestion of organic materials results in the production of biogas and nutrientrich digestate, which can be utilized as a liquid fertilizer in agricultural practices (Sheets *et al*. 2015). The treatment of LOF as a fertilizer contributes significantly to soil improvement and amendment, enhancing soil fertility and crop productivity (Sudibyo *et al.* 2022). Although LOF has the potential for cascade valorization, it is primarily used as an organic fertilizer in agriculture, underscoring its role in sustainable soil management (Weckerle *et al.* 2022). Research has demonstrated that LOF is an effective nutrient source for plant growth, with the capacity to enhance crop performance and nutritional status. The utilization of LOF as a liquid fertilizer can improve soil fertility, supporting the growth of various crops and enhancing agricultural sustainability (Valentinuzzi *et al.* 2020). Furthermore, the treatment of LOF in agriculture can help mitigate the environmental impact of organic waste by converting it into a valuable resource for crop production. Through managing LOF effectively as a liquid fertilizer, agricultural systems can benefit from improved nutrient cycling, reduced waste generation, and enhanced soil health (Tampio *et al.* 2016). The utilization of LOF in agricultural waste recycling exemplifies a holistic approach to sustainable agriculture, emphasizing the importance of nutrient recovery and resource efficiency in farming practices (Riya *et al.* 2021). Several studies have been conducted on stressed plants using liquid organic fertilizer. One of these studies examined the effects of fertilization on oat seed germination, plant growth, enzyme activity, and soil physical and chemical properties under moderate salt and alkaline stress. The results showed that 80% of liquid organic fertilizer applications significantly promoted oat seed germination and plant growth. After chemical fertilizer and liquid organic fertilizer application, root activity reduction intensity in oat roots, APX, POD, CAT, and SOD activities, proline and soluble sugar content in oat leaves increased in all application groups, while malondialdehyde content decreased (Zhao *et al*. 2023). In another study, liquid organic fertilizer (LOF) research showed benefits for plant production and soil fertility. These benefits include greater soil water holding capacity and plant nutrition availability. These changes may improve a plant's drought tolerance. Under 100% water need, foliar LOF application at 1.50 L/fed was more effective for the analyzed parameters. LOF boosted sunflower yield characteristics greatly over the control. The irrigation treatments with LOF foliar spray boosted sunflower water usage efficiency. LOF at 1.50 L/fed improved sunflower seed yield components, carbohydrates, protein, oil, flavonoids, and antioxidant activity (DPPH radical scavenging) during water stress. This dosage may maximize sunflower output (El Sayed *et al*. 2022).

Arugula (*Eruca sativa*) holds economic importance due to its widespread cultivation in Mediterranean countries and increasing consumer demand, especially in the Central Europe region (Kavga *et al.* 2018). This leafy vegetable is extensively consumed for its nutritional value, as an aphrodisiac, and as a medicinal herb (Yildirim *et al.* 2019). Its adaptability in both open field and greenhouse cropping systems contributes to its economic significance, with its popularity leading to its cultivation in various regions worldwide (Kavga *et al.* 2018). The high nutritional content of arugula, including glucosides, flavanols, mineral salts, vitamins A and C, and antioxidants, adds to its economic relevance (Plaksenkova *et al.* 2019). Additionally, arugula's use in culinary applications and its potential health benefits, such as anti-inflammatory properties and antioxidant activity, further enhance its economic value (Fratianni *et al.* 2014; Fuentes *et al.* 2014). The economic importance of arugula is underscored by its role as a valuable crop with diverse applications in the food, pharmaceutical, and agricultural industries, making it a significant component of the global market (Bell *et al.* 2015). The volatile components of arugula, affect its profile and health advantages. Isothiocyanates, glucosinolates, and flavonoids provide arugula its scent, flavor, and possible medicinal benefits (Michell *et al.* 2020). Arugula's organosulfur isothiocyanates have anticarcinogenic, anti-inflammatory, and antiproliferative properties (Ku *et al.* 2016). Glucosinolates and isothiocyanates in arugula provide bitter and acidic aromas, as noted for cruciferous vegetables (Bajpai *et al.* 2012). These chemicals may have antioxidant and antibacterial activities (Antonious 2023; Amanpour *et al.* 2024). Additionally, arugula's volatile components have been researched for sensory qualities and food safety uses. For example, flavonoids such quercetin, kaempferol, and isorhamnetin give arugula antioxidant properties (Corleto *et al.* 2018; Rostami *et al.* 2022). Arugula's volatile chemicals have been studied for their function in plant defense mechanisms, such as glucosinolate-derived nematicidal action (Veloso *et al.* 2019). A major volatile compound considered in this study was 4-(methylthio) butyl isothiocyanate, also known as erucin. This aliphatic, sulfuric compound is found in brassica vegetables and has demonstrated antitumor activity both *in vitro* and *in vivo*. It is considered a hydrolytic product from *E. sativa* and has been studied for its potential in modulating pathways related to cancer, such as the PI3K/Akt/mTOR pathway (Singh *et al*. 2021). Additionally, it has been associated with chemo-preventive and chemo-therapeutic activities, particularly in liver cancer and cancer stem cells (Lamy *et al.* 2013; Li *et al*. 2014).

In this study, the effects of liquid organic fertilizer (1%, 5%, and 15%) obtained by fermentation on various germination and emergence parameters of arugula seeds at various salt levels (0, 75 mM, and 150 mM) was first investigated. Then, the effect on the growth parameters and volatile components of the arugula seedlings at the end of the $60th$ day were analyzed.

EXPERIMENTAL

Materials

Eruca sativa Mill. cv. 'Bengi' (known as arugula, rocket salad, rocket, true rocket, roquette, and white pepper) seeds were purchased from the Malçok Agriculture Company. Salt (NaCl) was purchased from Sigma-Aldrich, peat and perlite from Klasmann, liquid organic fertilizer (LOF) from Seleda, and NPK (15% N, 15% P2O5, and 15% K2O) fertilizer from Ege Gübre Company.

Anaerobic digestion to obtain LOF is a process in which organic compounds are biodegraded in an airless environment and biogas is obtained as a product. In general, the anaerobic digestion process takes place in four main reaction steps. These steps are hydrolysis, acidification, acetate production, and methane production. As a result of these steps, the organic matter, which will be referred to as raw material, is transformed into a smaller and degradable form. As a result, LOF is produced by grinding and suspending the enzyme-rich fermented organic liquid matter and multiple solid organic matter mixtures taken from the plant at a size of $2 \mu m$. LOF is obtained as a result of these reactions. Its analysis details are provided in Table 1.

Table 1. Liquid Organic Fertilizer Analysis Details

Experimental Design

Planting and application details

The study was designed in three different growth media.

- 1. Arugula seeds were pretreated with LOF (1%, 5%, and 15%) for 1 h. Then, the seeds were germinated in petri dishes in the dark under salt stress (0, 75, and 150 mM) for seven days in a climate chamber. At the end of the seventh day, various *germination parameters* were determined.
- 2. Arugula seeds were pretreated with LOF (1%, 5%, and 15%) for 1 h. Then, the seeds were germinated in peat $+$ perlite pots in the dark under salt stress $(0, 75, 0.5)$ 150 mM) for seven days in a climate chamber. At the end of the seventh day, various *emergence parameters* were determined.
- 3. Arugula seeds were placed in peat + perlite mixture pots without pretreatment and grown under salt stress $(0, 75,$ and 150 mM) for 60 days. The LOF $(1\%, 5\%,$ and 15%) and NPK were applied directly to pots. At the end of 60 days, various *growth parameters* were determined, and *volatile compounds* were analyzed in the seedlings in pots that had been outdoors and in daylight for 60 days.

The study was performed in 12 groups for the germination and emergence phases (7 days). These are 3 salts $(0, 75,$ and 150 mM), 4 pretreatments of LOF $(0, 1\%, 5\%,$ and 15%), and 3 repetitions in each group. The other study was performed in 15 groups for the

outdoor phase (60 days). These are 3 salts (0, 75, and 150 mM), 5 treatments (0, LOF (1%, 5%, and 15%), and NPK), and 3 repetitions in each group. *Germination and emergence parameters*

Arugula seeds were sterilized in 1% sodium hypochlorite (Shabana *et al.* 2021; Ali *et al.* 2023) for 10 min, then washed five times with pure water and left to dry on filter papers at room temperature. Petri dishes, filter papers, and other glass materials to be used in the study were dried and sterilized in the oven. Seeds were germinated in 12 different groups to obtain both germination and emergence data. Three repetitions were used in each group, and 25 seeds for germination and 50 seeds for emergence were used in each repetition. Seeds that were plump in appearance and generally equal in size were selected for pretreatment. Then, the selected seeds were kept in beakers containing pure water, 1% LOF, 5% LOF, and 15% LOF for 1 h before germination (Baştabak 2019). At the end of the period, some of the seeds were planted with distilled water (control) with 75- and 150 mM salt solutions (10 mL each). A total of 25 seeds were placed in each petri dish (with a diameter of 12 cm) with double-layer filter paper. Another part of the seeds was planted in 50 pieces each in peat-perlite (1:1) mixture 1-L pots were watered (50 mL each) with distilled water (control), in 75- and 150-mM salt solutions to determine emergence parameters. Petri dishes and pots were left to germinate in constant darkness in a plant growth cabinet set at 20 °C for seven days. The data were obtained at the end of the seventh day for germination/emergence parameters. Separate calculations were made for germination and emergence values in all formulas. These determinations were made with the following formulas.

Germination or emergence (%)

The total number of seeds that germinated (the radicle came out by piercing the seed coat) and emerged (the cotyledon leaves came out to the soil surface) was proportional to the number of seeds planted and calculated as a percentage (Ellis and Roberts 1981).

$$
Germanation % = \frac{Number of seeds germinated or emerged}{Total number of seeds} \times 100
$$
 (1)

Germination or emergence speed

The germination/emergence speed of the seeds counted daily was calculated according to Eq. 2 (Czabator 1962; Gairola *et al.* 2011),

$$
Speed = n_1/d_1 + n_2/d_2 + n_3/d_3 + ... \tag{2}
$$

where *n* is the number of seeds germinated or emerged and *d* is the number of days.

Mean germination or emergence time

Mean time (MT) was calculated according to Eq. 3 (Ellis and Roberts 1981),

$$
MT = n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + ... / Total days
$$
 (3)

where *n* is the number of seeds germinated or emerged and *d* is the number of days.

Mean daily germination or emergence

Mean daily (MD) germination/emergence was calculated according to Eq. 4 (Gairola *et al.* 2011):

 $MD = Total number of germinated or emerged seeds / Total days$ (4)

Peak value

The germination or emergence peak value (PV) was calculated with Eq. 5 (Czabator 1962; Gairola *et al.* 2011):

PV= (Highest germination or emergence) / Number of days (5)

Germination or emergence value

Germination or emergence value (*V*) was calculated using Eq. 6 (Czabator 1962; Gairola *et al.* 2011):

$$
V = \text{PV} \times \text{MD} \tag{6}
$$

Treatment of NaCl and LOF to arugula seedlings and obtaining data

Seedlings were grown in 15 different treatment groups for 60 days. Each group underwent three repetitions of the study, each involving six seedlings.

In the first stage of the study, arugula seeds were planted, 25 each, in pots prepared with a mixture of 50% perlite and 50% sterile peat. Then, after 10 days, the seedlings were reduced and six seedlings were left per repetition. The pots were left to grow and develop for 60 days. In salt concentrations, Tort and Türkyılmaz (2003) report that NaCl is generally mentioned in soil salinity and salt stress. For this reason, only NaCl was preferred for salinity stress in the study. Salt levels were planned at 0, 75, and 150 mM NaCl concentrations, in accordance with the literature previously studied with arugula seedlings (Fallahi *et al.* 2015). NaCl solutions were applied to pots with a perlite-peat mixture three times, with gradual increases, at seven-day intervals. The application started with a starting dose of 25 mM and was increased up to 150 mM. The LOF was applied twice, with an interval of 40 days, in three different doses of 1%, 5%, and 15%. The NPK (15:15:15) was applied to the pots as 0.4 g/L. The experiment continued for 60 days, and the following measurements and analyses were made on the seedlings in accordance with the literature.

Arugula seedlings were grown in peat-perlite mixed pots under salt stress for 60 days. In seedlings belonging to saline or non-saline groups applied with LOF, plant length (cm), plant diameter (cm), number of leaves (pieces/seedling), plant fresh weight (mg/seedling), plant dry weight (mg/seedling), water content $(\%)$, and leaf area (cm²) were determined (Turner 1981; Şehirali 1989; Steiner *et al.* 1989; Kırtok *et al.* 1994). At the end of the $60th$ day, the seedlings were removed from the pots before measurements. However, because the seedling roots could not be separated from the peat/perlite mixture in a healthy and whole manner, the root parts were separated, and the measurements were taken in the above-ground part.

First, the plant length and plant diameter of the seedlings were measured with a digital caliper. To determine the fresh weight of the plants, the seedlings were weighed on a precision scale and divided by the number of seedlings to calculate the fresh weight as mg/seedling. To determine the dry weight, the seedlings were dried in a 65 °C oven for 72 h until they reached a constant weight. Afterwards, it was weighed with a precision scale. It was then divided by the number of seedlings and calculated as mg/seedling, similar to fresh weight. Water content (WC) can be expressed in terms of dry weight (DW) or fresh weight (FW) (Turner 1981). In this study, water contents were calculated based on fresh weight according to Eq. 7:

$$
WC (FW) = [(FW-DW) / FW] x 100 \tag{7}
$$

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At the end of the study, leaf numbers and leaf areas were calculated in the samples taken from the plants in each repetition. Six samples representing the number of leaves (mean) per area of the plant were taken and photographed with a ruler, and then leaf areas were calculated as cm^2 using the ImageJ program (Easlon and Bloom 2014; Nasution and Gusrian 2019).

Analysis of volatile components

In the study, above-ground plant samples were taken for the detection of volatile components after 60 days. The samples were kept in locked packages at -20 °C until analyzed. Analyzes were made at SPME-GC at Burdur Mehmet Akif Ersoy University Scientific and Technology Research Center (BİLTEKMER). The oven's starting temperature was 60 °C. After waiting for 2 min at 60 °C, it was increased to 220 °C with an increase of 2 °C per minute. It was held at this temperature for 20 min (Baydar *et al.* 2013; Taherpour *et al.* 2017). The detector and injector temperatures were set at 250 and 240 °C, respectively. Analyses were carried out on the Agilent 5975 C Agilent 7890A device with a CPWAX 52 CB (50 \times 0.25 (0.2)) column.

Statistical evaluation of results

The statistical significance of the differences between the means in the data obtained was analyzed with the Duncan $(P < 0.05)$ multiple comparison test in SPSS (IBM-SPSS Inc., Armonk, NY, USA) version 25.0, and the values are given as mean \pm SD (standard deviation) (Zhang *et al*. 2022).

RESULTS AND DISCUSSION

The effects of LOF on physiological parameters and volatile components in arugula grown under salt stress are presented in Tables 2 and 3, and Figs. 1 through 8.

Results on Physiological Parameters

The results of physiological parameters were obtained from seedlings grown in three different media. On the seventh day, the germination and emergence parameters of the seedlings were examined. At the end of the $60th$ day, plant length (cm), plant diameter (cm), number of leaves (pieces/seedling), fresh weight (mg/seedling), dry weight (mg/seedling), water content $(\%)$, and leaf area $(cm²)$ were determined in the seedlings (Figs. 1 through 7).

The following three stages were taken into consideration when evaluating the findings regarding physiological parameters:

- 1. The lowest and highest result evaluations in each parameter column;
- 2. The lowest and highest results evaluation in the groups without salt (between the control, 1% LOF-0, 5% LOF-0, 15% LOF-0, and NPK-0 groups);
- 3. The LOF and NPK performance evaluation in salt stress (75 and 150 mM) groups. In the groups (three repetitions each) of trial pots where 15% of LOF was applied,

plants could not be obtained because fertilizer burn occurred; therefore, the results of 15% LOF were not included in the table.

Germination parameters

Germination parameters of arugula seeds exposed to salt stress after LOF pretreatment in petri medium were evaluated at the end of seven days, and the inhibition of salt stress on germination was clearly observed in almost all groups (except 15% LOF-150 mM NaCl) in all parameters (Table 2). In all germination parameters, the highest values were observed in the control group, and the lowest values were observed in 1% LOF-150 mM NaCl. The decreases between the highest and lowest values were 62% in germination rate, 80% in germination speed, 73% in mean germination time, 62% in mean daily germination, 73% in peak value, and 90% in germination value. Ince (2021) examined the effects of 150- and 250-mM salinity on arugula and found that germination rate and speed decreased approximately 56% and 72% at 250 mM, respectively. These data are consistent with the authors' findings.

Salinity stress has been found to strongly hinder the germination of *Eruca sativa* seeds. In a recent study it has shown that exposure to high levels of salt can have a negative impact on the germination process and rate of *E. sativa* seeds (Liu 2024). This inhibition of seed germination under salinity stress can be attributed to osmotic stress and ion toxicity, which disrupt the balance of nutrient uptake and can lead to germination delay, inhibition, or complete prevention (Al-Sanabani *et al.* 2018; Corti *et al.* 2023).

In addition, excessive salinity levels can completely prevent seeds from germinating if they exceed the species' tolerance limits (Alshammari 2017). In addition, the decrease in seed germination when exposed to high salinity levels is linked to the hindered mobilization of lipid storage during germination in *E. sativa* (Jia *et al.* 2022). Salinity stress has detrimental effects on plants, as it hampers water absorption and leads to the accumulation of sodium and chloride ions. This disrupts the balance of nutrient uptake and inhibits germination (Al-Sanabani *et al.* 2018). It is clear that *E. sativa* seeds show a high sensitivity to water and salinity stress, as evidenced by the failure of most seeds to germinate under high salinity conditions (Jia *et al.* 2022).

Liquid organic fertilizers obtained from fermentation processes have been shown to effectively promote *Eruca sativa* seed germination without causing phytotoxicity (Phibunwatthanawong and Riddech 2019). Unlike this study, there was no positive effect of LOF pretreatments on germination parameters under non-saline conditions. However, the high salinity (150 mM NaCl), 5% LOF was found to improve compared to distilled water. This improvement caused an increase of 30% in germination rate, 42% in germination speed, 44% in mean germination time, 28% in mean daily germination, 28% in peak value, and 66% in germination value. This suggests that germinating arugula seeds were more tolerant to salt stress with a 5% LOF pretreatment. Anaerobic liquid-fermented manure is thought to have a stimulating effect on seed germination at low concentrations (Emino and Warman 2004; Alburquerque *et al.* 2012). In this context, Baştabak (2019) examined the effects of anaerobic fermented manure on lettuce seed germination at different concentrations and times. At the end of the study, it was stated that the most appropriate pretreatment was 2 h at 1% concentration and 1 h at 5% concentration and that the effect of anaerobic fermented manure on seed germination in seed germination studies can be positive or negative, and this effect may vary according to the concentration of the fertilizer. Çetinkol and Cesur Turgut (2023) examined the effects of salt stress on some growth parameters (percentage of germination over time, germination-hypocotyl percentage, radicle-hypocotyl length, fresh and dry weight, and water content percentage) of arugula. Seven-day-old seedlings of arugula were pretreated with liquid anaerobic

digestate and then exposed to salt stress. Liquid anaerobic digestate was found to partially alleviate the effects of stress in different growth parameters and at different salt levels.

Fermented liquid organic fertilizers can potentially increase seed germination under salt stress through various mechanisms. One possible explanation is related to the impact of salt stress on seed germination inhibition and how the application of organic fertilizers can counteract these effects. For instance, salt stress can induce excessive hydrolysis of arginine-derived urea, leading to an increase in cytoplasmic pH within seed radical cells, triggering salt-induced inhibition of seed germination (Bu *et al* 2024). In this context, fermented liquid organic fertilizers may help maintain a more favorable pH balance within the seed cells, thereby potentially mitigating the inhibitory effects of salt stress on germination. Furthermore, the faster germination observed upon salt stress release in certain plant species suggests that high salinity exposure can trigger an osmo-priming-like effect on seeds, boosting seed vigor and seedling growth (Debez *et al.* 2018). Fermented liquid organic fertilizers may contribute to this priming effect by providing essential nutrients and promoting metabolic processes that support rapid germination under stress conditions.

Emergence parameters

The emergence values of arugula seeds exposed to salt stress after LOF pretreatment in pot (peat + perlite) medium (Table 3) decreased in all parameters at the end of the seventh day due to the increase in salt level. Similar to the current findings, Ince (2021) reported that seed emergence of arugula decreased with 150- and 250-mM salt stress, and at the highest salt dose, emergence rate, emergence speed, average daily emergence, emergence peak value, and emergence value decreased 36%, 53%, 35%, 46%, and 65%, respectively, compared to the control. In all emergence parameters, the highest values were observed in the control group, and the lowest values were observed in 15% LOF-75 mM NaCl. The reductions between the highest and lowest values were 96% in emergence rate, 98% in emergence rate, 97% in average emergence time, 96% in average daily emergence rate, 95% in peak value, and 99.7% in emergence value. No positive effect of LOF pretreatment on germination parameters was observed under non-saline conditions. In the presence of salt (75 mM NaCl), it was observed that a 5% LOF pretreatment significantly alleviated the inhibition effect compared to other pretreatments at the same salt level. Yaraşır (2018) reported that fermented fertilizer treatment of wheat under field conditions did not result in a statistically significant improvement in emergence rates. These results support the current findings because, in this study, the negative effects of salt stress on arugula emergence were significant, while LOF pretreatments did not have a positive effect in any of the stressed or non-stressed conditions.

Salinity stress significantly impacts *E. sativa* emergence parameters by disrupting osmotic balance, inducing oxidative stress, altering metabolic profiles, and reducing essential physiological processes. High salt concentrations in the soil can hinder water uptake by seeds, affecting germination (Corti *et al*. 2023). Additionally, oxidative stress induced by salinity stress can lead to a decrease in osmotic adjustment, impacting photosynthesis and growth (Waheed *et al*. 2022). Salinity stress can also alter the secondary metabolic profile of *E. sativa*, affecting physiological responses and growth (Sarri *et al*. 2021). Furthermore, it can reduce protein content in leaves, affecting overall growth and development (Suhani *et al*. 2023). Salinity stress also decreases plant biomass production,

forage quality, and nitrogen fixation, further inhibiting *E. sativa* emergence and growth (Wang *et al.* 2018).

Liquid fermented fertilizer may face challenges in effectively overcoming the inhibitory effects of salinity stress on emergence parameters due to various factors. The composition of liquid organic fertilizer, particularly in terms of nutrient content, may not always be optimized to counteract the specific effects of salinity stress. Although the fermentation process can enhance the nutrient content of the fertilizer, levels of key nutrients such as phosphorus and calcium may not be sufficient to effectively mitigate the inhibitory effects of salinity on seed germination and plant growth (Adharani *et al.* 2023). Additionally, salinity stress can inhibit nutrient absorption and physiological processes, such as chlorophyll production, posing challenges for liquid fermented fertilizer to support plant growth effectively under saline conditions. Salinity-induced reductions in chlorophyll and other essential compounds may limit the ability of the fertilizer to promote emergence parameters in plants exposed to high salt levels (Shah *et al.* 2017). Furthermore, high salinity levels in the soil can create unfavorable conditions for microbial activity and nutrient availability, potentially affecting the fermentation process and the quality of the liquid organic fertilizer produced. The salinization and acidification of the soil due to high salinity levels can limit the effectiveness of the fertilizer in promoting plant growth under saline conditions (Zhou *et al.* 2018).

Table 2. Effects of LOF Pretreatments on Germination Parameters of Arugula Seedlings Grown for Seven Days under Salt Stress

Table 3. Effects of LOF Pretreatments on Emergence Parameters of Arugula Seedlings Grown for Seven Days under Salt Stress

Seedling Growth Parameters

Arugula seeds were grown in pots (peat-perlite) under salt stress (0, 75, and 150 mM) for 60 days. Unlike the germination/emergence process, LOF (1%, 5%, and 15%) was applied directly to the soil of the seedlings with irrigation water instead of pretreatment in 60-day-old pots. The LOF applications were repeated twice at six-week intervals. Although the experiment was repeated twice, a 15% LOF application caused fertilizer burn in seedlings, and seedlings could not be grown. To compare the performance of LOF treatments, NPK (15-15-15), which is frequently preferred by growers, was added to the experiment as another treatment group. At the end of the $60th$ day, the findings were remarkable (Figs. 1 through 7).

Both LOF (1% and 5%) and NPKs showed positive effects on all growth parameters in all groups and overcame the damaging effect of salt stress. Although not as much as NPK in all growth parameters except water content, 1% and 5% LOF treatments also significantly overcame salt-induced inhibition. In fact, in the presence of 150 mM salt, which is the highest salt level in all parameters except water content, all fertilizer treatments (LOF-NPK) promoted plant growth as if there was no salt, and the results were even higher than the control values. In almost all parameters (except water content), the lowest values were found in the non-fertilized (NF) group at 150 mM, while the highest values were found in the NPK-0 mM group. In the presence of salt stress (75 mM and 150 mM), 5% LOF outperformed NPK in all parameters except leaf number and water content. The positive effects of LOF on 60-day seedling parameters were remarkable. For this reason, each parameter was analyzed one by one.

Plant length

The effects of fertilizer applications on plant elongation of arugula seedlings growing under salt stress were evaluated (Fig. 1). It was observed that salt stress decreased plant elongation in all groups compared to non-stressed conditions. Similarly, Yousif (2016) studied arugula (*Eruca sativa* Mill.) with different concentrations of NaCl (0.00, 0.02, 0.03, 0.10, and 0.13 M) and reported that with increasing salt concentrations, plant elongation was less affected by the two lowest salt concentrations (0.02 M and 0.03 M) but was greatly reduced by the two highest salt concentrations (0.10 M and 0.13 M).

Fig. 1. Effects of LOF application on plant length of arugula seedlings grown under salt stress for 60 days

In the presence of stress, especially 5% LOF was observed to increase plant elongation above the control value with a performance close to the most effective fertilizer, NPK, and all fertilizer treatments were successful and surpassed the control group. In other words, with LOF and NPK applications, seedlings behaved as if there was no salt in the environment, eliminating the stress. In the groups without salt stress, NPK increased plant length 131% compared to the control value, while 5% LOF showed the best performance among LOF treatments and increased plant length by 110%. In the salinity (75 and 150 mM) effect, 5% LOF outperformed NPK, showed the best performance, and was 128% and 123% more successful compared to the control, respectively. Baştabak (2019) reported that in 28-day-old lettuce seedlings, 15% and 5% fermented fertilizer applications increased stem length 17.9% and 8%, respectively, compared to the control.

Liquid fermented fertilizer may not significantly affect the germination rate and speed, but it can increase plant length due to its impact on promoting root growth, enhancing nutrient uptake, and improving overall plant vigor and development. The fertilizer may stimulate root growth and nutrient absorption, leading to increased plant height without directly influencing germination parameters (Ibañez Jr. *et al.* 2023). Factors such as chlorophyll content, total nitrogen, and potassium levels, crucial for plant growth, may be more influenced by the application of the fermented fertilizer, contributing to enhanced seedling height (Aispuro *et al*. 2020). The fertilizer may have a more pronounced effect on the physiological characteristics and growth of seedlings rather than on the initial germination process, resulting in increased plant height (Ebel and Kissmann 2019).

Moreover, the enhanced vigor and health of the seedlings due to the fertilizer may promote elongation and growth, leading to increased plant height even if the germination rate and speed remain relatively unaffected (Riddech *et al*. 2019). The fertilizer's impact on seedling height and the number of leaves may indicate a more substantial effect on later stages of plant development rather than on the initial germination process (Ebel 2020).

Plant diameter

In terms of plant diameter, the inhibition effect of salt was observed in almost all groups (Fig. 2).

Fig. 2. Effects of LOF application on plant diameter of arugula seedlings grown under salt stress for 60 days

Similar to the results, Fidan and Ekincialp (2017) examined the responses of 20 different bean genotypes to 25- and 50-mM salinity and found that shoot diameter decreased in addition to many parameters, especially at 50 mM. When all groups were evaluated together, the lowest plant diameter (0.3 cm) was found at NF-150 mM. The highest (0.68 cm) was observed in NPK-0, with a 127% increase. In the groups without salt stress, plant diameter increased 84% with NPK application, 57% with 5% LOF application, and 22% with 1% LOF application compared to the control. In the presence of salt stress, 5% LOF and NPK equally overcame the stress, and an increase of about 97% was observed at both salt levels compared to the control. The results are consistent with the studies conducted by Sarmento *et al*. (2019) and Bastabak (2019).

Salinity stress can impact plant diameter through various mechanisms, as supported by research on different plant species. Studies have demonstrated that salinity treatments can lead to a decrease in the dimensions of vascular bundles, bundle sheath thickness, phloem tissue, and xylem vessel diameter, all of which can influence overall plant diameter (Sabbour 2002). Additionally, salinity stress can affect stem diameter dynamics, resulting in increased daytime shrinking and inhibited stem growth, which in turn affects plant diameter (Suwa *et al*. 2006). Salinity stress can hinder plant growth by impacting root moisture uptake, reducing water and nutrient absorption, and causing osmotic and toxic effects on plant roots, ultimately affecting stem diameter (Ashour and Othman 2023). In the study, the decrease in plant diameter due to salt stress (Fig. 2) may have decreased due to the effects of these mechanisms.

Under salt stress, liquid fermented fertilizer may increase plant diameter by boosting root development, nutrient uptake, and vigor. Fermented fertilizer can increase plant diameter by stimulating root development, nitrogen absorption, and root system expansion. Under salinity, fertilizer-enhanced root system and vigor may boost nutrient and water uptake, supporting plant growth and diameter. Fermented fertilizer may also improve nutrient availability and water uptake under salt stress, increasing plant diameter. Even in stressful saline environments, fertilizer-facilitated nutrition and water uptake may boost plant growth and diameter (Zhu *et al.* 2022). These mechanisms likely explain why the study's LOF application led to an increase in plant diameter.

Number of leaves

In leaf number, the lowest value among all groups was 3.7 at NF-75 mM. The highest value was 8.4 in the NPK-0 group, with a 127% increase. It was observed that salt stress had a negative effect on leaf number in general (Fig. 3). Similarly, Afsar *et al*. (2020) applied salt stress (150 mM NaCl) to four-leaf plants of arugula grown in hydroponic culture for 2 weeks and reported that the stress significantly reduced the number of leaves. Ince (2021) also reported that 250 mM NaCl salt stress caused a 21% decrease in the number of leaves in arugula leaves compared to the control. In the groups without salt stress, NPK application increased the number of leaves in the control group 87%, 5% LOF application by 44%, and 1% LOF application by 29%. In the presence of salt stress (75 and 150 mM), NPK treatment outperformed LOF treatments, causing a 97% increase compared to 75 mM and a 100% increase compared to NF-150 mM. However, LOF treatments were also very successful. They overcame salt inhibition even at 150 mM and increased the number of leaves well above the control group values. Baştabak (2019) reported that fermented fertilizer application did not show a statistically significant improvement on lettuce leaf number in his study. Iocoli *et al*. (2019) reported that different anaerobic fertilizer contents caused significant increases in lettuce leaf numbers.

Fig. 3. Effects of LOF application on number of leaves of arugula seedlings grown under salt stress for 60 days

Studies have shown that salinity stress can lead to a reduction in the number of leaves, along with other growth parameters such as plant height and biomass (Romadhan *et al*. 2022; Aboh and Ikwa 2023). This reduction in leaf number is often associated with the plant's effort to allocate resources efficiently and prioritize essential functions for survival under stress conditions (Ehtaiwwesh and Emsahel 2020). The application of fermented liquid organic fertilizer can stimulate root growth, improve nutrient uptake, and enhance overall plant vigor, which are essential factors for promoting leaf production (Zhu *et al.* 2022).

Plant fresh weight

Similar to leaf number, the lowest value of plant fresh weight (281.7 mg/seedling) was found at NF-75 mM, and the highest value (2792.2 mg/seedling) was found in the NPK-0 (Fig. 4).

Fig. 4. Effects of LOF application on plant fresh weight of arugula seedlings grown under salt stress for 60 days

In the current study, salt stress caused inhibition in all treatment groups. Similarly, Afsar *et al*. (2020) and Ince (2021) studied rocket, and Fidan and Ekincialp (2017) studied various bean genotypes in the presence of salt stress, and all researchers concluded that fresh weight decreased in the presence of stress. In groups without salt stress, NPK application increased the fresh weight of the control group approximately 8-fold, 5% LOF application 4-fold, and 1% LOF application 2-fold. In the presence of salt stress, 5% LOF showed the best performance, outperforming the other fertilizer treatments. It caused a 7-fold (75 mM) and a 5-fold (150 mM) increase in plant fresh weight values. However, all fertilizer treatments showed much better results than the control group even at the highest salt concentration of 150 mM in plant fresh weight, not only alleviating but also completely overcoming salt stress. Similar to the authors' findings, Baştabak (2019) mentioned that root fresh weight increased significantly (especially at 5%) as a result of applying various concentrations of fermented fertilizer to lettuce.

Salinity stress causes osmotic stress, which reduces water and nutrient uptake and growth, affecting fresh weight. Increased saline levels can lead to lower biomass output and fresh weight due to changes in photo-assimilate distribution (Júnior *et al.* 2018). In the study, it was observed that the salt level affected the fresh weight (Fig. 4). Fermented liquid organic fertilizer improves root growth, nutrient uptake, and plant vigor, which are necessary for fresh weight gain, even under salt stress. The fertilizer's strengthened root system under salt conditions can boost nutrient absorption and plant health, increasing fresh weight. By improving nutrient availability and water uptake, the fertilizer may help plants grow and gain weight, even amid salt stress (Napoleon 2023). In the study, salt stress reduced fresh weight and eliminated the inhibition of LOF (Fig. 4).

Plant dry weight

In plant dry weight, the lowest value (14.3 mg/seedling) was found at the NF-75 mM and the highest value (300.6 mg/seedling) was found in the NPK-0 (Fig. 5). No significant differences were observed in plant dry weight values depending on salt. However, Ince (2021) reported a 22% decrease in plant dry weight and 43% decrease in root dry weight at 250 mM in arugula. In the current study, it is thought that this decrease was not reflected on arugula seedlings because the highest salt level was 150 mM.

However, in the non-saline groups, NPK application increased the dry weight approximately 14 times, 5% LOF application 10 times, and 1% LOF application 4 times compared to the control. These values are quite striking. In the presence of salt stress, 5% LOF showed the best performance, outperforming the other fertilizer treatments, causing 18-fold (75 mM) and 7-fold (150 mM) increase in plant dry weight. However, all fertilizer treatments showed much better results than the control group even at the highest salt concentration of 150 mM in plant dry weight, and not only alleviated but also completely overcame the salt stress. Similarly, Baştabak (2019) observed a 3-fold increase in root dry weight with 5% fermented fertilizer application applied to lettuce, and Ronga *et al*. (2019) reported that liquid anaerobic fermented fertilizer application caused more positive effects on root dry weight compared to standard fertilizer application. Alburquerque *et al*. (2012) stated that, as a result of fermented fertilizer applications at 20%, 10%, 1% and 0.1%, the values obtained from 1% and 0.1% applications in plant dry weight were higher than the control, 20% and 10% fermented fertilizer concentrations. These results in the literature are compatible with the findings.

Salinity stress, as in fresh weight, disrupts growth and development-related physiological processes, affecting plant dry weight. In another study, as in *Eruca sativa*, plant photoassimilate distribution changes reduced biomass production and dry weight in *Raphanus sativus* under salinity (Abeed *et al*. 2023). In a soybean study, it was demonstrated that fermented liquid organic fertilizer promoted root development, which enhanced nutrient absorption and raise dry weight as in our study. Increased nitrogen uptake by plants is one of the factors that affect the increase in plant dry weight (Hasnelly *et al*. 2021).

Fig. 5. Effects of LOF application on plant dry weight of arugula seedlings grown under salt stress for 60 days

Water content

The highest water content was 95.1% at NF-75 mM and the lowest was 86.6% at NPK-150 mM (Fig. 6).

Fig. 6. Effects of LOF application on water content of arugula seedlings grown under salt stress for 60 days

Although the presence of salt stress was not statistically significant, it caused a decrease in water content in the groups, and it was observed that fertilizer applications did not make a significant improvement on water content. Afsar *et al*. (2020) reported that 150 mM salt level did not cause a significant difference in water content in their study with arugula in hydroponic medium. Similarly, Hniličková *et al*. (2017) applied various salt levels (0, 50, 100, 200, and 300 mmol/L NaCl) to the Astro variety of arugula and mentioned that there was no decrease in relative water content up to 200 mmol/L. These findings support the current results, but studies with different results in different plants were also found. Özlem *et al*. (2021) reached different findings and reported that salt stress decreased leaf relative water content in pepper genotypes.

Fermented liquid organic fertilizer uptake may lead to plants requiring less water to maintain their physiological processes, potentially impacting the water content in plant tissues. Moreover, the organic matter and beneficial microorganisms in fermented liquid organic fertilizer can enhance soil structure and water retention capacity, reducing the plant's reliance on excess water for growth (Freire *et al*. 2014).

Leaf surface area

The lowest value of leaf surface area (1 cm^2) was found at NF-150 mM and the highest value (26.3 cm^2) in the NPK-0 group (Fig. 7). Salt stress generally decreased leaf surface area in all groups (except 5% LOF). Petretto *et al*. (2019) studied the effects of salinity (0, 65, and 130 mM NaCl) on six different arugula genotypes and reported significant reductions in leaf area. Similarly, Afsar *et al*. (2020) reported significant reductions in leaf area in a study in which salt stress (150 mM NaCl) was applied for 2 weeks to four-leaf plants of arugula grown in hydroponic culture. These studies support the current findings. In groups without salt stress, NPK application increased the leaf surface area of the control group approximately 8-fold, 5% LOF application 5-fold, and 1% LOF application 2-fold. In the presence of salt stress, 5% LOF showed the best performance, outperforming the other fertilizer applications. It caused a 9-fold (75 mM) and an 11-fold (150 mM) increase in leaf surface area values. However, the leaf surface area in all fertilizer treatments outperformed the control even at the highest salt concentration of 150 mM and overcame salt stress.

Salinity stress can lead to a decrease in leaf expansion, leaf area, and leaf growth, affecting the overall leaf morphology and development (Yu *et al*. 2019). The increase in leaf area of fermented organic fertilizer in various plants supports our studies (Sulok *et al*. 2020; Sanni 2016)

Fig. 7. Effects of LOF application on leaf surface area of arugula seedlings grown under salt stress for 60 days

Volatile Components

Volatile component analyses of arugula, above-ground samples collected at the end of the 60th day, were carried out by SPME-GC at Burdur Mehmet Akif Ersoy University Scientific and Technology Application and Research Center. The volatile component ratios were defined as percents (%), and the major components common to all groups were evaluated. Some volatile components other than the major component 4-methylthiobutyl isothiocyanate, shown in Fig. 8, were also detected at higher than 1%. These components were as follows: In the non-fertilized group, oxirane, (ethoxymethyl)- and 2-pentene, ethanethioamide at NF-75 mM, and 3,3 epoxymethano-6-(3'-isopropenylcyclopropen-1'-yl)-6-methyl-2-heptanone, 3-chloro-1-deuterohex-1-ene, ethanol, 2,2'-thiobis, and propane at NF-150 mM were detected. In the 1% LOF group, propane, oxirane, [(methylthio)methyl]- and butanenitrile, 4- (methylthio)- at 0 mM, trideuteroethene at 75 mM, trideuteroacetonitrile at 150 mM, and ethanol, 2-(methylthio)- were detected. In the 5% LOF group, acetaldehyde, hydroxyfuran, 2-methyl indole at 0 mM, oxirane, 2,2-dimethyl, and butanenitrile, 4- (methylthio) at 75 mM, and butyl glyoxylate, ethanol, 2-(methylamino), 1,2:8,9 diepoxy-p-menthane-7-yl acetate at 150 mM were found. In the NPK group, benzene, isocyano, 1-butanol, 2-methyl *at 0 mM*, propane, 1-(methylthio)- and propane, 1- (methylthio) at 75 mM, and disulfide, dimethyl and trisulfide, dimethyl at 150 mM were observed.

Fig. 8. Effects of LOF application on major volatile component of arugula seedlings grown under salt stress for 60 days

Jirovetz *et al*. (2002) and Yehuda *et al*. (2009) performed volatile component analysis in GC-SPME with *Eruca sativa* L., and as a result of the analysis, especially isothiocyanates, butane, hexane, octane, and nonane derivatives were detected as aroma-effective compounds. Abiotic environmental stresses essentially salinity and drought have the most effectiveness on aromatic and medicinal plants (Heidari *et al*. 2008). Based on the provided references, it is evident that salinity can have varying effects on volatile compounds in plants. Research has shown that soil salinity levels can significantly impact yield components, volatile oil yield, total carbohydrates percentage, and total chlorophyll content in plants such as common sage (Hegazy *et al*. 2021). Overall, the impact of salinity on volatile compounds in plants appears to be complex and context-dependent, with studies highlighting both positive and negative effects on plant physiology, biochemistry, and interactions with the environment. Research indicates that the fermentation of organic materials can lead to the synthesis of various compounds, including volatile organic compounds, which have the potential to influence plant growth and metabolism. These volatile compounds, such as organic acids and alcohols, may impact plant physiology and biochemistry, ultimately affecting plant growth, yield, and the production of bioactive compounds (Tuccillo *et al*. 2022). Moreover, studies have shown that the application of organic fertilizers can enhance plant growth, yield, and the content of bioactive compounds in plants. Organic fertilizers can improve nutrient availability, soil health, and microbial activity, which could influence the synthesis and release of volatile compounds by plants (Matłok *et al*. 2019). In this study because the ratios of the major component in the groups had a fluctuating distribution, no significant correlation could be found between the groups.

CONCLUSIONS

- 1. Various parameters were examined in arugula seedlings germinated after seven days in petri dish. Salinity-induced inhibition of germination parameters was significant and 5% liquid organic fertilizer (LOF) pretreatment at 150 mM alleviated the stress.
- 2. Various parameters were examined in arugula seedlings emerging after seven days in pots. The emergence values decreased in almost all parameters due to the increase in salt level. The LOF treatments could not eliminate the stressinduced inhibition. 5% LOF pretreatment at 75 mM alleviated the inhibition pressure compared to other pretreatments.
- 3. Various growth parameters were examined in arugula seedlings after 60 days in pots. In the non-fertilized group, salt-induced inhibition had a significant effect on all parameters. LOF (1% and 5%) and nitrogen/phosphorus/potassium (NPK) treatments had very positive effects in all groups and overcame salt stress in all parameters except water content. Although not as much as NPK, LOF applications had positive effects that were significant enough to surpass the control values. Because the pots with 15% LOF had fertilizer burn, the experiment was terminated in these pots.
- 4. The above-ground samples collected at 60 days were analyzed for volatile components, and 4-methylthiobutyl isothiocyanate was found to be the most common major component in all groups. No significant and consistent increase or decrease due to salt stress or fertilizer was observed in the major component.

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