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# Impacts of plant growth-promoting bacteria, compost and biodynamic compost preparations for alleviating the harmful effects of salinity on essential oil characteristics of lavender

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## Abstract

**Background** Biodynamic agriculture and the use of plant growth-promoting bacteria (PGPBs) have been demonstrated to offer various benefits for achieving agricultural sustainability. The aim of this study was to evaluate the effects of PGPBs *Azotobacter* and *Azospirillum*, compost, and compost with biodynamic preparations (BD) on the essential oil (EO) characteristics of lavender under salinity stress.

**Research methods** The experiment was carried out in a greenhouse for 2 years and involved three factors: four PGPBs, three types of compost, and three levels of salinity stress.

**Results** The results indicated that the essential oil (EO) characteristics increased with 50 mM NaCl but decreased with 100 mM NaCl. Salt stress reduced the cell membrane stability (CMS) and auxin content, while increasing proline contents. However, the application of PGPBs, compost, and compost with biodynamic preparations had an opposite effect on CMS, auxin, and proline parameters compared to salt stress. Based on the results, the treatment that combined compost + BD with *Azotobacter* was found to be the most effective in enhancing the EO characteristics under both mild and severe salinity stress conditions.

**Conclusions** The results of this study suggest that compost, biodynamic compost preparations, and PGPBs could be useful in enhancing the EO in medicinal plants and alleviating the adverse effects of salt stress on plants.

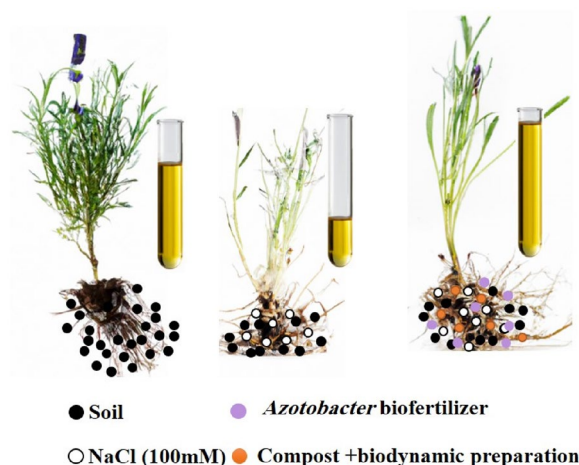
**Keywords** Abiotic stress, *Azotobacter*, Organic farming, NaCl, Sustainable agriculture

## Graphical Abstract

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## Background

Lavender (*Lavandula officinalis* L.) is a shrub cultivated in Europe and the Mediterranean region for its essential oils, which are used to produce cosmetic, aromatherapy, and pharmaceutical ingredients [1]. Medicinal plants have played a significant role in the treatment of diseases throughout the history of mankind [2, 3]. Soil salinity has severely limited agricultural sustainability in different parts of the world, particularly in arid and semi-arid regions [4–6]. Jamil et al. [7] reported that approximately 50% of arable lands worldwide will be affected by salinity by 2050. Currently, great consideration is being directed towards the application of different strategies, such as plant growth-promoting bacteria (PGPB) and biodynamic preparations, which are not only environmentally friendly but also promote healthy plant growth [8]. Therefore, these strategies are promising in terms of simultaneously mitigating salinity and enhancing plant production [9]. The PGPB is a new eco-friendly technology for mitigating biotic and abiotic stresses on plants. This method is useful in reducing the application of chemical fertilizers [10]. The PGPB involves the use of living microorganisms, such as rhizobacteria and fungi. Biodynamic agriculture, introduced by Rudolf Steiner, is an organic agricultural strategy [11]. The biodynamic (BD) preparations, produced using cow dung and some medicinal plants, can provide benefits in achieving agricultural sustainability [12]. In a study by Le Campion et al. [13], a combination of various organic farming

systems, such as PGPBs and biodynamic preparations, was found to produce high-quality agricultural produce.

The soil salinity is becoming a serious issue for agricultural sustainability worldwide. Lavender is a valuable crop used in various industries, but its production can be significantly affected by salinity stress. The use of environmentally friendly strategies, such as PGPBs and biodynamic preparations, can enhance plant growth and mitigate the negative effects of salinity [14, 15]. Investigating the effectiveness of these strategies on lavender production can provide insights into sustainable agriculture and the development of eco-friendly technologies. Therefore, the present study aimed to investigate the effectiveness of using PGPBs and biodynamic preparations to ameliorate the detrimental impact of salinity stress on the lavender essential oil.

## Methods

### Set-up experiment

The lavender seeds initially underwent a disinfection process, consisting of a 30-s alcohol (70 °C) treatment, followed by a 2-min exposure to sodium hypochlorite (2%). The experiment was conducted in a greenhouse setting and utilized a fully randomized factorial design with three replications. The study focused on three factors: biodynamic compost (control, compost, and compost + biodynamic preparations), PGPBs (control, *Azotobacter*, *Azospirillum*, and a combination of the two bacteria), and salinity stress in the form of hydroponic

application (normal conditions (control), 50 mM sodium chloride, and 100 mM sodium chloride).

### Greenhouse condition

In this study, a greenhouse experiment was conducted to investigate the growth and development of plants under controlled environmental conditions. The temperature and humidity inside the greenhouse were regulated using a split system and timed sprinklers, respectively, to maintain optimal growing conditions for the plants. The temperature was maintained at approximately 21 °C during the day and 15 °C at night, while the humidity was set at around 60%. In addition, the plants were arranged in pots with adequate spacing to prevent shading and ensure uniform light distribution.

### Composting process

The production of compost in this study involved the use of plastic barrels measuring 1.5 m in height. The process began with the preparation of natural and uncontaminated soil, which was then placed in the barrels at a depth of 5 cm. To provide carbon, a mixture of plant residues such as dried debris, mulch, leaves, twigs, and branches was used, with 70% of the material coming from this source. Platanus tree leaves were also included in the composting process. For nitrogen, 30% of the material was obtained from green leaves and stems of *Pelargonium* plants, as well as green leaves and stems of *Aspidistra elatior* plants. Lime was added to the compost to adjust soil pH and strengthen calcium levels, with 200-g packages added to each barrel. Chlorine-free distilled water was added to provide moisture for microbial activity. Livestock manure (60 kg) and leaf soil were included to layer the compost, which was then topped with a layer of loamy soil. The combined weight of all organic waste amounted to around 480 kg. The composting process lasted approximately 6 months and required frequent mixing and stirring every 10 days to enhance decomposition and oxygenation. The barrels used to hold the compost were equipped with holes to facilitate oxygenation, which were created with a long and sturdy stick. After each layer of compost was added, an additional layer of loamy soil was included. The barrels were sealed tightly to prevent the entry of any unwanted materials or compounds, thus raising the temperature of the compost, which typically runs 8 to 10 °C higher than ambient

temperatures. At the beginning of the composting process, the temperature and moisture level were 43 °C and 60%, respectively. The results of compost physical and chemical properties at the initial stage of study are presented in Table 1.

### Biodynamic compost preparations

This study utilized biodynamic compost preparations 502 to 507, in the form of a package containing six biodynamic agricultural preparations. Preparation 502 made from yarrow flowers to help regulate the decomposition process; preparation 503 made from chamomile flowers to stimulate the growth of beneficial microorganisms; preparation 504 made from stinging nettle to provide a source of nitrogen; preparation 505 made from oak bark to balance the mineral content; preparation 506 made from dandelion flowers to break down silicates; and preparation 507 made from valerian flowers to promote nutrient absorption by the plants [16–18]. The biodynamic preparations were purchased from the Josephine Porter Research Institute in Virginia, USA ([www.jpibiodynamics.org](http://www.jpibiodynamics.org)). It comprised packs of six biodynamic preparations that could be used for 15 tons of compost. In this study, 1.5 g of each of the six preparations was added in each barrel. After completing the composting process and allowing it to sit for 10 days in each barrel, six 5–10 cm holes were created in each barrel, and one teaspoon of each biodynamic compost preparation was added into the holes. The production of biodynamic compost follows the same composting process as regular biodynamic compost, and all the necessary requirements for biodynamic compost production must be met. The components of biodynamic compost, including soil and plants, adhere together completely, resulting in a cohesive mixture that holds together when compressed. In contrast, soil lacking biodynamic material does not adhere together as effectively, causing its components to scatter.

### PGPBs

The second variable examined in this experimental design was the use of PGPBs. The study utilized bacteria from the genus *Azotobacter* and *Azospirillum* sp. The PGPBs used in this study were acquired from the Golestan Agricultural and Natural Resources Research Center located in Gorgan, Iran. The bacteria were grown in a nutrient

**Table 1** Physical and chemical properties of compost at the initial stage of study

EC (dS m <sup>-1</sup> )	pH	T.N.V (%)	O.M (%)	P (mg kg <sup>-1</sup> )	K	Fe	Mn	Zn	Cu
2.52	7.34	2.07	79.59	10.8	656	3570	146.2	48.75	17.96

broth medium at a temperature of  $27 \pm 2$  °C for 24 h, with continuous shaking at 150 rpm, and their density was measured at 600 nm [19]. The bacterial inoculum used in this study contained an estimated  $10^8$  bacteria per gram. Prior to planting, the samples were inoculated with the bacterial solution by placing them in a plastic container 1 day in advance. The appropriate concentration of the PGPBs was added to the container, which amounted to 500 ml for 9 kg of seeds. To maximize seed inoculation, the container was subjected to shaking for an hour, allowing the bacteria to penetrate through the seed coat. The samples were then dried in a shaded environment and subsequently planted.

### Analysis performed

In this study, various parameters in the leaves of the plant were measured, i.e. the stability of the cytoplasmic membrane, proline content, auxin concentration, and essential oil content. To measure the stability of the cytoplasmic membrane, the selected leaves were placed in a mannitol solution with a potential of  $-2$  and incubated at 20 °C for 24 h. After the incubation period, the electrical conductivity of the solution was measured as an indicator of the membrane stability.

For the analysis of proline content, 0.5 g of fresh leaf samples were weighed, and 10 ml of 3% sulfosalicylic acid solution were added to the samples. The mixture was homogenized in a mortar, filtered through Whatman No. 1 filter paper, and collected in test tubes. To this, 2 ml of ninhydrin reagent and 2 ml of concentrated acetic acid were added to 10 ml of the extract. The test tubes were incubated at 100 °C for 1 h, followed by the addition of 4 ml of toluene. The toluene layer was separated from the liquid part, and its optical absorption at 520 nm was measured using a spectrophotometer (Perkin Elmer, USA).

To measure the concentration of auxin, 1 g of leaf tissue from the top of the stem and the root were separately boiled in 10 ml of 80% ethanol. After filtration, 1 ml of the extract was mixed with 2 ml of Salofsky's reagent, which was prepared by mixing 0.5 M FeCl<sub>3</sub> solution with 35% perchloric acid. The mixture was incubated at 40–50 °C for 15 min, and the optical absorption of the pink-colored extract was measured at 530 nm. The amount of indole-3-acetic acid (IAA) was calculated using a standard curve in the range of 0 to 40 mg/liter, which was drawn using pure IAA.

Finally, a total of 30 g of dried flowers were subjected to hydro-distillation in a Clevenger-type apparatus for 3 h. This procedure was repeated three times to ensure accuracy and consistency of the results.

### Statistical analysis

In this study, we evaluated the effects of three factors, salinity, compost, and plant growth-promoting bacteria (PGPBs), on lavender characteristics using a three-way ANOVA. Data analysis was carried out using the Statistical Analysis System (SASv9.4) software, and the least significant difference (LSD) test was used to compare the mean values at a significance level of  $P < 0.01$ . To visualize the data, graphs were generated using Microsoft Excel and GraphPad Prism version 5 for Windows.

### Results

The findings of this study indicate that all three factors investigated—compost, PGPBs, and salinity stress—had notable and statistically significant effects on all of the measured variables ( $P < 0.01$ ; Table 2).

However, the triple interaction between the factors was only found to be significant for the CMS character, while it was not significant for the other measured traits.

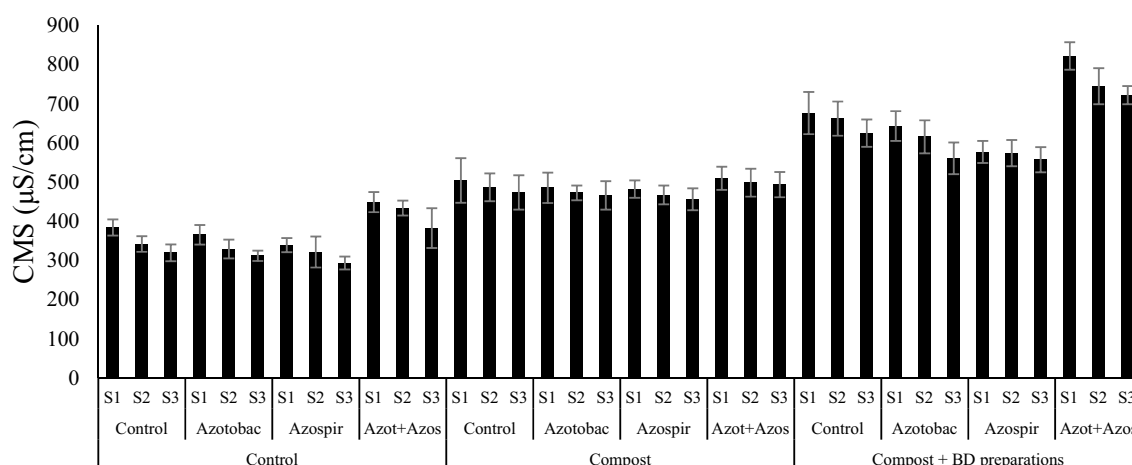
With increasing salt stress, cell membrane stability (CMS) and auxin contents decreased and proline

**Table 2** Three-way ANOVA for the measured characters in lavender

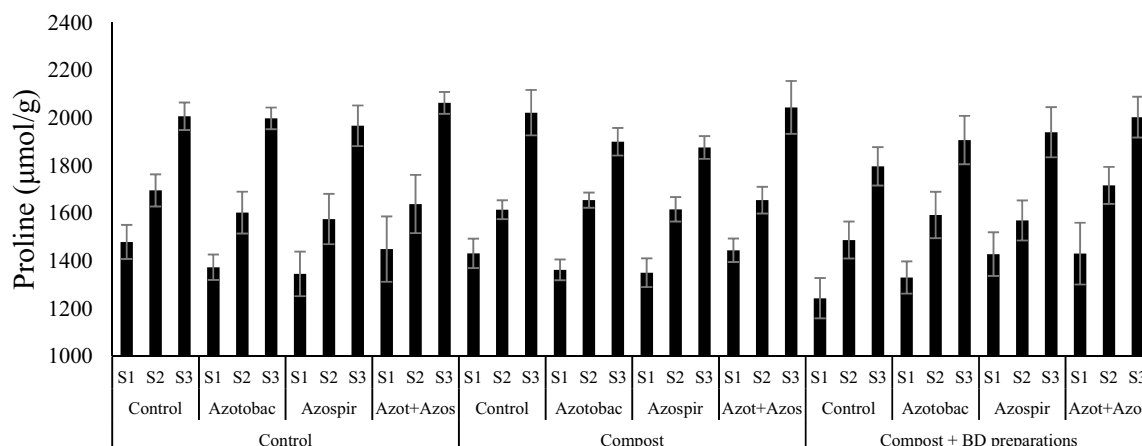
S.O.V	df	CMS	Proline	Auxin	EO weight	EO volume	EO percentage
Compost	2	**	**	**	**	**	**
PGPB	3	**	**	**	**	**	**
S	2	**	**	**	**	**	**
Compost *PGPB	6	**	ns	ns	ns	ns	ns
Compost *S	4	**	ns	ns	ns	ns	ns
PGPB *S	6	**	ns	ns	ns	ns	ns
Compost *PGPB*S	12	**	ns	ns	ns	ns	ns
Error	72	30.02	2463	13.65	3.8	0.05	0.02
CV (%)	–	1.1	2.99	11.34	16.27	14.43	9.32

ns no significant, S salinity

\*\* significant at  $P < 0.01$



**Fig. 1** Interaction effects of the three studied factors on CMS content of lavender. S1, S2, and S3 are salinity at 0, 50, and 100 mM, respectively. Azot *Azotobacter*, Azos *Azospirillum*



**Fig. 2** The effects of the three studied factors on proline content of lavender. S1, S2, and S3 are salinity at 0, 50, and 100 mM, respectively. Azot *Azotobacter*, Azos *Azospirillum*

contents increased (Figs. 1, 2 and 3). Under both mild and severe salt stress, the highest CMS values were observed with the interaction of compost + BD with *Azotobacter* + *Azospirillum*, as shown in Fig. 1. As indicated by Figs. 1 and 3, the levels of CMS and auxin increased in the order of control, compost, and compost + BD treatments. Additionally, based on the same rank order, there was a decrease in the proline content (Table 3).

In all treatments (except for non-salt stress conditions without any applied factors), moderate salinity (50 mM) resulted in significantly higher levels of essential oil characters compared to the control (Fig. 4). Conversely, at high salinity (100 mM), EO levels were significantly lower than those in the control (Fig. 4).

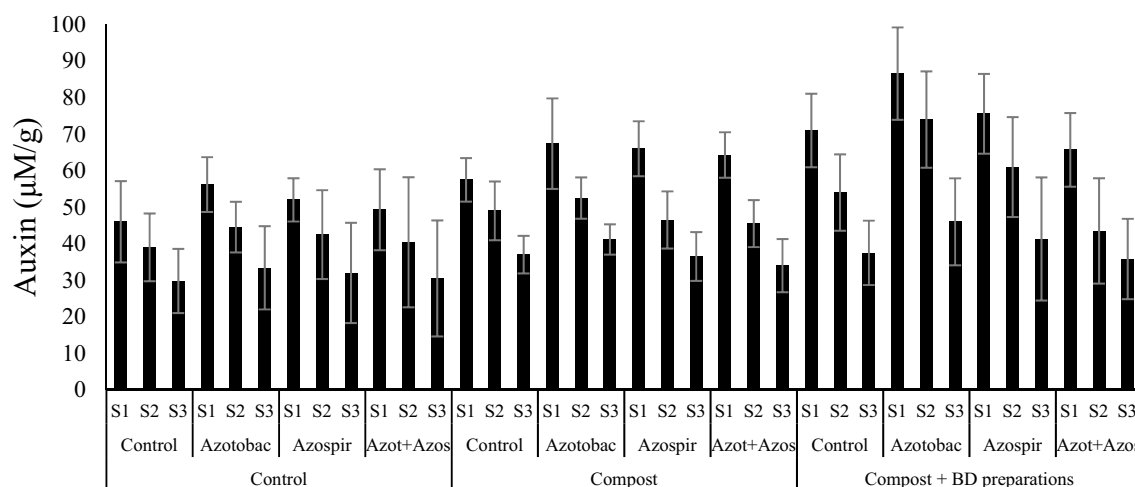
The application of *Azotobacter*, *Azospirillum*, and their combination resulted in significantly higher values of the essential oil (EO) characteristics compared to the control

(Table 3). However, the individual application of *Azotobacter* was found to be more effective in enhancing the EO characteristics compared to the application of *Azospirillum* or their combination (Fig. 4). The EO characteristics increased in the order of control, compost, and compost + BD, as shown in Table 3.

Based on the results, it can be concluded that the treatment combining compost + BD with *Azotobacter*, was the most effective in enhancing the essential oil characteristics under both mild and severe salinity stress conditions (Fig. 4).

## Discussion

The results of current study indicated that as salt concentration increased, the CMS decreased. High salt levels could cause nutrient imbalances and could also induce ROS production, which could further contribute



**Fig. 3** The effects of the three studied factors on auxin content of lavender. S1, S2, and S3 are salinity at 0, 50, and 100 mM, respectively. Azot *Azotobacter*, Azos *Azospirillum*

to cell membrane damage [20]. The CMS is an important physiological trait to investigate the effects of salinity stress on plants [21].

The results showed that the combination of compost + BD and *Azotobacter* had the highest values for CMS under both mild and severe salinity stress conditions. The literature indicated that various types of organic amendments, such as vermicompost, vermiwash, biochar, bio-fertilizer, and plant growth-promoting rhizobacteria, enhanced salinity tolerance, improved growth, and increased yield of plants [22]. This was achieved by modifying ionic homeostasis, enhancing the photosynthetic apparatus, improving antioxidant machinery, and reducing oxidative damage [22]. The application of organic amendments and PGPBs has been shown to improve soil fertility and increase the availability of micronutrients such as iron, zinc, and manganese, which are important for maintaining plant cell membrane stability [22, 23]. Additionally, *Azotobacter* is a nitrogen-fixing bacterium that can help to improve plant nitrogen status, which is important for maintaining cell membrane stability [24].

Proline serves as an osmolyte, a metal chelator, and a signaling molecule. It plays a crucial role in preserving membrane structure, preventing electrolyte leakage, and reducing the levels of ROS [25]. Proline enables plants to regulate osmotic adjustment and enhance tolerance to abiotic stressors [26]. In this study, it was found that the compost treated with biodynamic preparations resulted in lower proline content under both mild and severe salinity stress conditions, even without the use of PGPR, when compared to the compost without biodynamic preparations and the control group (Fig. 2).

Previous research has shown that the set of preparations in the BD compost can enhance the nutrient content of the compost and hasten the decomposition process [27]. Furthermore, it has been reported that when plants are treated with fertilizers, their proline content decreases [28]. Therefore, the observed decrease in proline content in the BD compost compared to the compost without BD under both mild and severe salinity stress could be attributed to the high nutritional values resulting from microbial activity promoted by the biodynamic preparations. In contrast with our finding, sprayed the tubers of coloured potatoes with BD preparation 501 caused to increase the concentrations of proline in the tubers of cultivars [29]. The proline did not differ between the biodynamic and conventional growing systems [30]. In same line with the results of present study, a significant decrease in proline content (~21.1%) was observed in 501 biodynamic-treated fruits compared to control, in Paiete vineyard [31].

Also, the results of this study showed that a combination of the two bacteria had the highest proline content. The proline was significantly enhanced with the application of nitrogen-fixing bacteria in organic fennel [32]. Inoculation with *Azotobacter* and *Azospirillum* improved the growth parameters, increased antioxidant activities in both control and salt stress conditions [33]. It has been reported that the application of *Azotobacter* and *Azospirillum* can lead to an increase in proline content in some plants under salinity stress [34, 35]. This could be due to the enhancement of nitrogen availability in plants, which can lead to increased protein synthesis and thus increased proline content [36]. Contrary to the present results, the application of *Azotobacter* and *Azospirillum*



**Table 3** Individual impacts of biodynamic preparations, PGPBs, and salinity on the plant traits

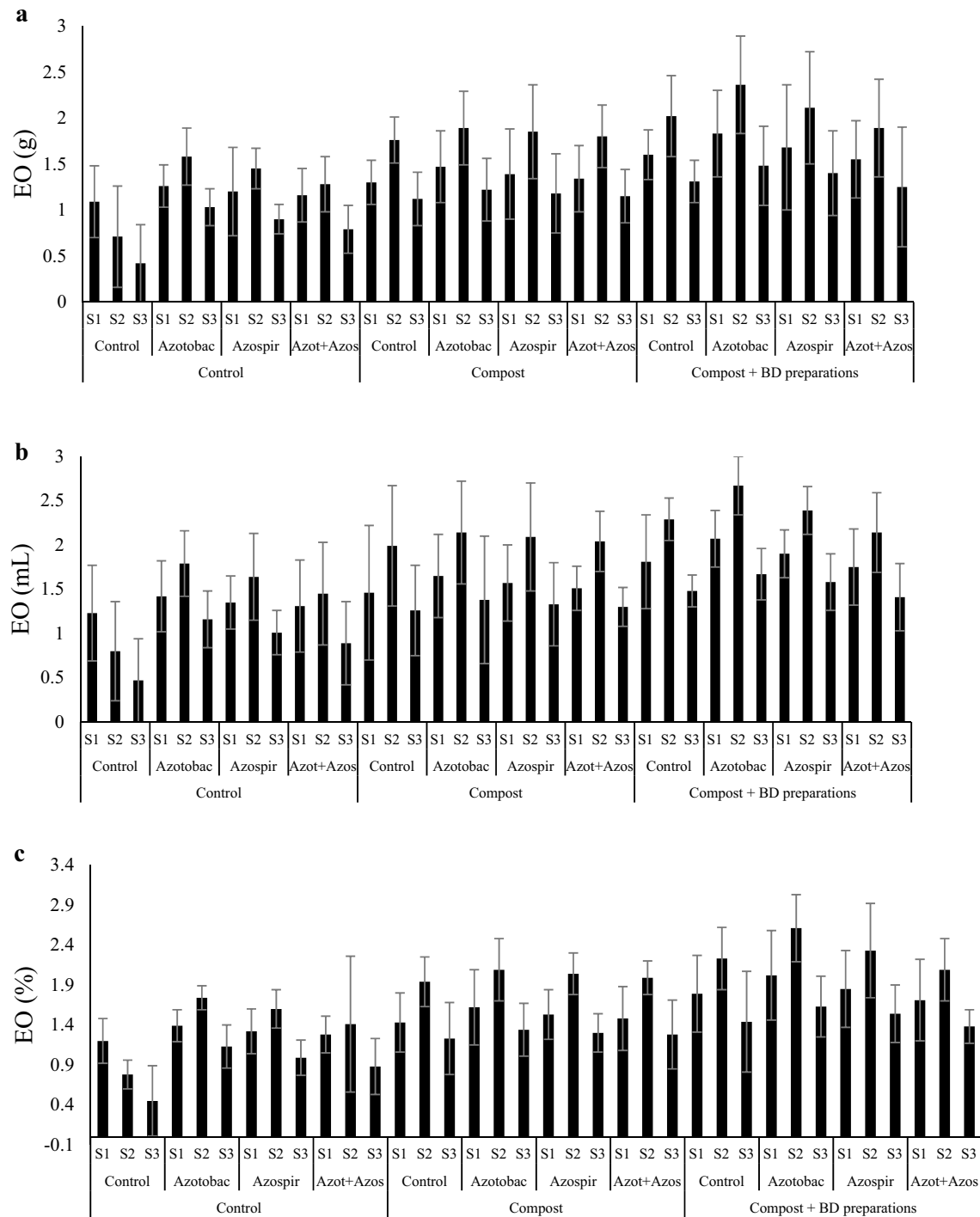
Factors	CMS ( $\mu\text{S}/\text{cm}$ )	Proline ( $\mu\text{mol}/\text{g}$ )	Auxin ( $\mu\text{M}/\text{g}$ )
Compost			
Control	355.58 c $\pm$ 29.92	1683.31 a $\pm$ 59.66	41.3 c $\pm$ 1.68
Compost	482.75 b $\pm$ 23.69	1664.68 b $\pm$ 45.77	49.78 b $\pm$ 2.12
Compost + BD preparations	648.35 a $\pm$ 33.57	1620.94 c $\pm$ 36.94	57.67 a $\pm$ 2.89
LSD (0.01)	3.42	30.95	3.51
PGPBs			
Control	496.83 b $\pm$ 45.66	1642.37 b $\pm$ 67.79	46.76 c $\pm$ 2.56
<i>Azotobacter</i> (A)	471.94 c $\pm$ 23.11	1636.11 b $\pm$ 53.44	55.76 a $\pm$ 3.32
<i>Azospirillum</i> (B)	451.94 d $\pm$ 36.46	1630.33 b $\pm$ 42.93	50.38 b $\pm$ 2.86
A and B	561.52 a $\pm$ 29.83	1716.43 a $\pm$ 54.65	45.45 c $\pm$ 2.53
LSD (0.01)	3.94	35.74	3.51
Salinity stress			
Control	519.67 a $\pm$ 16.53	1389.72 c $\pm$ 37.72	63.13 a $\pm$ 5.47
50 mM	495.42 b $\pm$ 25.82	1618.76 b $\pm$ 38.57	49.37 b $\pm$ 1.31
100 mM	471.6 c $\pm$ 35.57	1960.44 a $\pm$ 26.62	36.25 c $\pm$ 2.85
LSD (0.01)	3.42	30.95	3.51
	EO (g)	EO (mL)	EO (%)
Compost (BD)			
Control	1.07 c $\pm$ 0.1	1.21 c $\pm$ 0.08	1.18 c $\pm$ 0.25
Compost	1.46 b $\pm$ 0.06	1.64 b $\pm$ 0.06	1.6 b $\pm$ 0.16
Compost + BD preparations	1.71 a $\pm$ 0.12	1.93 a $\pm$ 0.07	1.89 a $\pm$ 0.07
LSD (0.01)	0.15	0.14	0.1
PGPBs			
Control	1.26 c $\pm$ 0.1	1.42 c $\pm$ 0.11	1.39 c $\pm$ 0.1
<i>Azotobacter</i> (A)	1.57 a $\pm$ 0.12	1.77 a $\pm$ 0.09	1.73 a $\pm$ 0.09
<i>Azospirillum</i> (B)	1.46 ab $\pm$ 0.08	1.65 ab $\pm$ 0.09	1.61 b $\pm$ 0.08
A and B	1.36 bc $\pm$ 0.07	1.53 bc $\pm$ 0.08	1.5 b $\pm$ 0.07
LSD (0.01)	0.17	0.17	0.11
Salinity stress			
Control	1.41 b $\pm$ 0.11	1.59 b $\pm$ 0.02	1.55 b $\pm$ 0.03
50 mM	1.73 a $\pm$ 0.08	1.95 a $\pm$ 0.06	1.9 a $\pm$ 0.06
100 mM	1.1 c $\pm$ 0.04	1.25 c $\pm$ 0.04	1.22 c $\pm$ 0.04
LSD (0.01)	0.15	0.14	0.1

CMS Cell membrane stability, EO Essential oil

has been reported to cause a reduction in proline content under salinity stress in tall fescue [37]. These contrasting results suggest that the effects of *Azotobacter* and *Azospirillum* on proline content in plants under salinity stress may be context-dependent and influenced by various factors such as soil type, plant species, and inoculum density.

The results demonstrated that increasing salinity levels led to a decrease in auxin content. Auxin acted as a plant growth regulator and was involved in most morpho-physiological processes [38]. Under saline conditions, decreased auxin levels hindered its transport within the plant [39]. In this study, both the compost

with and without BD and both *Azotobacter* and *Azospirillum* were effective in improving auxin production. Certain microorganisms, such as *Azotobacter* and *Azospirillum*, are capable of producing phytohormones-like auxin [40]. Hassouna et al. [41] observed the highest auxin production values in onion cultivars with some isolates of *Azotobacter* and *Azospirillum*. In the present study, compost with and without BD resulted in elevated auxin content compared to the control. However, the compost with BD was more effective in enhancing auxin production than the compost without them. This was likely due to the presence of efficient



**Fig. 4** The effects of the three studied factors on EO characters, i.e. EO weight (a), EO volume (b) and EO percentage (c) of lavender. S1, S2, and S3 are salinity at 0, 50, and 100 mM, respectively. Azot Azotobacter, Azos Azospirillum



microorganisms in the biodynamic compost that were capable of producing auxin [42].

The results of this study revealed that mild salt stress can increase the yield of essential oil (EO) compared to the control. Chrysargyris et al. [43] demonstrated that 100 mM salinity led to a reduction in the EO content of the plant compared to control conditions. Abiotic stresses, such as salinity and drought, commonly affect the essential oil characteristics of medicinal plants [39–42]. The increase in essential oil yield resulting from salt stress may be attributed to a higher density of oil glands and an increase in the total number of glands produced prior to leaf emergence [44]. The stress brought about by salinity can impact essential oil content by altering either net assimilation or the distribution of assimilates among different plant processes [45].

According to the results, there was an increase in essential oil (EO) in the rank order of control < compost < compost + BD. The biodynamic compost used in this study contained a set of seven preparations, designated as BD 502 to BD 507, each with specific nutritional benefits. BD 502 is high in Sulphur (S) and Potassium (K), BD 503 is rich in Calcium (Ca) and S, BD 504 is abundant in S, Ca, Potassium (K), and Iron (Fe), BD 505 is rich in Ca, BD 506 facilitates the absorption of Silicon (Si) and K into the soil, and BD 507 helps regulate the availability of phosphorus in the soil [42]. Therefore, the high concentration of essential oil in the plant by the compost with BD could be due to the high nutritional values in this treatment compared to the control and compost without the BD [46]. The present study demonstrated that the effects of biodynamic preparations, along with *Azotobacter*, enhanced the essential oil characteristics under 50 mM salinity stress. The interaction of Zn element with *Azotobacter* was found to be the most effective treatment in mitigating the harmful effects of salinity stress in soybean [47].

The findings of this study make a significant contribution to the EO industry by demonstrating the potential of biodynamic compost and the use of *Azotobacter* in improving plant growth and yield under salinity stress. The results suggested that the combination of these treatments can enhance the CMS, reduce proline content, increase auxin production, and ultimately lead to higher EO yield. These findings are particularly important for the lavender EO production industry where salinity can be a major issue.

In many parts of Iran, salinity and drought have become the most challenging agricultural problems due to climate change [48]. As a result, it has become increasingly important to develop sustainable agricultural practices

that can mitigate the negative effects of these stressors on plant growth and yield. The study demonstrates that the application of biodynamic compost and *Azotobacter* can be a promising strategy for enhancing plant growth and productivity in regions with high soil salinity.

## Conclusions

The present study demonstrated that the application of compost, biodynamic compost preparations, and PGPBs *Azotobacter* and *Azospirillum* could alleviate the adverse effects of salinity stress on plants. These factors improved cell membrane stability and auxin content of the plant under salinity stress. Therefore, the use of compost, biodynamic compost preparations, and PGPBs *Azotobacter* and *Azospirillum* can be considered as useful strategies for plant salt stress management.

## Abbreviations

BD	Biodynamic
S	Salinity
PGRB	Plant growth-promoting bacteria
EO	Essential oil
CMS	Cell membrane stability
ROS	Reactive oxygen species
IAA	Indole-3-acetic acid

## Acknowledgements

Not applicable.

## Author contributions

PK conceived and designed the research. SAK conducted experiments and wrote the manuscript. MO, HRTM, and FG elaborated on the results and discussion, while doing a critical reading of the manuscript. All authors read and confirmed the current manuscript.

## Funding

No grant was available for this project.

## Availability of data and materials

All data and materials used in this work were publicly available.

## Declarations

### Ethics approval and consent to participate

The ethical approval or individual consent was not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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Received: 27 April 2023 Accepted: 1 October 2023

Published online: 10 October 2023

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