



Article The Impact of Irrigation Intervals and NPK/Yeast on the Vegetative Growth Characteristics and Essential Oil Content of Lemongrass

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Abstract: The growth and quality of medicinal plants are greatly affected by environmental stress, with over half of the world's agricultural land facing water shortages. This research was conducted over two seasons in 2018 and 2019 and aimed to investigate the influence of varying irrigation intervals and partial replacement of mineral fertilizers with biofertilizer (yeast) on the growth, yield, and essential oil content of lemongrass. The study also looked at the effect of the partial substitution of NPK fertilizer with yeast on lemongrass's ability to withstand water deficit stress. The results showed that water deficit and reducing NPK levels led to a decline in growth characteristics and relative leaf greenness, which was accompanied by an increase in proline content and essential oil percentage. These findings suggest that lemongrass is sensitive to drought and requires adequate nitrogen fertilization. However, extending the irrigation intervals led to an improvement in essential oil content. To achieve high essential oil yield and maintain productivity while considering environmental factors, it is recommended to reduce mineral fertilizer to 50–75% NPK combined with biofertilizer, and space irrigation intervals every 10–15 days. Further research is needed to improve the growth of lemongrass in water-deficient conditions, particularly on newly reclaimed soils.

Keywords: mineral fertilization; biofertilization; *Cymbopogon citratus*; essential oil; water stress; proline; macronutrients

1. Introduction

Lemongrass (Cymbopogon citratus) is a widely distributed medicinal plant that is commonly found in tropical and subtropical regions of Africa, Asia, and America [1–3]. The herb of lemongrass is used for various medicinal purposes, such as treating digestive problems, lowering blood pressure and cholesterol levels, acting as an antioxidant and antifungal, and serving as an insect repellent, antidiabetic, diuretic, and nerve tonic. Its oil is used in the production of soap, cosmetics, and perfumes. Lemongrass is also an important agricultural export that generates significant income and foreign currency [4]. It has been traditionally used to produce essential oil, which is found in the herb at concentrations of 1-2% [4,5]. Studies on the essential oil of lemongrass have shown that it is rich in phenolic compounds, flavonoids, and tannins [6]. The major bioactive substances in the oil include geranial, neral, and myrcene, as well as other compounds present in lower concentrations, such as citronellol, geraniol, geranyl acetate, limonene, linalool, nerol, eugenol, etc. [1,4,7]. Essential oils are complex mixtures of chemicals, including monoterpenes, sesquiterpenes, diterpenes, and phenylpropane derivatives. Over 1500 compounds have been identified in the composition of different essential oils, including hydrocarbons, alcohols, aldehydes, ketones, esters, sulphur and nitrogen compounds, acetylene derivatives, coumarins, and organic acids [8–11]. The quality and yield of lemongrass can be influenced by various



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). factors such as genetic variety, maturity stage, temperature, light exposure, water availability, and farming practices [12,13]. Providing adequate nutrition is crucial for the growth and development of all crops, including medicinal plants that produce essential oils. Effective fertilization practices can significantly increase the yield and quality of essential oils produced by these plants [14–17].

Environmental factors such as heat stress, drought, flooding, salinity, and imbalanced nutrients can negatively impact crop growth, development, production, and quality [18]. Drought, in particular, poses a significant challenge for agriculture, as it is the most prevalent constraint on plant growth and output worldwide. It affects more than half of the world's agricultural lands, especially those in dry and semi-arid regions, and the situation is further worsened by global warming [19]. Water constitutes 80–95% of the fresh biomass of non-woody plants and is critical for various aspects of plant growth and metabolism [20,21]. A plant's response to water scarcity depends on various factors, including the plant species and growth stage, as well as the duration and intensity of the water scarcity [4,22]. However, plants have developed mechanisms to adapt to and withstand stress [23]. During drought, plants can conserve water by reducing water loss and increasing water intake, which helps to maintain normal cell function [21,22]. Despite its detrimental effects on the morphological, physiological, biochemical, and molecular properties of plants [24–26], there is limited research on the specific impacts of drought stress on plant metabolism [27].

Under stress conditions such as water deficit and salinity stresses, compatible solutes accumulate in plant tissue as a resistance mechanism in order to mitigate osmotic stress [21]. These solutes protect cells by maintaining osmotic balance, reducing the formation of reactive oxygen species (ROS), and regulating the cellular redox state [28,29]. In conclusion, the role of compatible solutes, such as proline, in the response of plants to environmental stresses is crucial for survival and adaptation. They provide protection against cellular damage and help maintain cellular homeostasis under stressful conditions [21].

In traditional agricultural systems, the excessive use of chemical inputs increased agricultural productivity, but adverse effects on the environment and human health from the long-term use of chemical fertilizers are well-documented [30]. The harmful effects of chemical fertilizers encouraged agrochemical companies to replace them with biofertilizers in sustainable agricultural structures in order to achieve adequate crop yields [31]. Biofertilizers are defined as substances or microorganisms containing active and beneficial microbes that can be used in sustainable agriculture to increase the efficiency of nutrient uptake by crops and improve soil fertilizers on the environment by decreasing the reliance on synthetic fertilizers and reducing the carbon footprint of agriculture [33]. Microorganisms have been found to play a crucial role in promoting plant growth and health. They are capable of improving plant growth in various ways, such as providing biologically fixed nitrogen, phytohormones, volatile compounds, protective compounds, and enzymes [34–36]. This makes them a valuable tool for sustainable crop production ensuring compatible food production with environmental sustainability [32].

Plant growth-promoting microorganisms (PGPMs), such as bacteria and fungi, have a mutually beneficial relationship with the plant. They colonize the root zone of the plant and form a symbiotic relationship with the plant that results in improved plant growth and increased crop yields [34]. The beneficial effects of PGPMs can also include increased nutrient uptake, improved water-use efficiency, and greater resistance to environmental stress, including drought and salinity stress [35]. The use of PGPMs can be a more sustainable alternative to traditional chemical fertilizers and can play an important role in achieving food security and environmental sustainability [36]. However, it is important to further study the specific mechanisms of action and potential risks associated with the use of PGPMs, in order to ensure their safe and effective use in agricultural systems [37].

Yeast is a promising biofertilizer that can enhance plant growth by providing essential vitamins and promoting plant vitality [34]. The effects of yeast extract are not well understood, but it has been shown to have advantages over traditional plant growth regulators and soil conditioners. Further research is needed to fully understand the impact of yeast extract on afforestation and its potential as a sustainable agricultural practice in semi-arid locations [35]. Yeast extract has been shown to have a positive impact on the growth, yield, and quality of various crops such as lettuce, eggplant, potato, cucumber, soybean, and turnip. It has been found to contain a variety of beneficial substances such as low-molecular-weight organic compounds, amino acids, nucleotides, peptides, nitrogen, phosphorus, and trace elements [38–43]. The application of yeast extract has been shown to decrease the harmful effects of drought and improve the growth characteristics and antioxidant enzyme activity of maize crops [44].

Lemongrass is considered one of the most important economic and export crops in Egypt; however, it did not find sufficient attention for improving the productivity efficiency of producers or find interest in the input productive elements affecting production [45]. The study aimed to investigate the impact of irrigation intervals and partial substitution of mineral fertilizers (NPK) with biofertilizer (yeast) on the growth, yield, and essential oil content of lemongrass (*Cymbopogon citratus*) in Egypt. The study also aimed to examine how the substitution of NPK fertilizer with yeast would affect the plant's ability to resist water deficit stress. The results of this study would have implications for the promotion and improvement of lemongrass production in Egypt, which is considered an important economic and export crop.

2. Materials and Methods

2.1. The Experimental Design

This study was conducted at the Agricultural Experimental Farm at Aswan University in Egypt over two consecutive seasons in 2018 and 2019. The experiment was designed as a randomized complete block split-plot design with three replicates. The main plots (A) were assigned to different irrigation intervals (5 days as the control, 10, 15, and 20 days) and the sub-plots (B) were assigned to different fertilization treatments (100% NPK, 75% NPK plus yeast, 50% NPK plus yeast, 25% NPK plus yeast, and yeast only). The 5-day irrigation interval is the best irrigation practice for lemongrass [46]. The yeast (*Saccharomyces cerevisiae*) treatment was applied at a rate of 20 mL of 0.6% yeast per plant.

2.2. Plant Materials and Experimental Site

Homogenous stalks of lemongrass, *Cymbopogon citratus* (DC.) Stapf, were obtained from the Tropical Farm, Agricultural Research Center, Kom-Ombo, Aswan, Egypt. The planting area was designed in the form of terraces, with each sub-plot consisting of two terraces 60 cm in width and 3 m in length. Organic compost was used in the preparation period by adding and mixing 25 m³ of compost per hectare in soils before planting. On the 20th of March in each year 2018 and 2019, the homogenous stalks of lemongrass were planted in the soil with a distance of 30 cm between each plant, resulting in a total of 40 plants per plot. The soil samples were tested for various physical and chemical properties according to the methods described by Jackson [47] and Black et al. [48] as shown in Table 1.

A drip irrigation system was used, and normal agricultural practices were followed. After two months of planting, fertilizers were applied, and the plants were irrigated according to irrigation intervals with 12 L per plant. The plants were harvested twice each season by cutting the shoots 10 cm above the ground. The first harvest was after two months of treatments. Then, the irrigation was applied every five days, followed by the same previous treatment for another two months, and the second harvest was completed. After harvesting, the vegetative growth data were recorded, including the number of tillers per plant, shoot fresh and dry weight per plant, and shoot fresh and dry weight per hectare.

2.3. Vegetative Growth Characteristics

Plant were harvested twice each season by cutting the shoots 10 cm above ground. At harvest time, number of tillers and fresh herb weight per plant were recorded. Then the herb was air-dried for a week, and the dry herb weight was recorded. Fresh and dry herb yield per hectare (ton) was calculated by multiplying shoot fresh weight per plant and plant number per hectare (50,000 plant).

2.4. Chemical Constituents

The SPAD chlorophyll meter (SPAD-502plus, Konica Minolta, INC., Osaka, Japan) was used to measure the relative greenness of lemongrass leaves. The readings were taken on the middle part of the leaf blade and provided an indicator of the level of chlorophyll present in the leaves.

1-Physical Analysis								
Sand %		94.67						
Silt %		2.27						
Clay %		3.07						
Soil Texture		Sandy						
2-Chemical Analysis								
рН	8.25	Electrical Conductivity (ds m ⁻¹)	0.25					
Soluble Cations meq L^{-1}	Soluble Anions meq L^{-1}							
Na ⁺	17.74	CO^{-3}	0.00					
K ⁺	7.51	HCO ⁻³	4.67					
Ca ⁺⁺	2.08	Cl ⁻	2.33					
Ca ⁺⁺ Mg ⁺⁺	0.53							

Table 1. Physical and chemical properties of the experimental soil.

This is a method for measuring the free proline content in lemongrass shoots. The dried shoots (0.2 g) were first homogenized in 10 mL of 3% aqueous sulfosalicylic acid for 10 min and then filtered. The filtered solution (2 mL) was mixed with 2 mL of glacial acetic acid and 2 mL of ninhydrin and heated in a water bath for 1 h. The developed color was extracted in toluene and measured colorimetrically at 520 nm. The concentration of proline is then calculated using a standard curve [49].

The chemical analysis of the lemongrass plant shoots involved wet-digestion with concentrated H_2SO_4 : H_2O_2 , followed by measuring the nitrogen (N) content using the semimicro Kjeldahl method [50], the phosphore content using the vanadate-molybdate-yellow method, and the potassium content using a flame photometer [51].

2.5. Essential Oil Percentage

The essential oil content was determined by dividing the weight of the essential oil obtained from the hydro-distillation by the weight of the fresh shoot and expressed as a percentage. The total essential oil yield per plant was calculated by multiplying the essential oil content by the fresh weight of the shoot [52]. The total essential oil yield per hectare was calculated by multiplying the essential oil yield per plant and plant number per hectare (50,000 plants).

2.6. Statistical Analysis

The study used a split-plot experimental design with a complete random block arrangement. In this design, the main plot was for the irrigation interval and the sub-plots were for the fertilization treatments. The data collected were analyzed using an "F" test and statistical analysis was performed using the Statistix 8.1 software [53]. Tukey's HSD was used to examine the differences among interaction treatments.

3. Results and Discussion

Analysis of variance (two-way ANOVA) showed the significant effects of water intervals and fertilization treatments on lemongrass growth and yield parameters as well as chemical and essential oil content (Table 2).

3.1. Vegetative Growth Characteristics

The water-deficit stress affects plant growth and development by causing loss of turgor, disordering enzyme activities, and decreasing energy supply from photosynthesis [54-57]. Lemongrass has been found to be sensitive to water-deficit stress [46]. The mineral macronutrients N, P, and K are important in improving the plant's vegetative development characteristics and play major roles in plant growth, development, and quality improvement. Nitrogen is a major component of amino acids, enzymes, and energy transmission elements such as chlorophyll, ADP, and ATP [58]. Phosphorus is essential for cell division, glucose metabolism, photosynthesis, and biological oxidation [59]. Potassium interacts synergistically with carbohydrates when it serves as an osmotic agent [60]. Yeast is a source of various plant development chemicals such as cytokinins, vitamin B, and microelements (P, K, S, Na, Ca and Mg) and can be useful in increasing the availability of nutrients for crop production [61,62]. The results of this study showed that water intervals and partial substituent of NPK fertilizers as well as the interaction coefficients between fertilization treatments and irrigation intervals had significant negative effects on growth characteristics such as the number of tillage, fresh and dry weights per plant and fresh and dry yield per hectare in both seasons and both cuts (Table 2). In general, the highest number of tillers has been obtained in plants irrigated every five days and fertilized with the recommended dose (100% NPK). In addition, the greatest fresh and dry biomass of lemongrass resulted from the five-day irrigation interval and 100% NPK treatment. Those values decreased with increasing irrigation intervals and decreasing NPK levels. The lowest number of tillers and lowest fresh and dry biomass were obtained when plants were irrigated every 20 days and fertilized with yeast only, 0% NPK (Figures 1–5).

Variables	F Value							
	Α	В	$\mathbf{A} imes \mathbf{B}$	Α	В	$\mathbf{A} imes \mathbf{B}$		
	First season (1st cut)			First season (2nd cut)				
Number of tillers	30.61 ***	72.27 ***	1.06 ^{ns}	27.11 ***	37.29 ***	2.36 *		
Plant fresh weight (g)	143.47 ***	39.50 ***	2.28 *	166.45 ***	207.34 ***	5.59 ***		
Plant dry weight (g)	23.45 ***	29.00 ***	1.30 ^{ns}	32.10 ***	15.17 ***	0.75 ^{ns}		
Fresh herb yield (ton ha^{-1})	143.47 ***	39.50 ***	2.28 *	166.45 ***	207.34 ***	5.59 ***		
Dry herb yield (ton ha^{-1})	23.45 ***	29.00 ***	1.30 ^{ns}	32.10 ***	15.17 ***	0.75 ^{ns}		
Relative leaf greenness (%)	2.62 ^{ns}	35.57 ***	2.75 **	22.30 ***	64.11 ***	2.22*		
Proline content (%)	27.78 ***	9.40 ***	1.61 ^{ns}	16.31 ***	11.85 ***	3.74 ***		
Nitrogen content (%)	26.41 ***	49.10 ***	1.44 ^{ns}	7.19 ***	65.48 ***	1.19 ^{ns}		
Phosphorus content (%)	482.29 ***	801.42 ***	663.92 ***	6.05**	6.23 ***	5.22 ***		
Potassium content (%)	293.74 ***	321.07 ***	715.38 ***	515.45 ***	367.40 ***	366.52 ***		
Essential oil percentage (%)	16.44 ***	8.48 ***	4.80 ***	9.45 ***	4.55 ***	3.55**		
Essential oil content (mL plant $^{-1}$)	53.60 ***	17.70 ***	2.26*	36.66 ***	77.20 ***	5.34 ***		
Essential oil yield (litter ha^{-1})	53.60 ***	17.70 ***	2.26*	36.66 ***	77.20 ***	5.34 ***		
	Second season (1st cut)			Second season (2nd cut)				
Number of tillers	38.07 ***	11.48 ***	0.63 ^{ns}	173.79 ***	85.15 ***	5.55 ***		
Plant fresh weight (g)	264.78 ***	101.07 ***	7.91 ***	896.28 ***	43.56 ***	8.35 ***		
Plant dry weight (g)	20.89 ***	12.50 ***	1.29 ^{ns}	178.29 ***	42.61 ***	12.45 ***		
Fresh herb yield (ton ha^{-1})	264.78 ***	101.07 ***	7.91 ***	896.28 ***	43.56 ***	8.35 ***		
Dry herb yield (ton ha^{-1})	20.89 ***	12.50 ***	1.29 ^{ns}	178.29 ***	42.61 ***	12.45 ***		
Relative leaf greenness (%)	118.01 ***	37.81 ***	2.30 *	98.89 ***	36.51 ***	3.80 ***		
Proline content (%)	42.19 ***	12.74 ***	19.05 ***	92.04 ***	27.33 ***	7.28 ***		
Nitrogen content (%)	7.47 ***	41.61 ***	0.64 ^{ns}	8.95 ***	163.79 ***	3.79 ***		
Phosphorus content (%)	0.87 ^{ns}	0.82 ^{ns}	2.41 *	5.95 **	1.45 ^{ns}	3.17 *		
Potassium content (%)	98.57 ***	653.78 ***	244.90 ***	498.13 ***	66.25 ***	287.61 ***		
Essential oil percentage (%)	67.08 ***	5.67 ***	3.74 ***	11.71 ***	2.82 *	2.45 *		
Essential oil content (mL plant $^{-1}$)	9.30 ***	21.22 ***	3.52 ***	93.17 ***	3.05 *	2.04 *		
Essential oil yield (litter ha^{-1})	9.30 ***	21.22 ***	3.52 **	93.17 ***	3.05 *	2.04 *		

Table 2. Analysis of variance (*F* value) of water intervals, fertilization treatments, and their interaction on the studied parameters of lemongrass in the first and second seasons.

A represented *F* value of difference among the water intervals, B represented *F* value of difference among the fertilization treatments, and A \times B represented *F* value of difference among the interaction between them. ^{ns}—not significant; *, ** and *** represented significant differences at probability level of 0.05, 0.01 and 0.001, respectively.

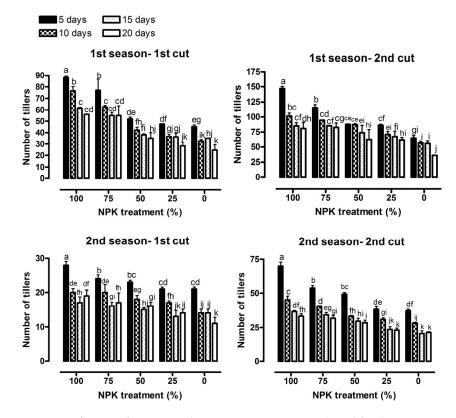


Figure 1. Influence of interaction between irrigation intervals and fertilization treatments on the number of tillers per plant. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK+ yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

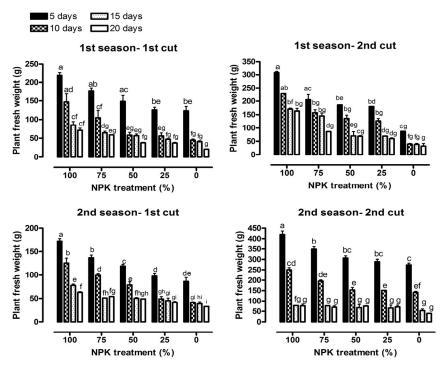


Figure 2. Influence of interaction between irrigation intervals and fertilization treatments on plant fresh weight. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

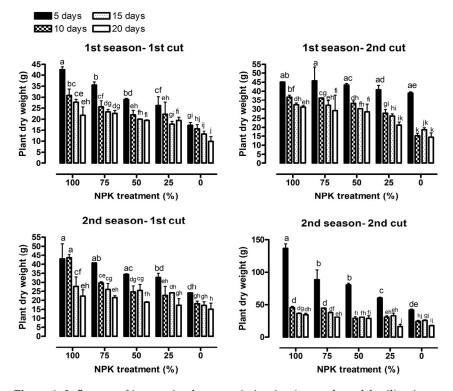


Figure 3. Influence of interaction between irrigation intervals and fertilization treatments on plant dry weight. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

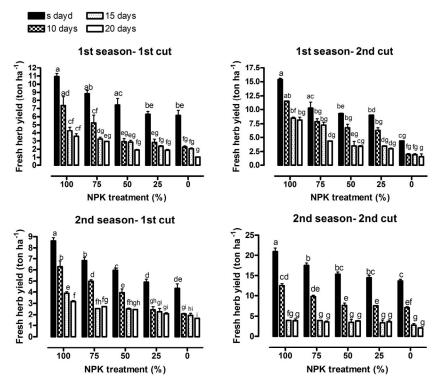


Figure 4. Influence of interaction between irrigation intervals and fertilization treatments on fresh herb yield. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

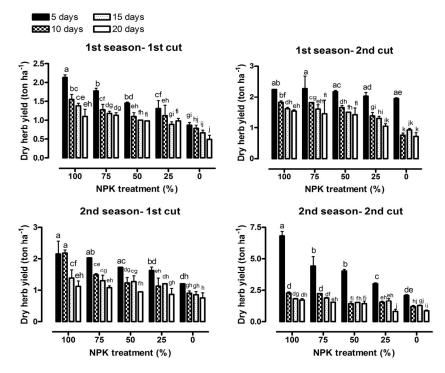


Figure 5. Influence of interaction between irrigation intervals and fertilization treatments on dry herb yield. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

Drought has a significant impact on both the quantity and quality of crop growth and yield [54,57]. Plant growth and development are dependent on factors such as cell division, elongation, and differentiation, and drought can cause a loss of turgor, enzyme activity disruptions, and a decline in energy supplied by photosynthesis [55–57]. The plants that received the recommended dose of NPK and were irrigated every 5 days showed the highest significant values for growth traits. However, decreasing NPK levels and increasing irrigation intervals resulted in a significant decrease in growth due to water and nutrient stress. This is consistent with a previous study which showed that 5-day irrigation intervals produced the largest number of tillers and heaviest herb yield of lemongrass, and these values significantly decreased with increasing irrigation intervals and the smallest number of tillers, and less herb yield was recorded with 15- and 20-day irrigation intervals [46]. On the other hand, dry yeast extract has been found to be an effective herbal biochemical with properties that promote growth, enhance nutrition, and provide protection [61–63]. It has been suggested that using yeast extract can improve vegetative growth characteristics, yield per plant, dry tuber percentage, and overall soluble solid content [64]. Furthermore, increasing the use of active dry yeast through foliar application has been found to improve the vegetative growth, productivity, and quality of potato tubers [65]. Inconsistent with the previous findings, the results of this study revealed that the recommended dose of mineral fertilization (100% NPK) gave the largest number of tillers and the highest values of herb yield of lemongrass which significantly decreased with decreasing the level of mineral NPK. These findings do not diminish the importance of yeast adding, but this is due to the lack of nutrients in sandy soil (Table 1) and its need for a long period of adding soil conditioners such as yeast, and also the fact that lemongrass is a nitrogen-hungry plant, which explains its negative impact with the lack of mineral fertilizer levels.

3.2. Chemical Constituents

Drought stress has negative effects on plant growth and development by reducing nutrient availability and uptake, leading to decreased root development, transpiration,

photosynthetic rates, and stomatal conductance, causing desiccation stress [66]. Yeast has been shown to be a rich source of phytohormones, nutrients, enzymes, amino acids, and minerals [67–70], and its use has been linked to increased growth, protein and nucleic acid synthesis, chlorophyll formation, and stimulation of cell division and growth [71]. Yeast is a natural bio-substance that contains various nutrient factors and semi-growth regulator compounds such as auxins, gibberellins, and cytokinins.

In the same line with vegetative characters, relative leaf greenness, which is reflected by the level of chlorophyll, was higher under control conditions and decreased with increasing watering intervals and nutrient deficit levels. These decreases are due to the negative effect of both water stress and nutrient-deficient stress on the photosynthetic apparatus and chlorophyll content (Figure 6). The greenest leaves were shown in plants irrigated every five days and fertilized with the recommended dose (100% NPK), and the greenness decreased with increasing irrigation intervals and decreasing mineral fertilizer (NPK) level. The lowest leaf greenness was shown in plants fertilized with yeast only without NPK. The F values showed that leaf greenness was affected mostly by NPK level in the first season, and with irrigation intervals in the second season (Table 2). This is in agreement with previous studies which reflected the negative impacts of environmental stresses on the photosynthetic system [46,55–57]. The negative effect of drought stress may be due to osmotic stress, which led to cell membrane leakage and stomatal closure causing a shutdown of the photosynthesis process [57,72]. Although yeast application enhances photosynthesis and improves plant greenness, in this study leaf greenness decreased with partial replacement of mineral fertilization (NPK) yeast. This is because yeast cannot compensate for the severe deficiency in mineral fertilizer (NPK) levels under such harsh conditions. However, it is possible to partially replacement of 25-50% NPK with yeast to partially compensate for the negative impact.

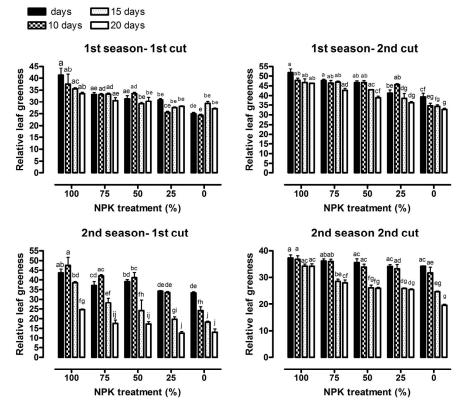


Figure 6. Influence of interaction between irrigation intervals and fertilization treatments on relative chlorophyll greenness. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

The increase in proline content under water-deficit stress is crucial for osmotic adaptation [28,29]. The proline content was raised with increasing irrigation intervals and the highest proline content was seen in plants irrigated every 15 and/or 20 days. The proline content also fluctuates with different levels of NPK, with the highest proline content seen in plants fertilized with 50% and/or 25% NPK combined with yeast. Proline accumulation is closely related to plant resistance to water-deficit stress and helps to preserve cell components from oxidative damage and maintain the energy balance between chloroplasts and mitochondria [21,28,29]. Proline has been reported to play an important role in osmotic adjustment, as seen in previous research on drought and salinity stress [57,72]. In this study, proline content was related to water stress, not to partial replacement of mineral fertilization. The increase in irrigation interval led to significant increases in proline content. This is consistent with previous findings of drought and salinity stresses, which have similar mechanisms [46,72]. The important role of proline was clarified in these studies where the plant under stress conditions accumulates compatible solutes in plant tissue as a resistance mechanism in order to mitigate osmotic stress. Compatible solutes are characterized by low molecular weight and can accumulate at high concentrations without negative impacts on the components and metabolism of cells. Compatible solutes, such as proline, led to raising the cellular osmotic pressure and water uptake maintaining the turgor pressure and water content of cells [28,66] (Figure 7).

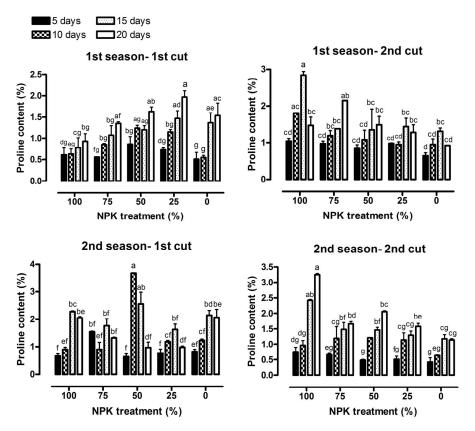


Figure 7. Influence of interaction between irrigation intervals and fertilization treatments on proline content. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

Macronutrients (nitrogen, phosphorus, and potassium) obviously decreased under nutrient deficit stress as a result of the shortage of nutrient inputs, but the response to water stress was not clear (Figures 8–10). The highest nitrogen, phosphorus, and potassium content were shown in plants fertilized with 100% NPK level regardless of irrigation intervals, and the lowest proline content was found in plants fertilized with yeast only (0% NPK) regardless of irrigation intervals. Mostly, potassium was more affected by irrigation intervals compared to nitrogen and phosphorus (Table 2). The decreased content of nitrogen, phosphorous, and potassium in the plant is normal with lowering levels of mineral NPK fertilizer. Many previous studies have shown the important role of mineral fertilizers, especially the major elements (nitrogen, phosphorus, and potassium) on the growth and development of plants, and that reducing the level of these macronutrients in the soil naturally leads to a decrease in their content inside the plant [58–62].

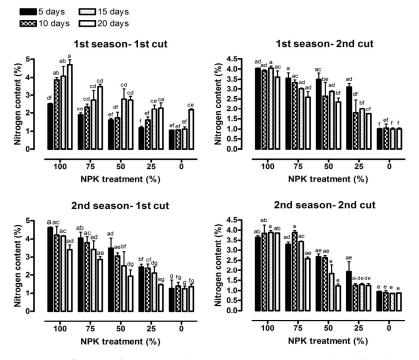


Figure 8. Influence of interaction between irrigation intervals and fertilization treatments on nitrogen content. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

3.3. Essential Oil Content

The yield of oil from herbs was impacted by various factors such as soil compost, weather, lifespan, mowing, and the quality of the distilled herb [73]. Changes in irrigation frequency may affect the metabolism of the plant, resulting in an increase in metabolites, particularly carbohydrates, which serve as the foundation for oil formation and output. This was also seen in lemongrass [74] and Trachyspermum ammi L [75]. Stressful conditions can trigger the secondary metabolism, leading to a rise in the production of volatile oil. This study found that reducing the NPK level resulted in a significant increase in essential oil percentage, with the highest amount observed in plants fertilized with a combination of 25% or 50% NPK and yeast. Meanwhile, increasing irrigation frequency also led to a rise in essential oil percentage, with the highest concentration seen in plants irrigated every 15 or 20 days. However, both a high water interval and low NPK fertilization led to a decrease in essential oil content. The greatest essential oil yield was achieved through 100% NPK fertilization and 5-day irrigation intervals, while the lowest yield was seen in plants treated with yeast alone (0% NPK) and under water stress (10-20 irrigation intervals). Although water stress raised the essential oil percentage in lemongrass, it caused a decrease in the overall oil yield due to decreased vegetation growth and herb output. Because of the interaction between irrigation frequency and fertilization treatment, the essential oil percentage increased but the total oil yield decreased (as shown in Figures 11–13). A prior study revealed that drought stress had a significant effect not only on the quantity of essential oil but also on its composition [46,47]. The main components of lemongrass essential oil were geranial (Citral A) and neral (Citral B). The content of these two compounds decreased in plants irrigated every 10 days but gradually increased with irrigation at 15 or 20-day intervals [46]. On the other hand, reducing the NPK fertilizer level did not have a significant impact on the essential oil percentage or its key components in lemongrass [76].

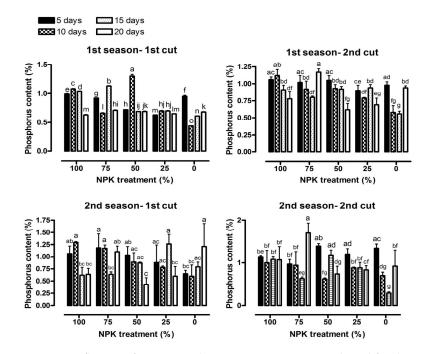


Figure 9. Influence of interaction between irrigation intervals and fertilization treatments on phosphorus content. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

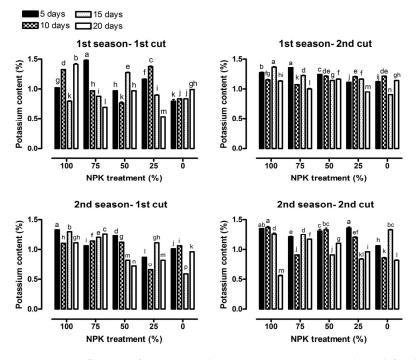


Figure 10. Influence of interaction between irrigation intervals and fertilization treatments on potassium content. Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

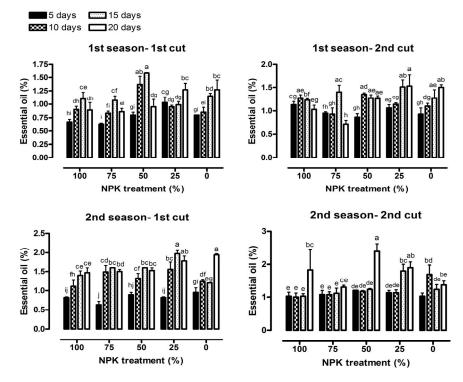


Figure 11. Influence of interaction between irrigation intervals and fertilization treatments on essential oil percentage (%). Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

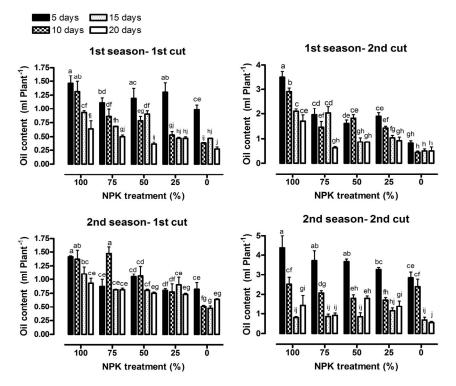


Figure 12. Influence of interaction between irrigation intervals and fertilization treatments on essential oil content (ml per plant). Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation, and different letters above represent significant differences.

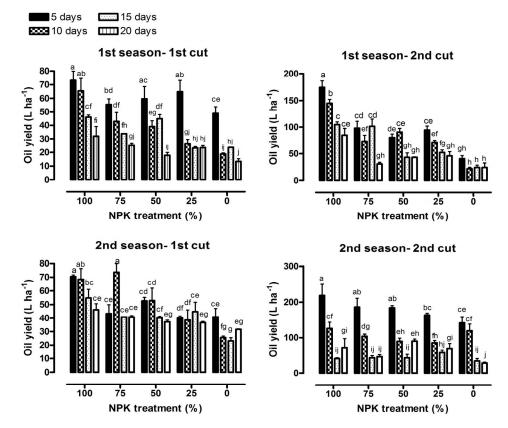


Figure 13. Influence of interaction between irrigation intervals and fertilization treatments on essential oil content (litter per hectare). Fertilization treatments were 100% NPK, 75% NPK + yeast, 50% NPK + yeast, 25% NPK + yeast, and 0% NPK (yeast only). Data represented means \pm standard deviation.

4. Conclusions

The findings of this study showed that partially substituting mineral NPK fertilizer with yeast and altering irrigation intervals, as well as the interaction between these factors had effects on growth characteristics, chemical composition, and essential oil content. The best results were observed in plants that received a recommended dose of NPK and were watered every 5 days. A decrease in NPK levels and an increase in irrigation intervals caused clear reductions in growth characteristics due to water and nutrient stress. Proline content, however, increased in response to these stress factors, particularly water shortage, highlighting its importance under stress conditions. Despite an increase in essential oil content due to water stress, the overall essential oil yield decreased because of reduced vegetative growth and overall herb yield. The interaction between fertilization and irrigation intervals had a positive effect on essential oil percentage but a negative impact on total oil production. Therefore, it is recommended to use a balance of 50–75% NPK fertilizer combined with biofertilizer and space irrigation intervals at 10–15 days for sustainable agriculture that prioritizes both productivity and environmental concerns, leading to high essential oil yield and content.

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