Effect of foliar application with aloe leaf extract (ALE) on vegetative growth, yield, and essential oil percentage of two species of lavender (*Lavandula* spp.)

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Abstract

Lavender (*Lavandula stoechas* L. and *Lavandula angustifolia* Mill.) is an important aromatic crop valued for its essential oil, and sustainable strategies are needed to enhance its productivity under semi-arid conditions. This study investigated the effect of foliar application of Aloe vera leaf extract (ALE) at 0, 40, 80, and 120 ml L^{-1} on vegetative growth, yield components, and essential oil percentage of the two species grown in Erbil, Iraq, using a randomized complete block design with three replications. Plants were sprayed at 15-day intervals, and essential oil was extracted by hydro-distillation and analyzed by GC–MS. Results showed that ALE significantly improved plant height, shrub diameter, number and length of inflorescences, flowering verticillasters, inflorescence yield, and essential oil yield compared with the control. The highest values were generally obtained at 80 and 120 ml L^{-1}, with essential oil yield reaching 29.9 kg ha^{-1} in *L. stoechas* L. and 27.9 kg ha^{-1} in *L. angustifolia* Mill., indicating a dose-dependent response with saturation at higher concentrations. Overall, foliar application of ALE proved effective in enhancing vegetative growth, floral productivity, and essential oil accumulation, highlighting its potential as a natural biostimulant for sustainable lavender cultivation.

Keywords: *Lavandula angustifolia* Mill, *Lavandula stoechas* L. *Aloe vera leaves extract ,essential oil yield*

Introduction

Among the many valuable crops cultivated across the globe, lavender is one of the most important essential oil crops worldwide, which in turn is used in the production of a number of products spanning cosmetics, medicine and aromatherapy [1, 2]. Three of these valuable compounds are linalool and linalyl acetate and camphor, which are characteristic of lavender oil. The concentration of these compounds, in addition to many others, is dependent on a number of factors including species and cultivar, as well as the conditions and methods used for cultivation [3, 4]. The most significant species is *L. angustifolia* Mill., which is greatly different in terms of vegetative traits, oil yield, and chemical composition [1, 2].

The innovative approaches to agriculture which focus on bio stimulants as sustainable alternatives to synthetic fertilizers and growth regulators have been receiving interest from practitioners and researchers alike [5]. Among them, plant-based supplements, and especially Aloe vera leaf extract (ALE), have demonstrated positive effects on growth, physiological activity, and yield of many horticultural and medicinal plants [6-8]. *Aloe vera* is endowed with bioactive polysaccharides, amino acids, enzymes, phenolics, vitamins, and phytohormone-like constituents, which afford natural growth promotion and stress alleviation [5, 9]. Vegetative growth, chlorophyll, and oil of several aromatics and medicinal plants were greatly enhanced through foliar application of ALE [6, 10, 11].

The lavender plant is a perennial herb known for producing essential oil and responds very well to the application of foliar nutrition along with the use of bio stimulants [12-14]. Foliar application of natural and some organic bio stimulator especially those derived from seaweed, amino acids, and chitin and chitosan has been shown to increase vegetative and reproductive growth along with the essential oil yield in several species of *Lavandula* [15-18]. Also, in lavender, some of the essential oil yields and compositions were increased using foliar bio stimulants with certain plant extracts, particularly *Malva parviflora* and *Moringa oleifera*, suggesting that these plant-derived bio stimulators can influence secondary metabolism and oil biosynthetic pathways [8, 19].There is still a very large knowledge gap with the use of *Aloe vera* leaf extract applied as a foliar bio stimulant and how it affects lavender growth and essential oil yield and production.

Considering the wide-ranging physiological differences in growth rate, flowering behavior, and secondary metabolite essential oil composition [1, 2], responses to bio stimulant application will most likely vary from vary between species. Knowing the influence of these traits on lavender cultivation oil yield and quality with a purposeful application of Aloe Vera leaf extract will enhance the sustainable practices in lavender cultivation [17, 20]

This research aims at determining the untapped ability of *Aloe vera* as a natural bio-stimulant for increasing productivity and essential oil yields in sustainable lavender production systems.

Materials and Methods

Experimental Site and Plant Material

The study was done in Erbil, in the Kurdistan Region-Iraq at (longitude 44.0316°E, latitude 36.1508°N and elevation ~ 417 MSL), from February 15 to August 15, during the Year 2024. The area is semi-arid, like the other lavender-producing areas of the Mediterranean Basin, experiencing hot dry summers and mild winters [3, 17]. The soil type was sandy clay loam with moderate fertility (Organic matter OM 0.01%, Electro conductivity EC 0.2 dSm^{-1} and slightly alkaline (pH 7.6).

At the Acacia Plant Nursery (Kirkuk Road – Erbil, Iraq), (one-year-old of *L. stoechas* L. and *L. angustifolia* Mill. cuttings propagated from mother plants) were used in the experimentation. The new plants were transplanted on February 15, 2024, and spaced in such a way to cover 70 cm x 40 cm of the area (around 35,700 plants). The criteria for selection for the two-valued species was the contrast in the profiles of the essential oil and the fact that these two species were [1, 2].

Design and Methodology of the Experiment

The Randomized Complete Block Design (RCBD) design was used for replicating each treatment three times. Plants in the field were treated with foliar sprays at trial amounts that included:

- 0 ml L^{-1} (Control)
- 40 ml L^{-1}
- 80 ml L^{-1}
- 120 ml L^{-1}

Fresh aloe vera leaves were collected from mature plants and thoroughly cleaned with distilled water. After removing the outer epidermis, the inner gel was carefully drained and blended to produce a homogenous mixture. The blended mixture was first filtered through muslin cloth and then through Whatman No. 1 filter paper to remove any remaining fibrous material. The resulting filtrate, which was regarded as the crude extract (100%) was diluted with filtered water to produce the experimental concentrations (0, 40, 80, and 120 ml L^{-1}). The strategies used to instrument the bio stimulants were derived from studies done by [6, 7]. After mixing the *Aloe vera* with purified water, it was sprayed on the plants in a modest amount until water began to flow off of them. Between March and June, the plants were simultaneously covered with the *Aloe vera* mixture, and these dosages were administered every 15 days. According to the suggestions of [8], who suggested that the watering timing be altered to dawn for better effectiveness, the watering was carried out at dawn.

Management of the Crop

All plants were subjected to the same management practices as described for the organic cultivation of lavender [14, 17]. Irrigation was delivered every 10-12 days via the furrow method and was dependent on climatic conditions. Weeding was done by hand and no synthetic fertilizers, pesticides, or herbicides were applied in order to avoid the *Aloe vera* extract's physiological effects [5].

Essential-Oil Extraction

The hydro distillation of fresh inflorescences was done with a 100 g sample and a Clevenger type apparatus with a 3-hour time limit simmering until a certain volume of oil was not retained anymore [3, 21]. The same way, lavender oil is extracted. The Essential Oil Percentage with respect to fresh weight basis can be calculated with the following formula:

Essential Oil Percentage= (Volume of Essential Oil (ml) / Fresh Weight of Plant Material (g)×100%

While;

Essential Oil Volume: is the amount of essential oil that has been measured and documented.

Fresh Weight of Plant Material: The fresh weight of the plant material which served as the source of oil was noted.

The essential oils which were left over were stored and dried by a temperature of 4 degrees Celsius. The storing vials are made up of amber colored glass [2].

Chemical Analysis of Essential Oils (GC–MS)

The chemical constituents of lavender species were determined by using an HP-5MS GC–MS system (Agilent Technologies, Santa Clara, CA, USA) with an HP–5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The carrier gas used was 1.0 mL min^{-1} of helium. The injection was performed in split mode (1:20) at 250 °C using a volume of 1 µL (1:100 v/v dilution in n-hexane). The oven was programmed to increase in temperature by 3 to 5 degrees Celsius every minute, from 50 degrees Celsius (kept for 3 minutes) to 250 degrees Celsius. The detector, the MS transfer line, was maintained at 280 °C. With the ionization energy set at 70 eV, a mass spectrum was recorded over time.

Identification of the compounds used two methods for each mass spectrum: retrieval of the NIST 17 and Wiley 11 databases, and mass spectrometry of respective retained indices, and the authentic standards. The methodology was set in place by [4, 19]. The measures for relative abundances were determined using peak-area normalization, and the principal constituents (linalool, linalyl acetate, 1, 8, cineole, camphor) were used as the measures to assay the quality of the oil [2, 22]. The Collaboratory tests were conducted in sets of three.

Data Collection and Measurements

Observations from the central rows of each plot were carried out to avoid border effects and were completed in August 2024, six months after the transplanting date. Measurements around vegetative and yield parameters considered the following criteria and were taken from the provided reports [13, 18]:

- **Plant height (cm)**: The tallest shoot from the soil surface were measured using a scale. The mean from a sample of three plants from each plot was taken for analysis [12].

1. **Shrub diameter (cm)**: The diameter (in cm) of the shrub at the complete vegetative stage was measured before to harvest. Each selected plant's greatest horizontal canopy spread (D_1) and a second measurement at a 90°

angle to the first axis (D_2) were measured perpendicularly using a flexible measuring tape. The mean shrub diameter was found using $(D_1 + D_2)/2$. The average value per plot was utilized for statistical analysis after five randomly selected plants per experimental unit were measured. [13].

- **Inflorescence yield (kg ha⁻)**: The harvested inflorescences were taken from the inner rows of each plot, and then the dry weight was measured using a digital balance. The ratio is then multiplied to obtain kilograms per hectare [12, 13].
- **Number of inflorescences per plant**: The flowering spikes on the tagged plants were counted when the plants reached full bloom, and the values were averaged from three plants to determine the mean per treatment [14].
- **Inflorescence length (cm)**: Inflorescence length was captured with a ruler starting from the base of the pedal and ending at the spike tip. Three representative inflorescences from each plant were captured and analyzed for their average [17].
- **Number of flowering verticillasters per peduncle**: Inflorescences were randomly sampled from three plants and the counts represent the total of verticillasters [8]. Data noted was the average of total counts per each treatment.
- **Essential-oil yield (kg ha^{-1})**: Essential-oil yield is earned by multiplying the inflorescence yield and the percentage of the oil obtained via hydro distillation (Essential-oil yield is kg / ha). [3, 17] with the following equation:

Essential oil yield (kg ha^{-1}) = Biomass of inflorescence yield (kg ha^{-1}) × Essential oil content (%)/100

Statistical Analysis

The variance and means were analyzed using the program SAS 9.4 (SAS Institute Inc. Cary, NC, USA). The fixed effects were two species of Lavender, ALE concentration, and their interaction, while the block was random. Mean comparisons were done using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ (as referenced in previous lavender bio stimulant studies by [12, 17]. The data were examined for normality and homogeneity before the analysis.

Results and Discussion

Vegetative Parameters

Effect of Aloe Leaf Extract on the vegetative growth characteristics of *Lavandula stoechas* L. and *L. angustifolia* Mill.

The interaction between species and ALE concentration was significant for plant height and shrub diameter. Foliar application of *Aloe vera* leaf extract (ALE) greatly improved plant height and shrub diameter in both lavender species in relation to the untreated control (Table 1). Most pronounced increase was at the highest concentration (120 ml L^{-1}) where *L. stoechas* reached maximum height (55.8 cm) and canopy diameter (47.1 cm) which translates to about 37 and 30 percent increase, respectively, in relation to control. *L. angustifolia* showed the same trends, which suggests that ALE foliar application improved vegetative growth in both genotypes.

The findings of the authors that *Aloe vera* extract applied to rosemary leaves promotes vegetative growth were also consistent with their results that these leaves contain amino acids, polysaccharides, phenolics, vitamins, and plant growth regulators like auxins, gibberellins, and cytokinins [5-7, 9].These substances stimulate cell growth and division, cell and leaf photosynthesis and nutrient absorption, which results in an increased height and branching of the plant [8, 23].

The increase in shrub diameter indicates that ALE also stimulated lateral branching and canopy expansion, which are typically associated with cytokinin activity and improved assimilate partitioning [5, 11]. The results of other aromatic and medicinal crops treated with natural or organic bio stimulants were similar, such as *Salvia officinalis*, *Rosmarinus officinalis*, and *Mentha spp*. that exhibited increased shoot length and biomass when treated with bio extracts from Aloe vera and similar plants[6, 8].

For lavender in particular, equally favorable changes in plant height and canopy expansion after the application of bio stimulants or foliar nutrients have been reported by [12, 13, 17], who attributed the response to improved nitrogen utilization and increased photosynthetic pigments. Also, [24] reported that bio stimulant-enhanced vegetative traits improve flowering and increase potential oil yield, which emphasizes the importance of these physiological changes in farming practice.

The similar reactions of both species in this study may be attributed to that *Aloe vera* extract works through broad, nonspecific metabolic and hormonal actions, rather than through pathways that are unique to each species.

The more marked response seen in *L. stoechas* could be attributed to its further advanced vegetative growth and its mechanism responsiveness to foliar bio stimulants, as recorded among lavender species with novel morpho-physiological traits [1, 2].

It is illustrated, as expected, that *Aloe vera* leaf extract is an organic bio stimulant that can increase the vegetative growth performance of lavender plants in semi-arid fields. The increases in growth performance which is in line with its multifunctional bio stimulant efficacy on nutrient uptake, hormonal and stress balance, and uptake moisture which, on a wider perspective lean on the eco-sustainable parameters to cultivate lavender [5, 17].

Table (1): Effect of Aloe Leaf Extract on the vegetative growth characteristics of the two lavender species.

Lavender species	Vegetative Parameters		
	ALE conc. (ml L^{-1})	Plant height (cm)	Shrub diameter (cm)
L. stoechas L.	0	40.8c	36.2c
	40	48.5b	39.6b
	80	53.3a	45.7a
	120	55.8a	47.1a
L. angustifolia Mill.	0	40.3c	34.7c
	40	45.5b	38.1b
	80	52.2a	44.2a
	120	54.1a	45.7a

**Means followed by different letters within a column are significantly different ($p \leq 0.05$) according to DMRT.*

Yield Parameters

Effect of Aloe Leaf Extract on Yield Components of *L. stoechas* L. and *L. angustifolia* Mill.

Foliar application of *Aloe vera* leaf extract (ALE) had a noticeable positive effect on the yield components of both lavender species (Table 2). The number of inflorescences per plant, inflorescence length, and number of flowering verticillasters per peduncle increased significantly in relation to the increasing ALE concentration. Consequently, the inflorescence yield increased from 317.3 kg ha^{-1} in the control to 513.4 kg ha^{-1} at 120 ml L^{-1} in *L. stoechas*, and from 299.3 kg ha^{-1} to 482.2 kg ha^{-1} in *L. angustifolia*. The absence of significant differences between 80 and 120 ml L^{-1} indicates that moderate concentrations (80 mL L^{-1}) may be sufficient to reach yield saturation, consistent with the dose–response pattern observed for bio stimulants in aromatic crops [25, 26].

The flowering and inflorescence traits are believed to increase due to physiological function Aloe vera bioactive compounds pack vitamins, amino acids, and phytohormones which promote assimilate partitioning and reproductive organ development [6, 7]. Aloe vera gel also possesses molecules similar to cytokinin and gibberellin which promote the differentiation of floral meristems, pedunculi elongation, and flower increase per spike [5, 9]. The present study's observation of a significant increase in inflorescence length is consistent with those of *Salvia officinalis* and *Rosmarinus officinalis*, in which ALE and other botanical bio stimulants improved spike elongation and floral biomass [6, 8].

In lavender, similar yield improvements under foliar bio stimulant treatments were documented by [12, 13, 17], attributing the increase in inflorescence number and length to improved assimilation of nutrients and chlorophyll biosynthesis. [27] similarly reported, that organic bio stimulant regimes enhanced floral spike biomass and spike density, yielding *L. angustifolia* Mill. at higher oil concentrations.

Increased flowering intensity, as observed in the current study, might be due to enhanced nitrogen and carbohydrate metabolism which are directly associated with the *Aloe vera* extract's hormonal and nutritive impacts [5, 11]

In addition, the plateauing of response beyond 80 ml L^{-1} is consistent with earlier findings that moderate amounts of most natural bio stimulants are the most beneficial, and that larger amounts do not further enhance growth or yield due to feedback control of hormone dose and metabolism activity [25, 26]. The similarity in yield response patterns for the two species of lavender suggest that ALE works through general physiological processes that are available to both genotypes. Nevertheless, *L. stoech*as L. had a slightly greater response, which could be the result of its more vigorous growth and abundant branching as also observed in species comparisons by [1, 2].

The application promotes eco sustainable crop management by increasing productivity through Green techniques, decreasing synthetic fertilizer dependence, and optimizing yield at low affordable levels [5, 17].

Table 2: Effect of Aloe Leaf Extract on floral characteristics and Yield Components of the two lavender species.

Lavender species	ALE conc. (ml L^{-1})	Yield Parameters			
		Inflo-rescence yield ($kg.ha^{-1}$)	No. of inflores-cences. $Plant^{-1}$	Length of in-florescences (cm)	No. of flowering verticillasters per peduncle
L. stoechas L.	**0**	317.3c	45.6c	12.6c	17.1c
	40	415.6b	57.6b	15.1b	19.5b
	80	489.1a	66.2a	18.2a	23.3a
	120	513.4a	69.3a	18.9a	24.5a
L. angustifolia	**0**	299.3c	41.7c	11.9c	14.9c
	40	386.6b	51.2b	13.8b	17.8b
	80	457.9a	62.9a	16.8a	20.3a
	120	482.2a	65.3a	17.2a	22.4a

**Means accompanied within the cells of a table with distinct letters differ significantly ($p \leq 0.05$) as per the DMRT.*

Essential Oil Yield

Effect of Aloe Leaf Extract on Essential Oil Yield of *L stoechas* L. and *L. angustifolia* Mill.

Foliar application of *Aloe vera* leaf extract (ALE) had a marked significant effect on the essential oil yield of both *lavender* species (Figure 1). In the case of *L. stoechas* L., the oil yield in the control group was 18.8 kg ha^{-1}, increasing to 29.9 kg ha^{-1} with 120 ml L^{-1} ALE, while *L. angustifolia* Mill. recorded 17.1 and 27.9 kg ha^{-1}, respectively. The positive effect on oil yield and ALE application on vegetative growth and floral biomass suggest more canopy and flowers, and thus, more essential oil [13, 17].

The rise in essential oil yield with exposure to ALE treatments is parallel to prior works whereby natural bio stimulants enhance the biosynthesis of volatile oils by stimulating the rates of photosynthesis, carbohydrate metabolism, and then the supply of carbons to be involved in the formation of terpenes [18, 20]. Aloe vera extract contains bioactive substances in bulk more than other ingredients and these include phenolic acids, amino acids, vitamins, and other phytohormones, all of which have to do with regulation of physiology and secondary metabolism [7, 9]. The salicylic acid, flavonoids, and ALE polysaccharides may act as elicitors of secondary metabolites by defense enzyme activation together with essential genes in terpenoid biosynthesis, causing the accumulation of vital oils [5, 28].

In lavender and other fragrant species subjected to organic or plant-sourced bio stimulants, the same and other favorable reactions were recorded. According to [12, 18] foliar spraying of bio stimulant applications improved the concentration and yield of essential oils of *Lavandula × intermedia* “Grosso” (and possibly other species) due to boosted photosynthesis and enzymatic activity of the oil gland cells. With the use of Aloe vera or moringa oil foliar sprays, the essential oil yield and composition of monoterpenes changed in positive and increased levels in *Rosmarinus offici-*

nalis and *Salvia officinalis* [6, 8]. The improvements in oil composition were attributed to increase the uptake of nutrients and the enzymatic stimulation of the methylerythritol phosphate (MEP) pathway that provides monoterpene precursors [29].

The current study's dose-dependent increase, which peaked at 120 ml L^{-1} and did not differ significantly from 80 mL L^{-1}, suggests that moderate ALE concentrations can maximize oil biosynthesis[25, 26]. Higher metabolite concentrations do not add any additional benefits and are presumed to be the result from saturation or regulation in negative feedback of secondary pathways. The similarity in response of *L. stoechas* and *L. angustifolia* suggests that the ALE positive stimulatory effects that range across species are in these case, general physiological processes. The *L. stoechas* case does offer itself more to the idea that the species has more dense cross sections of glandular trichomes and more active oil glands which would explain the species differences in yield [1, 2].

These findings, however, highlight the conclusion that *Aloe vera* leaf and its derivatives are not simply passive elicitors and growth stimulators, but can synergies to promote the accumulation of essential oil in lavender.

Integrating lavender cultivation systems could help produce lavender essential oil, reduce the necessity for chemical fertilizers, and improve phytochemistry in a green way [5, 17, 20].

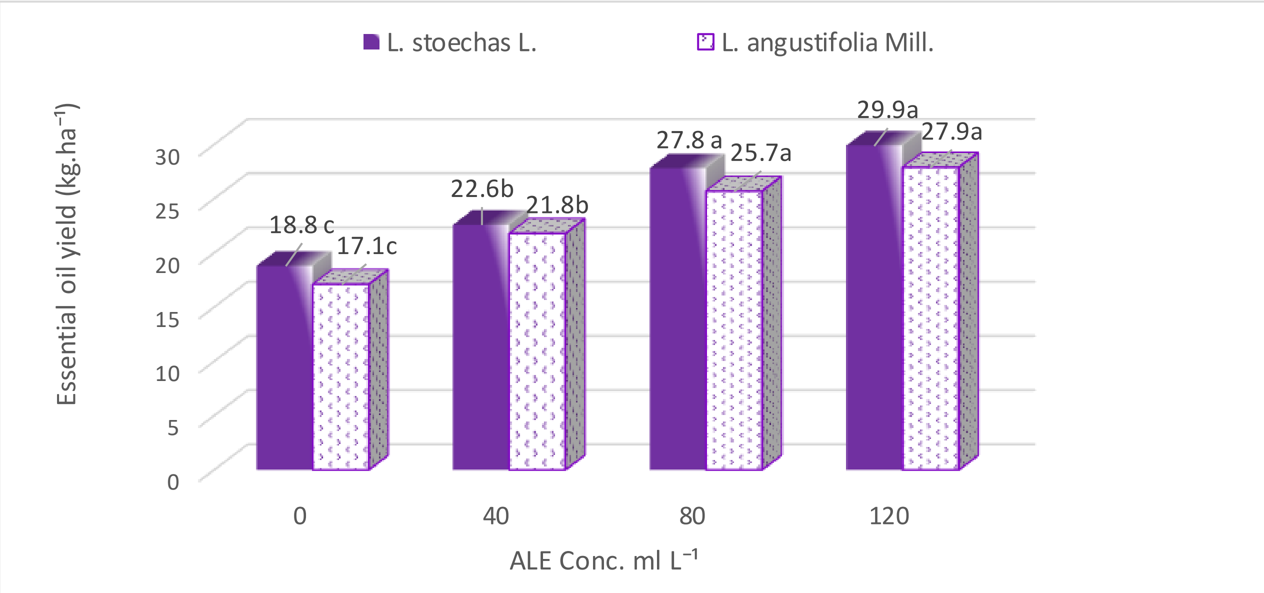

Figure (1): Effect of Aloe Leaf Extract on Essential Oil Yield of the two lavender species.

Conclusion

According to this study, when applied topically, Aloe vera leaf extract (ALE) considerably increases the yields of flowers, vegetables, and essential oils in two lavender species. When compared to controls, optimal concentrations of 80 and 120 ml L^{-1} enhanced plant height, inflorescence length, and number, resulting in higher biomass and essential oil yields. The vitamins, bioactive phenolics, amino acids, and phytohormone-like compounds found in ALE are thought to have positive effects on cell division and nutrient absorption. Interestingly, *L. stoechas* L. responded better to ALE, suggesting that it is sensitive to biostimulants. The quality and value of lavender essential oils may be improved by using aloe vera as a biostimulant in lavender production, especially in low-input and organic systems, which may lessen dependency on toxic fertilizers.

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