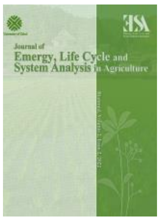
University of Zabol

ELSA

Emergy, Life Cycle and System Analysis in Agriculture

Journal of Emergy, Life Cycle and System Analysis in Agriculture

Biannual, Volume 2, Issue 2, December 2022



Journal of Emergy, Life Cycle and System Analysis in Agriculture (JELSA)

Volume 2, Issue 2, December 2022

Publisher: University of Zabol

Editorial Office: ***Faculty of Agriculture, University of Zabol, Zabol, Iran, P.O. Box 538-98615***
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Editor's Note

New Title, New Approach

Dear readers,

As the editor-in-chief of Journal of Emergy, Life Cycle and System Analysis in Agriculture (JELSA), I am pleased to address our valued readership with a new, more specialized approach and exciting news about our journal's evolution. Previously, known as Agriculture, Environment and Society (AES), we have refined our focus and clarified our scope of work to better serve our academic community.

As part of this evolution, JELSA has narrowed its scope to delve deeper into topics related to emergy, life cycle, and system analysis in agriculture. This change reflects the evolving scientific landscape within this field and allows us to deliver more specialized content to our readers. JELSA will now transition to a regular publication schedule, releasing two issues per year. This streamlines the review and publication process, ensuring our readers have access to the latest research findings.

In line with the specialization of JELSA, the composition of the Editorial Board has also been strengthened with the presence of prominent experts in the fields of emergy, life cycle, and system analysis in agriculture.

I would like to take this opportunity to express my sincere gratitude to the Dr Peyman Afrasiab, President of University of Zabol, Dr Majid Erfanian the Vice-President for Research of the University, and Dr. Daniel E. Campbell, the Honorary Editor-in-Chief of JELSA, who have been constant supporters of this journal.

We are also deeply appreciative of the authors, reviewers, and contributors to this current issue of JELSA. Your tireless efforts have significantly enhanced the quality of our journal.

With its renewed approach, JELSA aspires to be a leading reference for topics related to emergy, life cycle, and system analysis in agriculture. We aim to deliver valuable services to the scientific community and enthusiasts in this field.

Sincerely,
Mohammad Reza Asgharipour
Editor-in-Chief, JELSA

Aims and Scopes

Journal of Emergy, Life Cycle and System Analysis in Agriculture is an international journal that deals with interactions between agricultural systems and the life-supporting environment on which human wellbeing ultimately depends. The journal publishes original article, short communications and review article. The journal's focus should capture the current needs of the agricultural systems with the goal of advancing the well-being of the people. The papers in the journal should address the critical issues that will move agricultural systems forward and improve the living conditions of the people. In this regard, the three critical systems that we need to understand to accomplish this end are environment, agriculture and society. The role of Journal is to provide a forum to agricultural scientists to deliberate on important issues of agricultural research, education and extension and present views of the scientific community as policy inputs to planners, decision/opinion makers at various levels.

Journal of Emergy, Life Cycle and System Analysis in Agriculture honors scientists at various levels, and encourages cutting edge research in a variety of agricultural disciplines. The journal's mission is to publish papers on new and emerging disciplines and concepts in order to provide future directions for agricultural research across the world. It is a unique journal that promotes inter-disciplinary research by encompassing all fields of crop sciences, animal sciences, fishery sciences, forestry sciences, agricultural machinery and natural resources management sciences, to stimulate interest in inter-disciplinary research.

The following should be included in all manuscripts submitted to *Journal of Emergy, Life Cycle and System Analysis in Agriculture:*

- ***Generally, should focus on the critical issues that will move agricultural systems forward and improve the living conditions of people.***
- ***Substantial natural science material (particularly farm- or landscape-level, sometimes coupled with social sciences), and***
- ***A thorough examination and discussion of the interconnections between agricultural system components and other systems.***

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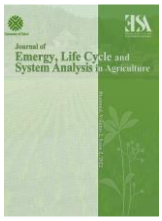
Journal homepage: jelsa.uoz.ac.ir



Effect of different irrigation treatments on physiological traits of milk thistle (*Silybum Marianum*) at different stages of growth

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ARTICLE INFO

Article history:
Received: 27 March 2022
Accepted: 24 April 2022
Available online: 27 April 2022

Keywords:
Malondialdehyde
Oxidative stress
Photosynthetic pigments
Water requirement

ABSTRACT

Drought stress is the primary constraint on crop and medicinal plant yields in arid and semi-arid regions. Milk thistle is a medicinal plant with antioxidant secondary metabolites (flavonolignans). The effect of drought stress was evaluated in this study at three growth stages (6, 13, and 20 weeks after planting) using four different levels of irrigation (100, 75, 50%, and 25% of water requirement, respectively, as non-stress, mild stress, moderate stress, and severe stress). The experiment was conducted in a greenhouse located in Shandol village, Hirmand city, Iran, as a factorial experiment with a completely randomized design. The following agronomic and physiological characteristics were determined: fresh weight, dry weight, photosynthetic pigments, proline, carbohydrates, malondialdehyde, relative water content, and ion leakage. The results indicated that the effect of various irrigation levels, harvest time, and their interaction were significant for the majority of traits, except for the relative water content and ion leakage traits, indicating that these traits react differently at various growth stages. Fresh weight, dry weight, photosynthetic pigment content, and relative water content all decreased under drought stress conditions, to the point where the lowest amount was observed under severe drought stress (25% of water requirement). Drought stress results in thylakoid protein hydrolysis, chlorophyll a and chlorophyll b reduction, and pigment and photosynthetic structure loss. With increasing stress intensity, the concentrations of proline, carbohydrates, malondialdehyde, and ion leakage increased. As a result, the highest amount was discovered under severe drought stress conditions. As a result, this increase indicates that the plant is suffering from oxidative stress as a result of the drought. Proline content increased proportionately to the severity of the stress, reaching its maximum value under severe drought stress (25% water requirement). Thus, under drought stress conditions, milk thistle responds to oxidative stress by increasing the accumulation of this osmolyte.

Highlights

- The study examines how drought stress affects milk thistle across three growth stages.
- The paper shows that drought stress reduces milk thistle biomass and photosynthetic pigment and increases proline and carbohydrates, indicating oxidative stress.
- The paper shows that milk thistle plant physiological traits vary significantly with irrigation level, harvest time, and their interaction, suggesting that the plant adapts to drought stress differently at different growth stages.

1. Introduction

Milk thistle belongs to the *Asteraceae* family, annual or biennial (Abenavoli et al., 2010). The genus Silybum includes two species, *S. marianum* and *S. eburneum* (Adzet *et al.*, 1993). This plant is native to Western, Central, and Northern India and today grows as a spontaneous plant in

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https://doi.org/10.22034/aes.2022.335343.1026

Southern Europe, Australia, China, South America, Africa, and Asia (Yanive and Palevitch, 1982). Due to its increasing use in the pharmaceutical industry, it is widely cultivated in several countries. It is an essential medicinal plant in European countries and has recently become more important in North America (Karkanis *et al*., 2011). A substance called silymarin has been extracted from the fruit of the milk thistle. The medicinal importance of this plant is due to the presence of this substance in it. Extraction of this substance and related chemical studies were first performed by Wagner *et al*. (1968). Silymarin is a flavonoid compound consisting of five different flavonolignans, including Silybin A and B, Silyadine, Silydianine, Silicristine, and Dihydrosilicin. About 4% of Silymarin is present in the seeds of this plant (Subramanian *et al*., 2008).

Drought refers to the insufficient amount of water available during the plant growth period and is one of the most critical environmental stresses, as a result of which the economic performance of the plant is reduced (Mitra, 2001). Drought stress reduces photosynthesis due to closed stomata. Carbon dioxide stabilization is further reduced by drought stress due to biochemical changes in chloroplasts (Bhattacharjee, 2005).

Photosynthesis, as a physiological indicator, is one of the most essential processes in growth and production, and maintaining the rate of carbon stabilization under stress is essential for plant yield. Under moisture stress conditions, photosynthesis is one of the first processes that is affected by stress, and its amount decreases with decreasing amount of available water (Hosseinian *et al*., 2020). Numerous researchers have reported a decrease in chlorophyll content due to drought stress. Caser *et al*., (2018) observed a decrease in chlorophyll content under drought stress conditions in sage. In another study in carrots, a decrease in the content of chlorophyll a, chlorophyll b, and carotenoids were reported under different moisture stress conditions (Razzaq et al., 2017).

Reduction of growth parameters as a result of drought stress in milk thistle has been reported by several researchers. Elsayed et al., (2019) found that drought stress led to a decrease in fresh weight, dry matter, and chlorophyll content and an increase in proline content, antioxidant enzyme activity, malondialdehyde content, and silymarin content. Mohammad Pour et al., (2017) observed a decrease in seed yield of milk thistle under drought stress conditions and stated that this decrease was due to reduced photosynthesis and material formation in the plant. Merwad et al., (2018) reported an increase in electrolyte leakage in the kidney bean plant under stress conditions, indicating membrane damage. Khaleghi *et al*., (2019) observed that the amount of soluble carbohydrates in *Maclura pomifera* increased with increasing drought stress, but decreased under severe stress (30% of field capacity).

Mechanisms for coping with drought stress have particular importance in medicinal plants. Identifying enzymes involved in stress tolerance in genetic manipulation of this plant will be effective. Also, by identifying the resistance of this plant to stresses, it is possible to increase the amount of effective compounds in these plants by using various types of stresses, including drought, and take advantage of their valuable properties. Because effective compounds in this plant increase with developmental stage, the resistance of this plant to various irrigation treatments at various developmental stages was investigated. The main purpose of this study was to investigate the effect of different irrigation treatments on the physiological traits of milk thistle (Silybum Marianum), a medicinal plant with antioxidant flavonolignans, at different stages of growth.

2. Materials and methods

2.1. Plant material

The seeds of the milk thistle plant were prepared from Pakan Bazr Isfahan Company (Iran, Isfahan province). The experiment was conducted in the greenhouse of Hirmand city, Shandol village (Sistan and Balouchestan province, Iran).

2.2. Germination and cultivation of seeds

First, the seeds were immersed in 5% sodium hypochlorite solution for 2 minutes, and after surface washing with 70% alcohol, they were disinfected for 30 seconds and washed again with sterile distilled water. Then, for germination, it was transferred to Petri dish containing filter paper and placed in a germinator at 25 °C.

Pots of the same size are selected and about one third of the pots are filled with sand, and the rest are filled with light soil containing granular fertilizer. The germinated seeds are transferred to the pots in the greenhouse under controlled temperature and humidity (temperature 19 to 21 °C and relative humidity 60 to 65%). This study was performed as factorial experiment based on the completely randomized design with three replications in July 2020 to December 2020. Experimental factors included four irrigation levels (100, 75, 50, and 25% of plant water requirement) and three levels of harvest time (6, 13, and 20 weeks after planting).

The pots were normally watered and given Hoagland nutrient solution every two days. Four weeks after planting, the irrigation regime was changed, and different irrigation treatments were applied. To apply drought stress, we used the weighting method using a digital scale with an accuracy of 0.001 g (Elsayed et al., 2019). In this way, after mixing the soil and adding it to the pots, the pots were completely saturated and then the pots were covered to prevent water evaporation. Gravity outflow from the bottom of the pot was measured at regular intervals until gravity outflow stopped. The weight of the pot in this case was considered as the weight in the field capacity (100% plant water requirement). To apply stresses of 75%, 50% and 25% of water requirement, the volume of water obtained n 100% of water requirement of the plant, was multiplied by these numbers (25, 50 and 75%) and the volume obtained was given to the pots as stress treatment. To measure traits, at different stages of development, including early development (6 weeks), medium development (13 weeks), and final development (20 weeks) of all treatments as well as control treatment, was collected leaf sample and were divided into two parts. One part was used to calculate fresh weight and dry weight, and the other part was used at -80 °C for further evaluations.

2.3. Measurement of physiological traits

2.3.1. Fresh and dry weight: To measure the fresh weight of the plant, the entire shoot and root of the plant were wholly separated from the soil, and after removing the soil from the roots, the plant was measured with an accurate scale. The samples were then placed on filter paper and dried at room temperature for 48 hours with an accurate scale.

2.3.2. Chlorophyll a, chlorophyll b, and carotenoids: 0.5 gr of fresh plant material was poured in a porcelain mortar, then liquid nitrogen was used to grind it to a fine powder. Twenty ml of 80% acetone was added to the sample and centrifuged at 6000 rpm for 10 minutes. The upper phase was transferred to a glass balloon, and some of the samples inside the balloon was poured into a cuvette spectrophotometer, and then the absorbance values were read separately at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids. Finally, using the following formulas, the amount of chlorophyll a, chlorophyll b, and carotenoids in milligrams per gram of fresh weight of the sample is obtained (Litchtenthaler and Wellburn, 1985).

$$\text{Chlorophyll a: } [(12.7\times A_{663}) - (2.69\times A_{645})]\times V/1000\times W$$
$$\text{Chlorophyll b: } [(22.9\times A_{645}) - (4.69\times A_{663})]\times V/1000\times W$$
$$\text{Carotenoids: } [1000(A470) - 1.8\,(chla) - 85.02(chlb)]/198\}\times V/1000\times W$$

where A is the absorption rate the desired wave length, V is the final volume of the acetone 80% in milliliters and W is the leaf size based on grams.

2.3.3. Measurement of leaf proline: The amount of proline was performed by the method of Bates et al. (1973), and was measured by spectrophotometer with a wavelength of 520 nm and calculated using a standard curve in milligrams per gram of fresh weight.

2.3.4. Carbohydrate concentration: The total carbohydrate content of the leaf solution was measured by Sheligl's (1986) method. The amount of extracted carbohydrates based on micrograms of glucose in grams of wet weight was extracted from the standard table.

2.3.5. Relative leaf water content and ion leakage: Sampling was performed of the last developed leaf of all experimental treatments, and the relative water content of the leaf was measured by Ritchi and Nguyen, (1990) method. The initial electrical conductivity (LT) and the final electrical conductivity (LO) were measured by EC meter, and the percentage of ion leakage was calculated according to the formula (LT / LO) × 100 (Lutts et al., 1995).

2.3.6. Measurement of membrane lipid peroxidation: Malondialdehyde (MDA) concentration was used to measure membrane lipid peroxidation. The extinction coefficient of 155 $mM^{-1}cm^{-1}$ was used to estimate the MDA concentration, and its values were calculated in terms of nanomoles per gram of fresh weight (Heath and Paker, 1969).

2.4. Data analysis

The Kolmogorov-Smirnov test was used to assess the normality of the data. Analysis of variance of data related to all measured traits was performed using a factorial experiment model based on the completely randomized design, with three replications. Analysis of data was performed using SAS software version 9.2. Data were compared by the Duncan method. Excel software version 2017 was used to draw the charts.

3. Results and discussion

3.1. Wet weight, dry weight, relative water content, ion leakage, and malondialdehyde content

The analysis of variance showed the irrigation treatment factor had a significant effect on these traits, which indicates that these traits had different reactions under different soil moisture conditions. There was a significant difference between harvest time factors for these traits, except for the relative water content and ion leakage traits, which indicates the difference in the reaction of these traits at different growth stages. Also, the interaction between irrigation treatment factor and harvest time was significant for these traits, except for the relative water content and ion leakage (Table 1).

According to our results, Belits and Sams (2007) reported that the growth parameters of milk thistle, such as capitol diameter, stem height, and the number of seeds, were affected by irrigation treatments. In another study, it was observed that drought stress, plant growth stages and their interaction had a significant effect on the fresh weight and dry weight of milk thistle (ElSayed et al., 2019).

The significant effect of drought stress on relative water content, ion leakage, and malondialdehyde content in milk thistle has been reported by other researchers (ElSayed et al., 2019). In other plants, the effect of drought stress on these traits has been observed by several researchers (Sajedi et al. 2012, Yousefzadeh and Ehsanzadeh, 2017; Zhang *et al*., 2017).

The highest and lowest fresh weight (3.31g and 1.48 g, respectively) and dry weight (1.02 g and 0.237 g, respectively) belonged to the irrigation treatment of 100 and 25% of water requirement, respectively. These results showed that drought stress has a detrimental effect on plant growth parameters and ultimately leads to fresh and dry weight loss. According to our results, Zahir et al., (2014) reported that drought stress significantly affected root development and shot growth in the milk thistle plant. Therefore, root growth, seedling height, leaf development, fresh weight, an dry weight decreased in drought stress compared to non-stress conditions. Other researchers have reported a decrease in the growth parameters of milk thistle under drought stress conditions (Beltis and Sams, 2007; ElSayed et al., 2019).

Table 1. Analysis of variance for fresh weight, dry weight, relative water content, ionic leakage, and malondialdehyde content of milk thistle under 4 irrigation treatments (A) and 3 harvest times (B)

Source of variation	d.f.	Mean of Squares				
		Wet weight	Dry weight	Relative water content	Ionic leakage	Content of M.D.A
A	3	5.95**	1.11**	393.6**	780.8**	3.25**
B	2	24.5**	6.16**	1.43n.s	84.1n. s	0.302**
A×B	6	1.11**	1.02**	6.45n.s	74.3n. s	0.042**
Error	24	0.035	0.0004	4.97	47.6	0.002
CV	-	7.52	3.98	3.88	12.4	6.58

ns: non-significant, * and ** significant at 5% and 1% probability levels, respectively.

In the present study, moisture stress significantly increased ion leakage, and malondialdehyde content, indicating oxidative damage to the plant under drought stress. This increase is 14.6%, 23.5% and 32.9% for ion leakage, and 44.2%, 83.1%, and 87.2% for malondialdehyde content in 75, 50 and 25% water requirements, in comparison 100% water requirement (non-stress), respectively (Table 2). In this study, increasing the amount of ion leakage and malondialdehyde content indicates that the peroxidation of cell membrane lipids has increased under drought stress, and as a result, the stability of the cell membrane has been lost. Under drought stress conditions, reactive oxygen species are produced, which leads to the peroxidation of cell membrane fatty acids and the production of malondialdehyde. Therefore, malondialdehyde levels can indicate membrane lipid peroxidation and are an important measure of stress sensitivity (Lata *et al*., 2011). When the relative water content of cells and tissues is maintained, better conditions for metabolite activity are provided through osmotic regulation, and therefore plants will grow better. A direct relationship has been observed between maintaining the relative water content and drought tolerance in safflower (Alizadeh *et al*., 2019). Reduction of relative water content, increase of ion leakage, and malondialdehyde content under drought stress conditions by other researchers in different plants such as carrots (Razzaq *et al*., 2017), wheat (Zhang *et al*., 2017), and cowpea (Abdel- Rahman *et al*., 2018) have also been reported.

Table 2. Mean of fresh weight, dry weight, relative water content, ionic leakage and malondialdehyde content of milk thistle under 4 irrigation treatments

Treatment of irrigation	Wet weight (gr)	Dry weight (gr)	Relative water content (%)	Ionic Leakage (%)	Content of M.D. A (nmol/gr of wet weight)
100% water requirement	3.31a	1.02a	65.2a	44/9c	0.150d
75% water requirement	2.98a	0.429b	59.2b	52.6b	0.269c
50% water requirement	2.23b	0.350c	56c	58.8b	0.890b
25% water requirement	1.48c	0.237d	49.4d	66.9a	1.45a

For each trait, the averages that have at least one common letter, do not differ significantly according to Duncan's test at the 5% probability level.

The results of the present study showed that at different stages of plant growth, relative water content and ion leakage were not significantly different, but malondialdehyde content increased with increasing plant growth. The highest and lowest values were related to the growth stage twenty weeks after planting (final development) and six weeks after planting (initial development), respectively (Table 3). The interaction effect of irrigation treatment and harvest time was not significant for the relative water content and ion leakage traits, indicating that the response of these traits is not different at different growth stages under different moisture conditions. Comparison of the mean interaction of irrigation treatment and harvest time of Malondialdehyde content revealed that in all stages of plant growth, its amount increased in drought stress compared to non-stress conditions. The lowest and highest were in the growth stage six weeks after planting, in the condition of 100% water requirement (non-stress), and twenty weeks after planting to 25% water requirement (severe stress) respectively.

3.2. Photosynthetic pigment content and proline content

The analysis of variance showed the irrigation treatment factor had a significant effect on these traits, which indicates that these traits had different reactions under different soil moisture conditions. Also, a significant difference was observed between harvest time factors for these traits, which indicates the difference in the reaction of these traits at different stages of development. Their interaction was significant for these traits except for the content of chlorophyll a and carotenoids (Table 4).

Table 3. mean of traits fresh weight, dry weight, relative water content, ionic leakage and malondialdehyde content of milk thistle in different time harvest

Time of harvest	Wet weight (gr)	Dry weight (gr)	Relative water content (%)	Ionic leakage (%)	Content of M.D. A (nmol/gr of wet weight)
6 weeks after planting	1.19[c]	0.059[c]	57.8[a]	52.9[a]	0.555[c]
13 weeks after planting	2.30[b]	0.134[b]	57.3[a]	56.6[a]	0.649[b]
20 weeks after planting	4.02[a]	1.34[a]	57.2[a]	60[a]	0.865[a]

For each trait, the averages that have at least one common letter, do not differ significantly according to Duncan's test at the 5% probability level.

Table 4. Analysis of variance for photosynthetic pigment content (chlorophyll a, chlorophyll b and carotenoids) and proline of milk thistle under four irrigation treatments (A) and three harvest times (B)

Source of variation	d.f.	Mean of Squares			
		Chlorophyll a	Chlorophyll b	Carotenoid content	Proline
A	3	5.35**	0.776**	0.632**	7.08**
B	2	90.2**	9.64**	2.20**	18.8**
A×B	6	0.776[ns]	1.62**	0.018[ns]	3.96**
Error	24	0.358	0.023	0.053	0.064
CV%	-	10.8	7.64	19	5.37

ns: non-significant, * and ** significant at 5% and 1% probability levels, respectively.

3.2. Photosynthetic pigment content and proline content

The analysis of variance showed the irrigation treatment factor had a significant effect on these traits, which indicates that these traits had different reactions under different soil moisture conditions. Also, a significant difference was observed between harvest time factors for these traits, which indicates the difference in the reaction of these traits at different stages of development. Their interaction was significant for these traits except for the content of chlorophyll a and carotenoids (Table 4).

According to the results of the present study, a significant difference between different growth stages (6, 12, and 18 weeks after planting), and different water stress treatments (75 and 50% water requirement) for photosynthetic pigment traits and proline content in milk thistle by ElSayed *et al*., (2019) observed. Other researchers have also shown a significant effect of drought stress on the content of photosynthetic pigments and proline in different plants such as sage (Caser *et al*., 2018), fennel (Gholami and Ehsanzadeh, 2018), and safflower (Alizadeh *et al*., 2019) have reported.

The amount of photosynthetic pigments decreased in proportion to the increase in stress intensity. At 100% water requirement (non-stress) the highest, and at 25% water requirement (severe stress), the lowest amount of photosynthetic pigments was obtained. Similar to our results, other researchers have shown a decrease in total chlorophyll content in milk thistle under drought stress conditions compared to non-stress conditions (Mazarei *et al*., 2017 and Elsayed *et al*., 2019). Under moisture stress, hydrolysis of thylakoid proteins, reduction of chlorophyll a and chlorophyll b, and loss of pigments and photosynthetic structures occur. Therefore, the amount of chlorophyll damage in leaves can be one of the critical indicators of environmental stress, which ultimately leads to reduced plant yield (Nikan and Ghorbani, 2007). Merwad *et al*., (2018) reported that moisture stresses significantly reduced photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid content) in cowpea. Decreased chlorophyll under drought stress was also observed by Yousefzadeh and Ehsanzadeh, (2017) in sesame, and Caser *et al*., (2018) in sage.

In the present study, the content of photosynthetic pigments showed different reactions to different stages of plant growth, so that the highest content of chlorophyll a, chlorophyll b and the content of carotenoids was observed in the growth stage thirteen weeks after planting (medium development) and twenty weeks after planting (final development), respectively (Table 6).

Proportional to the increase in stress intensity, the amount of proline increased, so that it had the highest value in severe drought stress (25% water requirement). The amount of proline was 14.2%, 29.7%, and 34.6% in 75, 50, and 25% water requirements, compared to 100% water requirements respectively. Therefore, milk thistle responds to oxidative stress by increasing the accumulation of this osmolyte under drought stress conditions.

According to our results, an increase in proline content was observed by Amiri *et al*., (2017) in the aromatic geranium plant and in the carrot plant by Razzaq *et al*., (2017). Numerous studies have reported that under water stress conditions, proline content accumulation in drought-tolerant cultivars was higher than drought-sensitive cultivars (Gholami & Ehsanzadeh, 2018 and Alizadeh *et al*., 2019). Therefore, proline has a protective role against oxidative stress and increases plant yield and tolerance to drought stress. Comparison of the mean interaction of irrigation treatment and harvest time of proline content showed that proline content increased in all growth stages. The lowest and the highest were observed in the growth stage 6 weeks after planting at 100% water requirement and the growth stage 20 weeks after planting at 25% water requirement.

Table 5. mean of photosynthetic pigment content (chlorophyll a, chlorophyll b and carotenoids) and proline content of milk thistle under irrigation different treatment

Treatment of irrigation	Chlorophyll a	Chlorophyll b	Carotenoid content	Proline
unit		Mg/gr wet weight		Mmol/gr wet weight
100% water requirement	6.38[a]	2.34[a]	1.46[a]	3.69[d]
75% water requirement	5.89[a]	2.02[b]	1.43[a]	4.3[c]
50% water requirement	5.21[b]	2.03[b]	1.03[b]	5.25[b]
25% water requirement	4.62[c]	1.62[c]	0.94[b]	5.65[a]

For each trait, the averages that have at least one common letter, do not differ significantly according to Duncan's test at the 5% probability level.

Table 6. mean of photosynthetic pigment content (chlorophyll a, chlorophyll b and carotenoids) and proline content of milk thistle in different time harvest

Time of harvest	Chlorophyll a	Chlorophyll b	Carotenoid content	Proline
		Mg/gr wet weight		Mmol/gr wet weight
6 weeks after planting	2.469[c]	1.072[c]	0.844[c]	3.465[c]
13 weeks after planting	2.770[a]	2.860[a]	1.113[b]	4.733[b]
20 weeks after planting	6.333[b]	2.075[b]	1.683[a]	5.971[a]

For each trait, the averages that have at least one common letter, do not differ significantly according to Duncan's test at the 5% probability level.

4. Conclusion

The results of this study showed that with increasing intensity stress, traits such as photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid content), fresh weight, dry weight, and relative water content decreased. As at 100% water requirement (non-stress) the highest, and at 25% water requirement (severe stress), the lowest amount was obtained. Therefore, the amount of chlorophyll damage in leaves can be one of the critical indicators of environmental stress. These results showed that drought stress has a detrimental effect on plant growth parameters and ultimately leads to wet and dry weight loss. Moisture stress significantly increased ion leakage and malondialdehyde content, indicating oxidative damage to the plant under drought stress. Increased ion leakage and malondialdehyde content in this study showed that under water stress conditions, peroxidation of cell membrane lipids increased, and as a result, cell membrane stability was lost. Under drought stress conditions, reactive oxygen species are produced, which leads to the peroxidation of cell membrane fatty acids and the production of malondialdehyde.

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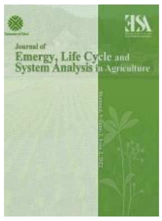

Investigation of the phytochemicals produced by the eucalyptus (*Eucalyptus viminalis*) throughout several growth seasons

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ARTICLE INFO

Article history:
Received: 10 May 2022
Accepted: 19 August 2022
Available online: 29 August 2022

Keywords:
Climate
Essential oils
Flavonoids
Phenolic compounds

ABSTRACT

Medicinal plants contain flavonoids and phenolic compounds, which are the primary antioxidants produced by plants. Antioxidants are chemical compounds that protect the body from damage by counteracting free radicals. The Myrtaceae family comprises eucalyptus, a medicinal plant. At the Lorestan Research Center, this study investigated how harvesting times affected the phytochemical composition of eucalyptus (Eucalyptus viminalis) essential oil. Three replications of a randomized complete block design were utilized to conduct the experiment. The concentrations of flavonoids and total phenol were ascertained by employing Folin-Ciocalteu's regent and aluminum chloride, respectively. By means of GC/MS analysis, the hydrodistilled essential oils of eucalyptus leaves were evaluated. In general, the results indicated that the highest concentrations of phenolic and flavonoid compounds were detected during the spring season. During the spring, summer, and fall, leaf essential oil contained 43, 14, and 17 components, respectively. 1,8-Cineol, alpha-pinene, and trans-pinocarveol exhibited the highest concentrations across all three seasons. Collectively, the results indicate that the duration of the harvest significantly influences the concentration of bioactive compounds in plants.

Highlights

- The study investigates the effects of harvesting time on the phytochemical composition of eucalyptus essential oil
- The study uses Folin-Ciocalteu's regent and aluminum chloride to measure the concentrations of phenolic and flavonoid compounds in the oil
- The study finds that the spring season yields the highest concentrations of these compounds, followed by the fall and summer seasons
- The study identifies 1,8-Cineol, alpha-pinene, and trans-pinocarveol as the major components of the oil across all seasons

1. Introduction

Eucalyptus trees are among the fastest-growing forest species. This plant is native to Oceania, especially Australia. They have been cultivated in countries other than Australia (their natural habitat) for about 200 years. So far, nearly 65 million hectares of different species of Eucalyptus have been cultivated in more than 200 countries, including Iran (Anonymous, 1979; Tewari, 1992). The genus Eucalyptus, with more than 700 species, belongs to the family Mirtaceae. Eucalyptus was first cultivated about 100 years ago. However, it has been almost 35 years since the study of its various species in the different provinces of Iran was started by executive bodies in arable areas and domestic research stations (Assareh and Sardabi, 2007).

Today, most uses of Eucalyptus essential oil are in the cosmetics and perfumery industries. Also, the essential oil of this plant is used in medicine as an antimicrobial and

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https://doi.org/10.22034/aes.2022.341837.1037

antiseptic to improve the taste of medicines. In addition, the components of the essential oil can be used as a flavoring in food. As mentioned, essential oils have antimicrobial properties that are used in a variety of ways, such as dental fillings, disinfectants, and feed supplements for livestock. In addition, today's few preservatives contain essential oils that are commercially available (Burt, 2004). Phenols and flavonoids play different roles in plants, for example, anthocyanins. Anthocyanins in the petals are responsible for creating different colors, which are very important in attracting pollinators and plant survival. Flavonoids in leaves have a protective role against ultraviolet rays. Accordingly, flowers and leaves are the two main storage organs for these compounds. On the other hand, due to photosynthesis in leaves, the precursors of the flavonoid biosynthesis pathway are more abundant in leaves than in other organs. This is another factor in the higher flavonoid composition of leaves than flowers and other organs (Davis et al., 2004).

In 2005 and 2006, a study was conducted on seasonal changes in the quantity and quality of essential oils of three species of Eucalyptus in southern Iran in the tropical regions of Shushtar and Dezful (Esfahanianfard et al., 2010). The results of this study showed that the highest essential oil yield was in *E. melliodora* species and was about 1.3-3.9%. Also, the highest amount of 1,8-cineole compound was measured in the Shushtar region in winter, at about 62-73%. According to other results from the above research, the highest yield of essential oil in *E. kingsmilli* in the Shushtar region in winter is about 2.8–3.1%, and the highest 1,8-cineol composition is about 68.4–77.3 %. In another study, the effect of different seasons on the composition and amount of essential oils of *E. maculate* leaves was investigated. The results of their research showed that the amount of essential oil of this species is different in different seasons. The highest production of 1,8-cineole was obtained in late spring and early summer (Assareh et al., 2010). This condition can be due to seasonal changes in location. In one study, the essential oil compounds of *E. globulus* were investigated by the gas chromatography-mass spectrometry (GC / MS) technique. The results showed that of the 32 compounds identified in the essential oil of the plant leaves (including 95.51% of the total essential oil), the compounds 1,8-cineole (39.59%) and alpha- phellandrene (15.83%) contained the highest percentage. Also, 25 compounds were identified in the fruit of the plant (including 94.68% of the total essential oil), among which alpha-pinene (57.82%), alpha-terpineol (6.76%), alpha-camphollenal (4.80), and ortho-cement (3.82%) were identified as the main compounds (Pashazanousi et al., 2012). In another study, by studying the constituents of the essential oils of the leaves of four species of Eucalyptus in Kashan, it was found that the compound 1,8-cineole is the main component of the essential oils of the leaves of all studied species (Batooli et al., 2012).

In other words, the main chemical composition in the essential oils of the leaves of all species of the genus Eucalyptus is 1,8-cineole. It should be noted that the amount of this compound is different in different species of Eucalyptus. Since Eucalyptus grows in different arid and semi-arid regions of Iran and environmental factors affect the amount of essential oil in this plant, it is very important to study the composition of active ingredients in different seasons to select the appropriate time for leaf harvest. This study was conducted to evaluate the phytochemical compositions and essential oils of Eucalyptus leaves in different seasons in Lorestan climatic conditions.

2. Materials and Methods

The leaves *of E. viminalis* were harvested from the Lorestan Agricultural Research Station in different growing seasons. The leaves were dried under shade and without moisture. For this purpose, first the waste was removed from the leaves, and then, after washing with water, they were dried in laboratory conditions. Then the leaves of each season were powdered separately by the mill and then used for extraction (Naznin and Hasan, 2009).

2.1. Measurement of total phenol

The amount of total phenol compounds was measured by the Folin-Ciocalteu's regent, and the results were expressed in terms of mg of gallic acid per gram of extract (Slinkard and Singleton, 1977). The basis of this method is the reduction of folate reagent by phenolic compounds in an alkaline environment and the formation of a blue complex. In this method, the following compounds were first added to the test tube and mixed:

- 200 μl of extract (1 mg/ml) or standard ethanolic solution of gallic acid or another phenolic acid
- 400 microliters of fullene cyclate reagent (diluted with distilled water) in a ratio of 1 to 10
- 400 microliters of 7% sodium carbonate

In the next step, after 30 minutes of storage at laboratory temperature, its light absorption was read by a spectrophotometer at 765 nm. It should be noted that the blank was also prepared by the above method, but 70% methanol was used instead of the extract.

2.2. Measurement of total flavonoids

In this study, the aluminum chloride colorimetric method was used to determine the amount of flavonoids (Chang et al., 2002). For this purpose, first, each of the plant methanolic extracts (0.5 ml) was separately combined with 1.5 ml of methanol, 0.1 ml of aluminum chloride (10% methanol), 0.1 ml of potassium acetate (1 M), and 2.8 ml of distilled water. Then, after storing the solutions at room temperature for 40 minutes, the adsorption of each reaction compound was measured at 415 nm relative to the blank with a spectrophotometer. It should be noted that blank contained all of the above ingredients, but instead of the extract, the same volume of 80% methanol was added.

2.3. Extraction and analysis of essential oils

In this study, essential oils were extracted by distillation with water (Clevenger) for 2 hours. The essential oils were then identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) with the following characteristics. For this purpose, the essential oil extracted from the studied plant was injected into the GC/MS device by water distillation. The constituents were separated based on boiling point and polarity along a 30 m long column. In all given GC/MS spectra, the inhibition index for each peak was calculated from the normal alkane exit pattern and the spectrum inhibition index. By comparing the obtained indexes with the information available in the computer library and other sources, the spectra related to each object were interpreted. With the help of these results, the constituents of essential oils and their chemical formulas were identified. The relative percentage of each of the constituents of the essential oils was obtained according to the area under its curve in the chromatogram spectrum. The results were compared with the values published in different sources (taking into account the inhibition index).

2.4. Statistical analysis

The data obtained from the above sections was analyzed based on a one-way analysis of variance (ANOVA) using SPSS 26 and Excel software. The means were compared using Duncan's multiple range test at the statistical level of P 0.05.

3. Results and Discussion

3.1. Investigation of phenolic and flavonoid content:

The total amount of total phenolic compounds in *E. viminalis* leaves is shown in Figure 1. The results of comparing the total phenol content of Eucalyptus leaves in three different seasons showed that the highest phenol content is related to spring and the lowest phenol content is related to autumn (with an average of 30.05 mg GA/ g D.W.). It can be said that the amount of phenolic compounds under the influence of climatic factors in the region has been studied. These results indicate the effect of climatic factors on the total phenol content of Eucalyptus leaves.

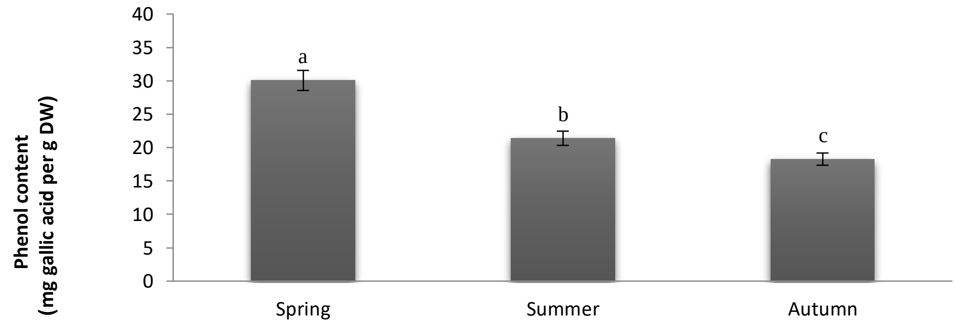

Figure 1. Total phenol content of E. viminalis leave

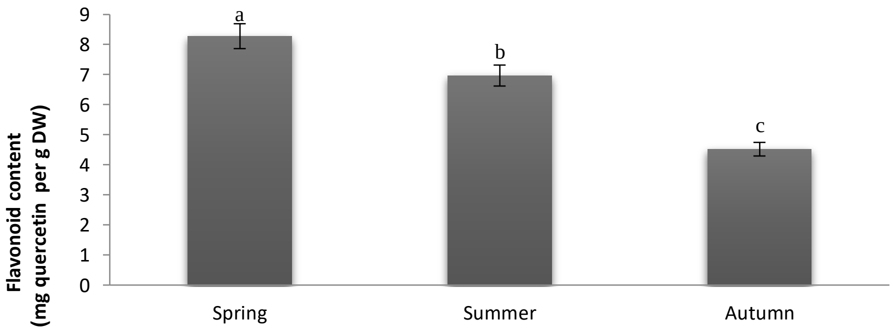

Figure 2. Total flavonoid content of *E. viminalis* leave

The total amount of total flavonoid compounds in *E. viminalis leaves* is shown in Figure 2. Analysis of the data indicates that the amount of total flavonoid compounds of this Eucalyptus leaf in spring has the highest amount (8 mg quercetin per g DW), and the lowest amount of flavonoids (about 4 mg quercetin per g DW) is related to autumn.

3.2. Chemical composition of Eucalyptus essential oil

The results of the constituents of *E. viminalis* essential oil during the three seasons of spring, summer, and autumn are given in Tables 1, 2, and 3. The results obtained from the analysis of Eucalyptus leaves collected from the Lorestan region in spring showed that this season, with 43 compounds, has the highest number of essential oil compounds compared to the other two seasons. The highest percentages of compounds in this season are related to 1, 8-cineole (54.51%), paracetamol (6.31%), and alpha-pinene (4.61%), respectively (Table 1). As shown in Table 2, there are 14 compounds in the summer, of which the three main compounds of this season are 1,8-cineole (59.58%), alpha-pinene (19.54%), and trans-pinocarol (13.2%). The results of Table 3 show the essential oil compositions in autumn. In this season, 17 compounds were observed, of which 1,8-cineole (51.42%), alpha-pinene (14.76%), and trans-pinocarol (6.01%) were the major compounds.

Table 1. Essential oil content of *E. viminalis* leave in spring

Row	Compounds	Retention time (RT)	Percentage of compounds
1	1,8-cineole	8.24	54.51
2	p-Cymene	5.93	6.31
3	α –pinene	5.63	4.61
4	trans-Pinocarveol	11.40	3.61
5	*β*- Pinene	6.72	2.93
6	L-Alloaromadendrene	23.64	2.80
7	Crypton	12.87	2.19
8	Naphthalene, 1,2,3,4,4a,7-hexahydro	24.59	1.75
9	α-Phellandrene	7.38	1.59
10	Terpinene-4-OL	12.47	1.54
11	Cembrene	23.56	1.23
12	δ –cadinene	21.66	1.13
13	Spathulenol	23.41	1.02
14	Tert-butydimethylsilyl ether	28.54	1.01
15	Isoledene	24.94	0.84
16	p-Cumic aldehyde	14.41	0.71
17	γ –terpinene	8.76	0.52
18	α-terpinene	15.75	0.48
19	α-4-trimethylbenzyl carbar	12.73	0.48
20	Torreyol	21.18	0.47
21	(+)-Ledene	23.86	0.35
22	(-)-Calamenene	21.83	0.34
23	β –myrcene	6.82	0.33
24	β –selinene	25.36	0.33
25	γ –Muurolene	25.05	0.32
26	Phellandral	15.43	0.32
27	Myrtenal	13.04	0.29
28	α-Terpinene	7.65	0.29
29	Trans-Sabinene hydroxide	10.80	0.28
30	Isovaleric acid	9.98	0.27
31	B-Phelladrene	6.56	0.25
32	α- Cadinen	25.01	0.25
33	Carvomenthol	13.94	0.24
34	Epi-Bicyclosquiphell	12.10	0.22
35	Myrtenol	12.98	0.22
36	Butanal, 3-methyl	2.17	0.21
37	Terpinolene	9.52	0.20
38	Trans-Carveol	11.47	0.15
39	Caryophyllene	19.12	0.14
40	Cis-carvyl acetate	13.61	0.13
41	Carvacrol	15.92	0.12
42	Cyclohexene, 1-butyl	11.62	0.11
43	α-Campholene aldehyd	10.89	0.10

Table 2. Essential oil content of *E. viminalis* leaves in summer

Row	Compounds	Retention time (RT)	Percentage of compounds
1	1,8-cineole	17.23	59.58
2	α –pinene	12.50	19.54
3	trans-Pinocarveol	21.98	2.13
4	Borneol	23.13	0.64
5	Fenchol	21.07	0.47
6	Pinocarvon	22.89	0.39
7	4-Terpineol	23.51	0.33
8	γ-Terpinene	18.19	0.26
9	*β*- Pinene	14.51	0.25
10	Camphene	13.20	0.16
11	p-Cymene	16.75	0.18
12	Myrcene	15.07	0.18
13	Campholenal	21.37	0.12
14	α -Pinene oxide	20.11	0.10

Table 3. Essential oil content of *E. viminalis* leave in autumn

Row	Compounds	Retention time (RT)	Percentage of compounds (%)
1	1,8-cineole	17.33	51.42
2	α –pinene	12.47	14.76
3	trans-Pinocarveol	22	6.01
4	α –terpineole	24.15	1.48
5	Pinocarvon	22.87	1.40
6	Cis-Carveol	25.54	1.15
7	Borneol	23.26	0.92
8	Fenchol	21.03	0.69
9	Camphene	13.19	0.46
10	p-Cymene	16.74	0.23
11	4-Terpineol	23.47	0.19
12	Campholenal	21.32	0.17
13	*β*- Pinene	14.49	0.14
14	Trans-Carveal	23.85	0.12
15	Verbenone	23.84	0.12
16	E-2-Hexenal	9.24	0.11
17	α -Pinene oxide	19.72	0.08

The amount of 1,8-cineole monoterpene varies between 51.42 and 59.58% in the studied Eucalyptus species. This monoterpene has been reported as the main compound in *E. globulus* (85.6%) (Barazandeh, 2005*)*, *E. porosa* (6.58%) (Assareh et al., 2005), *E. caesia* (4.69%) (Assareh et al., 2007), *E. spathulata* (72.5%) and *E. torquata* (9/66%) (Sefidkon et al., 2007*)*, *E. sargentii* (7.56%), *E. stricklandii* (71.2%) (Abravesh et al., 2007) and *E. globulus* (60 to 70%) (Pereira et al., 2005). Other studies have been performed on the essential oil components of *E. tereticornis* smith and *E. resinifera* smith (grown in Cuba). The results of their study showed that the composition of 1,8-cineole accounted for 68% of the essential oil of *E. resinifera*. This compound also contained 23.3% of the essential oil of *E. tereticornis.* In addition, paracetamol constitutes 13.8% of the essential oil of the above species, so paracetamol, along with 1,8-cineol, were the main components of the essential oil in this species (Batooli et al., 2012). It seems that the reason for the difference in the amount of monoterpene depends on the geographical conditions, climatic conditions, collection season, and the conditions of essential oil collection and phytochemical decomposition of essential oil. Alpha-pinene is the second major essential oil compound in *E. viminalis*, with the highest amount in summer (19.54%) and the lowest in spring (4.61%). Alpha-pinene is the second major essential oil compound in the species (*E. viminalis*). The highest amount of this compound is related to summer (19.54%) and the lowest amount is related to spring (4.61%). According to research, this monoterpene has been reported as the main component of essential oils in *E. camaldulensis* (12.8%), *E. alba* (20.1%) (Samate et al., 1998), *E. coleziana* (6.46%) (Ogunwande et al., 2005), *E. microtheca* (10.7%), *E. spathulata* (12.7%) (Sefidkon et al., 2007), *E. stricklandii* (9.2%), *E. brockwagii* (14%), *E. kruseana* (15.9%) (Abravesh et al., 2007) and *E. porosa* (12.8%) (Assareh et al., 2005). The main constituents of eucalyptus leaf essential oil studied in this study are consistent with the major constituents of eucalyptus cultivated in Kashan Botanical Garden (Batooli et al., 2012). Jaimand et al. (2012) reported the major constituents of *E. stricklandii* Maiden essential oil from northern Khuzestan as 1,8-cineole (72.7%) and alpha-pinene (12.2%). The third known compound is transpinocarol. This compound has shown the highest (6.01%) in autumn and the lowest (2.13%) in summer. Other compounds, such as para-cement, beta-pinene borneol, and phenolic, also have an acceptable amount of essential oil compounds. In addition, the results show that the amount of 1, 8-cineole and alpha-pinene (the two main constituents of essential oils in the present study) in the summer is greater than in the spring.

The increase in these compounds in the summer may be due to the drought stress of this season. In addition to increasing the amount of essential oil compounds in summer, the highest amounts of flavonoids and antioxidant activity are also observed in this season.

As can be seen in Table 1, spring has a higher number of identified compounds than summer and autumn. These results are probably related to seasonal changes (temperature conditions, water availability, etc.) that have led to the formation of new and more compounds in the essential oil. These findings can also be explained by the fact that in the summer, a high percentage of compounds related to 1, 8-cineole (59.58%) are present.Therefore, this can reduce the percentage of other compounds in the essential oil. On the other hand, in spring, the amount and percentage of compounds in the essential oil are lower and this has caused more compounds in the plant essential oil to be identified in this season.

This result is consistent with the results of Sefidkon et al. (2009), who showed that for obtaining the highest number of compounds of essential oil from *E. porosa* leaves, the best harvesting time is spring. In another study, the effect of region and harvest time on the type and amount of constituents of Eucalyptus essential oil was investigated. The results of that study showed the highest number of essential oil constituents (39 compounds) and the highest amounts of the main constituents of this plant (i.e., 1,8-cineole, alpha-pinene, and alpha terpinene) are in the Qasr Shirin and Gilangharb regions in summer (Gerdakaneh et al., 2018). One of the most important factors influencing the amount of active compounds in medicinal plants is the harvest time of plant organs.In this study, the highest concentration of the main components of Eucalyptus essential oil was obtained in summer. In some species, the best season for essential oils is hot and sunny weather.

These results are consistent with the results of the present study. The results of other researchers also showed that harvest time and seasonal changes also had a significant effect on the components of mint essential oil (Kofidis et al., 2006) and basil (Hussain et al., 2008).

4. Conclusion

One of the most important factors influencing the amount of active ingredients in medicinal plants is harvesting time. The results of the study showed that the highest amounts of essential oils and main compounds of Eucalyptus were obtained in summer.

Acknowledgments

This research was financially supported by the University of Zabol, Iran. [grant number IR-UOZ-GR-5521].

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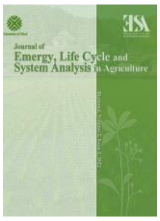
Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir



Energy and economic analysis of quinoa production in Iran: A case study in Iranshahr Region

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ARTICLE INFO

Article history:
Received: 2 July 2022
Accepted: 28 August 2022
Available online: 29 August 2022

Keywords:
Benefit-cost ratio
Energy productivity
Energy use efficiency
Net energy gain
Specific energy

ABSTRACT

Food and energy shortages, as well as environmental pollution, are the three fundamental challenges of many countries. On the one hand, optimal energy utilization decreases input consumption, while on the other hand, it reduces environmental damage. Energy analysis is a scientific tool for gauging the stability of an ecosystem devoted to crop production. The objective of the present study was to assess the amount of input and output energy, the proportion of direct, indirect, renewable, and nonrenewable energy types, and the energy use efficiency of the quinoa production system in the Iranshahr region of southern Iran. In 2020, the required information was gathered by administering a questionnaire and conducting an interview with the quinoa farmer at the Agricultural Research and Agricultural Jihad Center. This study collects data from 35 farms and is based on two outputs (grain and straw yields) and eight inputs. Evaluations of energy and economics revealed that the total energy input and output for quinoa production were 39122.99 MJ ha^{-1} and 90741.78 MJ ha^{-1}, respectively. For the cultivation of one hectare of quinoa, the values for energy use efficiency (2.32), energy productivity (0.17 kg MJ^{-1}), specific energy (5.83 MJ kg^{-1}) and net energy gain (51618.79 MJ ha^{-1}) were determined. Diesel fuel accounted for the largest portion of energy usage (26.39%). The results of the economic research also revealed that the average consumption expenses for producing one hectare of quinoa were \$1668.93 ha^{-1}, while the average net income of the farmer was approximately \$1451.86 ha^{-1}. In terms of energy consumption and profitability, this plant is therefore suited for cultivation in the research region. Compared to wheat and barley, quinoa requires significantly less energy to produce in this location. However, with improved management, the energy efficiency of quinoa can be increased and the proportion of nonrenewable energy used in production can be decreased.

Highlights

- This study assessed the input and output energy, proportion of direct, indirect, renewable, and nonrenewable energy kinds of the quinoa production system in Iranshahr.
- The energy consumption efficiency, energy productivity, specific energy, and net energy gain of quinoa were determined to be 2.32, 0.17, 5.83, and 51618.79 MJ/ha, respectively.
- The economic investigation found that the average cost to produce one ha of quinoa was $1668.93, whereas the average farmer netted $1451.86.
- With better management, quinoa's energy efficiency and nonrenewable energy use can be boosted.

1. Introduction

Today, food and energy resources shortages and environmental pollution are the three main problems in the world (McGuire, 2013). As the population grows, the demand for food and energy resources also increases, so the need for new knowledge to study the effects of agricultural

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https://doi.org/10.22034/aes.2022.349964.1040

production systems in the form of sustainability criteria is vital (Ruviaro et al., 2012). In this regard, agriculture must have minimal negative effects on climate, soil, water, air, biodiversity, and human health, as well as adhere to the principles of sustainable agriculture (De Boer, 2003). Cultivating drought and salinity -tolerant plants is one of the approaches that help human beings achieve sustainable agricultural goals. In this regard, the quinoa plant has the ability to grow in harsh conditions (Amiryousefi et al., 2021). Quinoa (*Chenopodium quinoa* Willd) is an annual broadleaf plant, one to two meters high, and part of the cereal-like plants native to Latin America. This plant is resistant to a variety of environmental stresses such as salinity, drought, and cold. Also, due to the high efficiency of this plant in water resource use (Präger et al., 2018), it can be a suitable plant for areas with limited water resources and very saline soils (Amiryousefi et al., 2021) Therefore, quinoa cultivation as a plant that can grow in poor soils (Jacobsen et al., 2009) and marginal lands (Präger et al., 2018), causes diversity in crops, increases production sustainability, farmers' incomes, and food security. Also, because quinoa is a medicinal and gluten-free plant, it is a valuable food and will also contribute to community health (Bonales-Alatorre et al., 2013). One of the most important approaches to achieving sustainable agriculture is the optimal use of energy in agriculture, which leads to reduced fossil fuel consumption, reduced environmental pollution, and also economic savings (Uhlin, 1998). According to economic principles, producers achieve sustainable production and maximum profit if they use inputs optimally (Cetin & Vardar, 2008). But a research has shown that with the development of agriculture, energy consumption in this sector has increased significantly (Abbas et al., 2020). In other words, most farmers use more energy to increase production (Ozkan et al., 2004). The reason for this can be attributed to the strong dependence of agricultural activity on various inputs such as fossil fuels, electricity, machinery, seeds, fertilizers, and chemical pesticides (Hamedani et al., 2011; Sefeedpari et al., 2014). Due to the improper use of energy and natural resources and its adverse effects on human health and the environment, one of the vital issues in the agricultural sector is the study of energy consumption patterns (Hatirli et al., 2005). The trend of energy changes in the agricultural sector can be monitored and managed by calculating energy efficiency and effectiveness. Therefore, the analysis of input and output energies is necessary for the optimal management of scarce resources in order to determine efficient and economical production activities. In this way, the amount of energy consumed at each stage of the production process can be determined (Chaudhary et al., 2006). This will determine how much energy has been used effectively (Moghimi et al., 2013). Energy circulation is one of the topics of agricultural ecology and so far many researches in this direction has been done by researchers all over the world, including Iran (Amiryousefi et al., 2021; Lotfalian Dehkordi & Forootan, 2020) quinoa, (Abbas et al., 2020) maize, (Nasseri, 2019) wheat, (Kazemi et al., 2018) cotton, (Jafari et al., 2018) pistachio, (Yildizhan, 2018) strawberry, (Zangeneh et al., 2010) potato, (Lu et al., 2010) rice and (Ghorbani et al., 2011) wheat.

Due to the fact that agriculture and the production of horticultural and agricultural products are the main activities of rural communities in Iran, most of the energy consumed in rural areas is spent on agriculture (Amiryousefi et al., 2021). In this regard, it seems necessary to analyze the pattern of energy consumption and its efficiency in the agricultural system and finally provide solutions for optimal energy consumption. Quinoa is well cultivated in different parts of Baluchestan, which has limited water resources and lands with low fertility and relatively high salinity and is able to produce a suitable crop. So far, very limited studies have been conducted in the fields of energy consumption and economic analysis of quinoa. Therefore, considering the adaptation and cultivation of this plant in the Iranshahr region and its great importance in the fields of food security, energy efficiency, increasing farmers' incomes and production sustainability, the purpose of this study is to evaluate the energy of inputs and outputs and determine the share of direct, indirect, renewable and non-renewable energy types in the quinoa production system in the Iranshahr region.

2. Materials and methods

The present study was conducted in the Iranshahr region, located in southeastern Iran. Iranshahr city (60 degrees and 41 minutes' east longitude and 27 degrees and 12 minutes' north latitude) is located in the central part of Sistan and Baluchestan province. Figure 1 shows the location of the study area on a map of Iran.

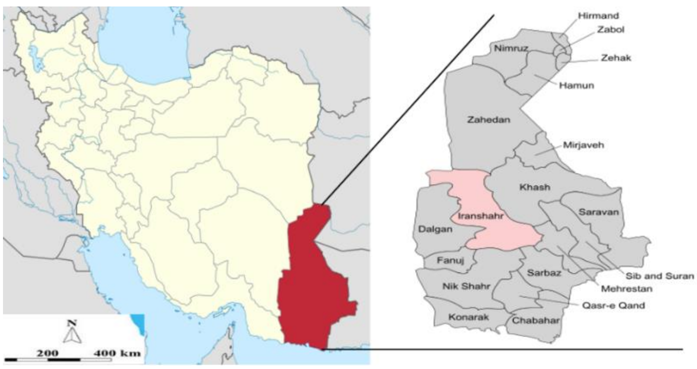

Figure 1. Location of the study region on the map of Iran

The necessary research data were gathered through the completion of a questionnaire and face-to-face interviews with quinoa producers, the Agricultural Research Center, and the Baluchestan Agricultural Jihad. The statistical population in this study was 35 quinoa farmers. In these fields, seed sowing is started in the second half of November and continued until the end of December, and the harvest is done after 140-120 days on average, depending on the type of cultivars. Input energies for quinoa production in the study area included seeds, chemical fertilizer, manure, insecticide, machinery, diesel fuel, water for irrigation and labor, and output energies included grain and crop residues (the production process is summarized in Figure 2). In order to calculate the input and output energies, the input and output data were converted to MJ ha^{-1} using the energy equivalents listed in Table 1.

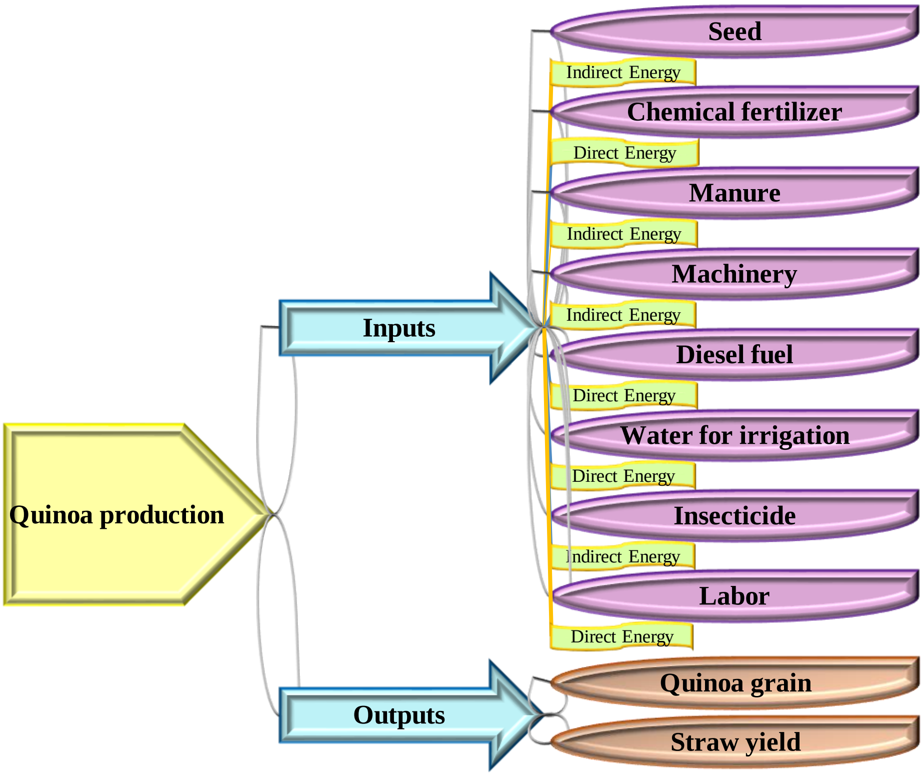

Figure 2. Inputs and outputs for the production of quinoa in Iranshahr, Iran

Table 1. Energy equivalents of inputs and outputs for the production of quinoa in one hectare

Particulars	**Energy equivalent (MJ unit^{-1})**	**Refs**
A. Inputs		
1. Seeds (kg)	17.21	(Lotfalian Dehkordi & Forootan, 2020)
2. Chemical fertilizer (kg)		(Abbas et al., 2020)
(a) Nitrate (N)	78.1	
(b) Phosphate (P2O5)	17.4	
(c) Potassium (K2O)	13.7	
3. Animal manure (kg)	0.3	(Mobtaker et al., 2010)
4. Insecticide (kg)	101.2	(Elhami et al., 2016)
5. Machinery (h)		(Lotfalian Dehkordi & Forootan, 2020)
(a) Tractor	93.61	
(b) Machinery (plows and discs)	62.70	
(c) Combine	87.63	
6. Diesel fuel (L)	47.8	(Elhami et al., 2016)
7. Water for irrigation (m3)	1.02	(Pishgar-Komleh et al., 2012)
8. Labor (h)	1.96	(Elhami et al., 2016)
B. Outputs (kg)		
1. Quinoa grain	17.21	(Lotfalian Dehkordi & Forootan, 2020)
2. Straw yield	12.13	(Lotfalian Dehkordi & Forootan, 2020)

2.1. Energy analysis

After collecting energy data and equivalence of units, the most important variables and energy indices were calculated. In general, energy consumption in agriculture is divided into four groups: 1) direct energy (labor, diesel fuel, irrigation water, and electricity), 2) indirect energy (chemical fertilizer, manure, insecticide, seed, and machinery), 3) renewable energy (labor, manure, seed, and irrigation water); and 4) non-renewable energy (diesel fuel, machinery, chemical fertilizer, insecticide and electricity) (Kazemi et al., 2015).

Also, energy indices in this study include energy use efficiency, energy productivity, specific energy and net energy gain, which were calculated according to relationships 1 to 4 (Abbas et al., 2020).

$$Energy\ use\ efficiency = output\ energy\ (MJ\ ha^{-1})\ /\ input\ energy\ (MJ\ ha^{-1}) \quad (1)$$

$$Energy\ productivity = quinoa\ output\ (kg\ ha^{-1})\ /\ input\ energy\ (MJ\ ha^{-1}) \quad (2)$$

$$Specific\ energy = input\ energy\ (MJ\ ha^{-1})\ /\ quinoa\ output\ (kg\ ha^{-1}) \quad (3)$$

$$Net\ energy\ Gain = output\ energy\ (MJ\ ha^{-1}) - input\ energy\ (MJ\ ha^{-1}) \quad (4)$$

2.2. Economic analysis

For economic analysis of farms in the study area, conventional economic indicators including net return, gross return, benefit - to - cost ratio, productivity, gross value of production, and total cost of production according to relationships 5 to 10 were used (Asgharipour et al., 2012). The fixed cost of production included one year's rent of arable land and water for irrigation, and the variable cost of production also included chemical fertilizer, insecticide, diesel fuel, labor, and economic yield, including quinoa grain and straw yield. The price of input and output was based on the average price in 2020 (226000IRR-1USD).

$$Net\ return = Gross\ value\ of\ production\ (\$\ ha^{-1}) - Total\ cost\ of\ production\ (\$\ ha^{-1}) \quad (5)$$

$$Gross\ return = Gross\ value\ of\ production\ (\$\ ha^{-1}) - Variable\ cost\ of\ production\ (\$\ ha^{-1}) \quad (6)$$

$$Benefit\text{-}to\text{-}cost\ ratio = Gross\ value\ of\ production\ (\$\ ha^{-1})\ /\ Total\ costs\ of\ production\ (\$\ ha^{-1}) \quad (7)$$

$$Productivity = Quinoa\ yield\ (kg\ ha^{-1})\ /\ Total\ costs\ of\ production\ (\$\ ha^{-1}) \quad (8)$$

$$Gross\ value\ of\ production = Quinoa\ yield\ (kg\ ha^{-1}) \times Quinoa\ price\ (\$\ kg^{-1}) \quad (9)$$

$$Total\ cost\ of\ production = Variable\ cost\ of\ production\ (\$\ ha^{-1}) + Fixed\ cost\ of\ production\ (\$\ ha^{-1}) \quad (10)$$

3. Results and discussion

3.1. Energy analysis

The energy content of the consumed inputs and the share of energy of each of them in the total input energy are presented in Table 2 and Figure 3. A total of 39122.99 MJ ha^{-1} input energy and 90741.78 MJ ha^{-1} output energy were calculated. The highest share of energy consumption was observed in diesel fuel (26.39%), nitrate fertilizer (25.95%) and manure (19.17%), and the lowest share of energy consumption was observed in seeds (0.33%), insecticide (0.39%) and combined (0.52%). Due to the higher consumption of nitrate among the chemical fertilizers used in the quinoa production system, this fertilizer had the highest energy share (25.95%). As can be seen in Table 3, about 45.53% of the total input energy for the production of one hectare of quinoa is direct energy and 54.47% is indirect energy. Among them, the share of renewable energy is 38.64% and non-renewable energy is 61.36% of the total input energy. The reason for the high share of indirect energy and non-renewable energy in this study is the high energy consumption of nitrate fertilizer and diesel fuel (Figure 4).

Table 2. Consumed and produced energy for the production of quinoa in one hectare

Particulars	Average quantity (unit ha^{-1})	Consumption energy (MJ ha^{-1})
A. Inputs		
1. Seeds (kg)	7.50	129.08
2. Chemical fertilizer (kg)	259.00	
(a) Nitrate (N)	130.00	10153.00
(b) Phosphate (P2O5)	68.00	1183.20
(c) Potassium (K2O)	61.00	835.70
3. Manure (kg)	25000.00	7500.00
4 Insecticide (kg)	1.50	151.80
5. Machinery (h)	17.30	
(a) Tractor	7.00	655.27
(b) Machinery	8.00	501.60
(c) Combine	2.30	201.55
6. Diesel fuel (L)	216.00	10324.80
7. Water for irrigation (m3)	7100.00	7242.00
8. Labor (h)	125.00	245.00
Total inputs energy		39122.99
B. Outputs (kg)		
1. Quinoa grain	1850.00	31838.50
2. Straw yield	4856.00	58903.28
Total outputs energy	6706.00	90741.78

Source :research findings

The results of the present study are consistent with the results of other researchers who have reported that in crop systems, the ratio of direct energy to indirect energy is higher and the rate of consumption of non-renewable energy is higher than renewable energy (Asgharipour et al., 2012; Baran & Gokdogan, 2016; Lotfalian Dehkordi &

Forootan, 2020). Also, Unakıtan and Aydın (2018), Abbas et al. (2020), Ghorbani et al. (2011), GÖKDOĞAN and SEVİM (2016), Imran and Ozcatalbas (2021), Lotfalian Dehkordi and Forootan (2020), Nabavi-Pelesaraei et al. (2018)) obtained similar results in their studies and reported chemical fertilizers and diesel fuel as inputs with the highest share of energy consumption.

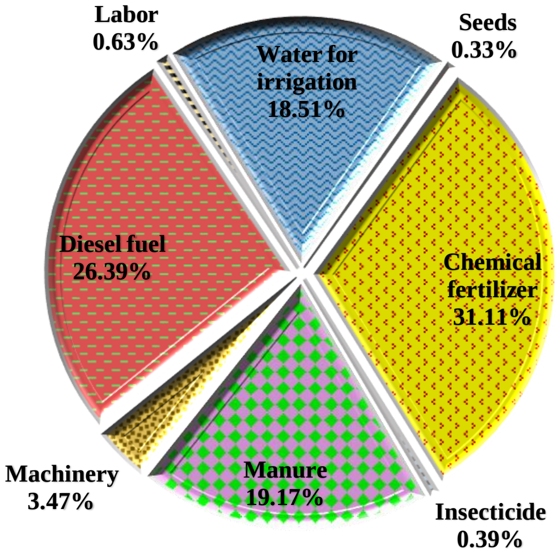

Figure 3. The share of energy input for quinoa production of Iranshahr, Iran

In this study, an energy use efficiency of 2.32 was calculated to produce one hectare of quinoa. In other words, the total output energy was greater than the total input energy. Therefore, it can be said that the production of quinoa in this region is profitable. Energy productivity was 0.17 kg MJ^{-1}, which means that this crop system produces 0.17 kg of quinoa for every megajoule of energy consumed.The specific energy in this system is 5.83 MJ kg^{-1}. The implication of this indicator is that 5.83 MJ of energy is consumed to produce one kg of quinoa. The net energy gain of 51618.79 MJ ha^{-1} was calculated. Therefore, considering the positive rate of net energy gain in this study, it seems that the cultivation of quinoa in this region can be justified in terms of energy balance (Table 3).

Table 3. Total energy input-output in the form of direct, indirect, renewable energy and calculated energy indices for quinoa production in Iranshahr, Iran.

Indices and types of energy	Units	Quantity of energy indices	Contribution of energy Forms (%)
Energy input	MJ ha^{-1}	39122.99	100
Energy output	MJ ha^{-1}	90741.78	100
Direct energy	MJ ha^{-1}	17811.80	45.53
Indirect energy	MJ ha^{-1}	21311.19	54.47
Renewable energy	MJ ha^{-1}	15116.08	38.64
Non-renewable energy	MJ ha^{-1}	24006.92	61.36
Energy use efficiency	-	2.32	
Energy productivity	kg MJ^{-1}	0.17	
Specific energy	MJ kg^{-1}	5.83	
Net energy gain	MJ ha^{-1}	51618.79	

In this regard, a previous study compared the energy consumption of wheat and barley in Sistan and Baluchestan province in Iran. In that study, energy use efficiency for wheat and barley was calculated as 1.49 and 1.94, and energy productivity was 0.056 and 0.066 kg / mJ, respectively (Ziaei et al., 2015). Based on this, it can be stated that less energy is consumed in this region for quinoa production compared to wheat and barley production. In other words, for every megajoule of energy consumed in this region, more quinoa is produced than wheat or barley. Therefore, quinoa can be introduced as a good alternative for these products in this region.

In other studies, the energy use efficiency index for irrigated wheat was 1.44 (Ghorbani et al., 2011) and 1.92 (Naderloo et al., 2012) and 2.3 (Rahman & Hasan, 2014), for paddy production 1.28 (Nabavi-Pelesaraei et al., 2018) was obtained. These results also indicate that quinoa consumes less energy than irrigated wheat and paddy production.

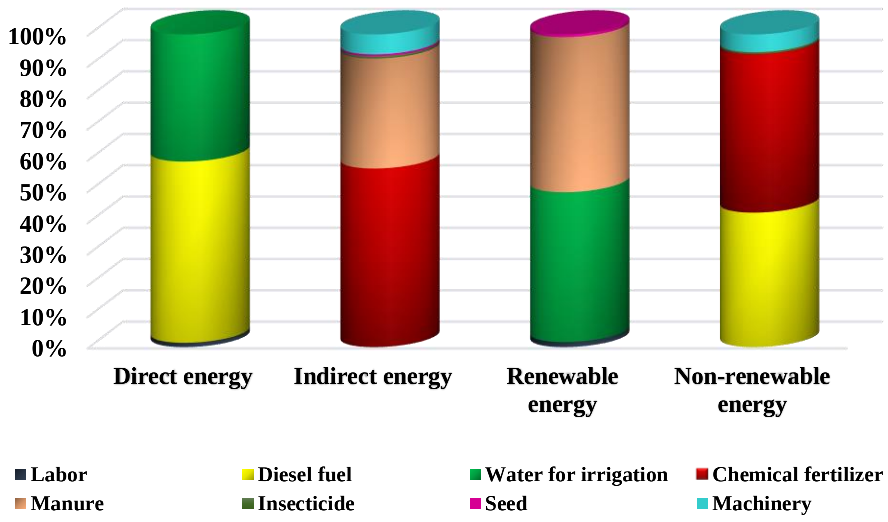

Figure 4. Contribution of inputs to consume energy forms in quinoa production of Iranshahr, Iran

3.2. Economic analysis

Maximizing profits and a successful production process is one of the most important motivations for farmers. Economic analysis is used to calculate the profitability of the agricultural system. As shown in Table 4, the total cost of producing one hectare of quinoa was 1668.93$ ha^{-1}. Among these, the highest costs were related to land rent, machinery, and labor. The results of the economic analysis of quinoa production in the Iranshahr region are presented in Table 4.

Table 4. Economic analysis of Quinoa production in Iranshahr, Iran

Cost and return components	Unit	Value
Grain yield	Kg ha^{-1}	1850.00
Sale price	$ kg^{-1}	1.11
Straw yield	Kg ha^{-1}	4856.00
Sale price	$ kg^{-1}	0.22
Gross value of production	$ ha^{-1}	3120.80
Variable cost of production	$ ha^{-1}	952.32
Fixed cost of production	$ ha^{-1}	716.62
Total cost of production	$ ha^{-1}	1668.93
Gross return	$ ha^{-1}	2168.48
Net return	$ ha^{-1}	1451.86
Benefit - to - cost ratio	-	1.87
Productivity	kg $$^{-1}$	1.11

4. Conclusions

In the present study, the status of energy consumption in the quinoa production system in southeastern Iran was evaluated. The results showed that this plant is suitable for cultivation in the study area in terms of energy consumption and profitability. Agricultural activity is energy dependent. In this regard, the most important inputs are water, fuel, pesticides, and chemical fertilizers that have made the agricultural sector energy-intensive. The results of this study showed that the total input energy was 39122.99 MJ ha^{-1}, of which diesel fuel, with 26.39%, was the most energy consumed. urea and manure were also in the next ranks. Also, of the total input energy, the share of indirect energy was higher than direct energy, and non-renewable energy was higher than renewable energy. In this regard, farmers on most farms do not use chemical fertilizers, particularly urea, based on soil tests and scientific evidence. Rather, they use it based on experience and the notion that the higher the application of chemical fertilizer, the higher the yield. Most of the agricultural machinery and tools used in this area were worn and old. This in turn leads to increased fuel consumption as well as environmental pollution. Therefore, in order to increase the share of direct energy and renewable energy, chemical fertilizers can be used optimally or in combination with organic fertilizers to increase renewable energy and plant yield on the one hand and reduce biological risks on the other hand. In addition, with timely repair and proper maintenance of agricultural machinery, the amount of diesel fuel consumed and environmental hazards caused by its use can be reduced. Generally, it is recommended to reduce the use of chemical fertilizers and fossil fuels by using crop residue management, conservation agriculture, and low tillage methods as sustainability strategies. Also, the value of the energy productivity index (0.17 kg MJ^{-1}) obtained in this study indicates that if management methods, product production, and energy consumption are monitored and efforts are made to increase production per unit area, Productivity can be increased.

Data availability: The data is accessible from the corresponding author (Mahmoud Ahmadpour Borazjani) upon request.

Consent to participate: Not applicable.

Consent for publication: The authors have provided consent to publish this work.
Competing interests: The authors declare no competing interests.

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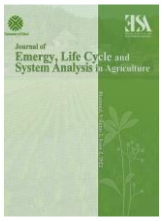
Journal of Emergy, Life Cycle and System Analysis in Agriculture (2022) Volume 2, Issue 2, P. 135-144

Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir



Contrasting effects of chelated zinc and nanoscale zinc oxide on barley growth and salinity tolerance

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ARTICLE INFO

Article history:
Received: 3 August 2021
Accepted: 30 January 2022
Available online: 3 February 2022

Keywords:
Foliar application
Germination
Proline
Seedling
Vigor index

ABSTRACT

An experiment was carried out to determine the effects of chelated zinc and nanoscale zinc oxide particles on tolerance salinity of barley. In the experiment, barley seeds were treated with different concentrations of chelated zinc (Zn-Chelated) and nanoscale zinc oxide (Nano-ZnO), and the effects of these treatments on seed germination, seedling vigor, plant growth, grain filling, and yield were studied. The inhibitory effect of nanoparticles and chelated zinc (1.5 ppm) was discovered. The results emphasize that water can be supplied to the barley followed by Zn-Chelated application with 0.5 ppm to get the desired results. With increasing salinity stress, seed germination and seedling vigor decreased sharply, so the highest obtained from control treatment and the lowest obtained from a salinity level of 18 dS m^{-1}. The genotypes respond differently to salinity levels and alkaline soils. It seems that the Khatam genotype has more tolerance to salinity conditions. Consequently, an experiment was conducted in a strip-plot design with three replications. Based on the correlation coefficients, the kernel number per spike (KNS) showed the highest correlation with the grain yield in barley genotypes, followed by grain filling rate (GFR), maximum grain weight (MGW), thousand-kernel weight (TKW), number of spikes (NS), and saturation water deficit (SWD), respectively. Thus, not only a higher KNS and TKW, but also GFR, MGW, and proline in aboveground plant parts are crucial for successful tolerance in barley. These findings indicate that these agrophysiological traits could be key factors and useful tools for screening many samples in a short time.

Highlights

- Barley seed germination and seedling vigor were significantly reduced by increasing salinity levels.
- Water followed by low-dose chelated zinc significantly enhanced barley germination, shoot and root length, and seedling vigor compared to other treatments.
- Chelated zinc application reduced the negative effects of salinity on barley growth and yield.
- Higher nanoscale zinc oxide concentrations inhibited plant growth, emphasizing the need for proper zinc source and concentration.
- Salinity increased barley leaf proline content, suggesting a stress response mechanism.
- Chelated zinc application improved plant water status under salinity stress by reducing saturation water deficit.

1. Introduction

More than 50% of the cultivated area might be salinized, and it is estimated to reach 9 billion people by 2050. Salinity is a major stressor on crop production in the world (Poustini et al., 2020), affecting 19.5% and 2.1% of irrigated land and dry land in the world, respectively (Sonia et al., 2019). Also, the total salt soil land is 932 m ha (sodic and saline are 581 m ha and 351 m ha, respectively) (Hasanuzzaman et al., 2014). Salt stress causes a nutritional imbalance through lowering phosphorus (Evelin et al., 2009), nitrate and calcium (Hu and Schmidhalter, 2005), zinc absorption, and the accumulating of sodium and chloride ions (Khoshgoftarmanesh et al., 2004). Plant

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https://doi.org/10.22034/aes.2022.298103.1006

growth is not increased by increasing nutrient concentration in saline soils because uptake is low (Mahlooji and Pessarakli, 2017).

Zinc is required for chlorophyll production, germination (Pandey et al., 2006; Cakmak, 2008), biomass production (Kaya and Higgs, 2002), crop productivity (Signorell et al., 2019) and seedling vigor (Rashid et al., 2019). The ability of a seed to germinate at a high salt concentration is important for the survival of a plant (Bojović et al., 2010). It is imperative to keep salinity low during germination and development. Furthermore, responses to salinity vary with germination, seedling vigor (Cuartero et al., 2006), agronomic factors, soil and water management, climate, genotypes, and elements of nutrition such as zinc (Tao et al., 2018). Measurement of traits, including germination (Atak et al., 2006), seedling vigor, relative water content (RWC), saturation water deficit (SWD), grain filling, yield, and yield component, can be used to monitor plant responses to salt stress (Izadi et al., 2014; Mahlooji and Pessarakli, 2017). Salinity was shown to decrease RWC and increase SWD (Ebrahimian and Bybordi, 2011). Trials can be rapidly screened for genotypes which maintain high leaf RWC and low leaf SWD values during stress (Gholinezhad et al., 2009).

In calcareous soils, zinc precipitates in unavailable forms for plants (Degryse et al., 2020). Soil salinity is also associated with zinc efficiency in alkaline conditions (Morshedi and Farahbakhsh, 2012). The high pH (Rengel, 2015; Mueller et al., 2012) and $CaCO_3$ content of these soils are usually considered the reasons for the low availability of Zn (Mahlooji, 2017). By reducing the amount of soil moisture in this area, Zn and Fe in soil solution reduced mobility. The lack of these elements in plants can be compensated by spraying with a solution (Cakmak et al., 2017). Many studies suggest that foliar micronutrient fertilizer could increase plant productivity (Schjoerring et al., 2019), plant resistance to environmental stresses (Dwivedi et al., 2016), shoot growth (Phuphong et al., 2020), nutrient uptake (El-Fouly et al., 2010), yields (Sarkar et al., 2007) of wheat and barley (Morshedi and Farahbakhsh, 2012; Keshavarz and saadat, 2016), and reduce the effect of salinity on yield (Mahlooji et al., 2018), Na concentration on roots and leaves (Thalooth et al., 2006), and nutritional disorders (El-Fouly et al., 2002). Nanomaterials are proposed to be the building materials for the new millennium. It indicates that different plants have different responses to the same nanoparticles and nanofoliar applications. Nanoparticles generate both additive effects (Hong et al., 2005; Yang et al., 2006; Lu et al., 2002; Prasad et al., 2012), and inhibitory effects (Nel et al., 2006; Qiang et al., 2008; Lin and Xing, 2008; Yang and Watts, 2005; Lin and Xing, 2007; Doshi et al., 2008) or not change yield (Knijnenburg et al., 2018) and need to be explored.

About two billion (Chen et al., 2017) or one-third (Zou et al., 2019) of people suffer from zinc malnutrition (Chen et al., 2017). Zn is an essential micronutrient, which is deficient in many regions worldwide (low solubility of Zn in soils rather than low total amount of Zn), such as in calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh et al., 2004), the fourth most important yield-limiting nutrient in India (Prasad et al., 2012), half of the cultivated soils (Phuphong et al., 2020) and 30% of the global soils (Babaeian *et al*., 2011). Zinc is an essential micronutrient for humans, animals, and plants, which acts either as the metal component of enzymes or as a functional structural or regulatory co-factor of a large number of enzymes. Zn is typically the second most abundant transition metal in organisms after iron and is the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) (Auld, 2001). In the developing world, cereal crops play an important role in nutrition, but zinc concentration in cereal grains is very low, particularly when grown on Zn-deficient soils. As a major solution to Zn deficiency, enrichment of cereal grains with zinc foliar application (biofortification) is the most sustainable, with low cost, and useful in improving Zn concentrations in grain. Farmers are using both sulfates and chelated Zn (with ethylenediamminetetraacetic acid, EDTA) for soil and foliar applications. Therefore, this study was conducted to find out the role of zinc fertilizer application in reducing salinity stress and to determine the agrophysiological traits of barley genotypes to selecting and screening. Two aspects were studied in this investigation: 1) the seed treatment with chelated Zn and nanoscale ZnO and study of seed characteristics; and 2) a field experiment with foliar application of chelated Zn and nanoscale ZnO.

2. Materials and methods

2.1. Preparation of Zn-chelate and ZnO nanoparticles and seed treatment

Zinc oxide nanoparticles were produced by China's Neutrino Company with a purity of 99%. The average particle diameter was less than 30 nanometers and the specific surface area was more than 30 m^2gr^{-1}. Due to the fact that Nano-ZnO is not soluble in water, first the Nano-ZnO was suspended directly in deionized water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. To avoid aggregation of the particles, magnetic bars were placed in the suspensions for stirring before use. Because ZnO will not dissolve in water and plants cannot absorb it, farmers are widely using Zn-chelate (EDTA-Zn). EDTA-Zn was produced by the Spanish Company TradCorp and had a 14% zinc element. Both chelated and Nano-ZnO suspensions were prepared at concentrations of 0.5, 1.0, and 1.5 ppm. A control was also maintained, corresponding to pure water.

2.2. Seeds

Barley genotype seeds (Morocco, Nosrat, and Khatam) were procured from the Isfahan Agricultural Research and Natural Resources Center, Iran. The average germination rate of the seeds was 99%. The seeds selected were of uniform size to minimize errors in seed germination and seedling vigor.

2.3. Laboratory experiments

An experiment was performed in the laboratory of the Isfahan Agricultural and Natural Resources Research

Center to investigate the effect of zinc compounds on germination and salinity tolerance indices. The experiment was conducted as a factorial experiment with a completely randomized design and three replications. The first factor was three barley genotypes, including Morocco, Nosrat, and Khatam. The second factor was seven zinc concentrations, including Nano-ZnO at three levels of 0.5, 1.0, and 1.5 ppm, Chelate-Zn at three levels of 0.5, 1.0, and 1.5 ppm, and control (without fertilizer consumption). The third factor was water quality, including 2, 10 and 18 dS m^1.

2.4. Seedling Vigor Index

First, petri dishes (100 x 15 mm) were disinfected. Also, in order to prevent contamination, the seeds were soaked in 1.5% sodium hypochlorite solution for 3 minutes and then washed several times with completely "distilled water". Then the seeds were placed separately in a petri dish with a fertilizer treatment with different concentrations for 3 hours, and the treated barley seeds were shade-dried for 1 hour. One piece of sterilized filter paper and five mL of quality water were added (as per the recommendations of the International Seed Testing Association, 2011). The Petri dishes were covered and placed in an incubator at 25 ± $1^{\circ C}$ for eight days. Seedlings were counted every day for 7 days after the start of the experiment. On the eighth day, the length of the radicle and the plumule and the percentage of germination were measured. Germination was calculated based on the number of seeds germinated in a petri plate and expressed as a germination percentage. The seedling Length Vigor Index (SLVI) was calculated by the formula described by Abdul-Baki and Anderson (1973).

Seed Length Vigor Index (SLVI) = Germination Rate (root length + shoot length)

2.5. Field experiment

The field experiment was conducted during 2013-14 at Esfahan Kaboutarabad Research Station. The experiment was conducted in a strip-plot design with three replications. Each plot consisted of six rows, each 4 m in length, and were spaced 20 cm apart. Three water irrigation quality, including W_1 = 1-2 dS m^{-1} (low salinity) as a check, W_2 = 10 dS m^{-1} (common salinity in the region), and W_3 = 18 dS m^{-1} (high salinity), were evaluated in vertical factors. The horizontal factors were spraying, including Nano-ZnO, Zn-Chelate and water spraying as a check. Three different barley genotypes, including Morocco (salt-sensitive), Nosrat (semi-salt-tolerant), and Khatam (salt-tolerant), are spilt within vertical factors. On November 5, Seeds were sown with a density rate of 450 seeds m^{-2} by a cereal row planting machine (Wintersteiger Plotman). To irrigate the plots, water was delivered from the channel (S_1 = 2 dS m^{-1}), a local well (S_2 = 10 dS m^{-1}), and mixed drainage water and local water well (S_3 = 18 dS m^{-1}). The application rates of Nano-ZnO and Zn-chelate were 100 and 1000 g ha^{-1}, respectively. Grain yield was measured in plots of 0.4 4 m^2. At the maturity stage, grain yield samples are harvested and weighed. Developmental stages were determined according to the method suggested by Zadoks et al. (1974). Saturation water deficit of the flag leaves was measured as described by Pask et al. (2012). In order to estimate, analyze, and interpret the parameters related to grain filling, a linear (two-piece) regression model based on the DUD procedure and Proc Nlin guidelines of SAS software was used as follows (Rondanini et al., 2004).

2.6. Statistical analysis

Data were subjected to analysis of variance by SAS (SAS Institute 2007). Means of treatments were compared by the least significant differences (LSD) test at ($P \leq 0.05$). The Pearson correlation between traits is determined by correlation analysis.

3. Results

3.1. Seed germination and seedling vigor

According to the mean comparison result, germination percentage (GE), length of shoot (LS), length of root (LR) and seedling length vigor index (SLVI) were significantly (p0.01) affected by Zn concentration, genotype and water quality, but no significant differences in LR were found among the water quality. The results showed that barley seeds responded positively towards the treatment at various concentrations of both Chelated-Zn and Nano-ZnO particles. However, the control treatment (Table 1) produced the highest germination percentage (GE = 63.28%), shoot length (LS = 23.19 mm), radicle length (LR = 22.23 mm) and seedling length vigor index (SLVI = 2968.26). Among the different Chelated-Zn and Nano-ZnO particle concentrations, 0.5 ppm showed the maximum and an increased concentration (1.5 ppm) showed decreased GE, LS, LR, and SLVI. Chelated-Zn showed more GE and SLVI and larger LR compared to Nano-ZnO, though Nano-ZnO had the least LS. Seed treated with 0.5 ppm Chelated-Zn, more GE (57.89%), LS (20.91 mm), LR (21.22 mm) and SLVI (2425.41) was obtained compared to other various Zn concentrations (1.5 ppm) (Table 1). Moreover, examination of the correlation coefficients of the studied traits showed that there was a significant and positive correlation was found between the percentage of germination with the SLVI (r = 0.95, p = 0.01), LS (r = 0.61, p = 0.01), and LR (r = 0.39, p = 0.01) under different salinity levels (Table 2). Despite of non-significant effect of water quality on LR (Figure 1), the highest GE, LS, LR and SLVI were observed in Khatam genotype and minimum salinity, whereas the lowest these traits were noted in Nosrat and high saline water quality (Figures 1-3).

Germination is the stage in a plant's development cycle most adversely affected by salinity. Barley, the fourth most important cereal crop, has prominent salinity tolerance relative to other cereal crops, but its salinity tolerance diverges among genotypes (Mwando et al., 2020). As the quality of water salinity levels increased, the seed germination characteristics decreased as well. The results showed that different levels of salinity had significant effects on germination percent and longitudinal traits of seed (root length, shoot length, and seedling vigor index).

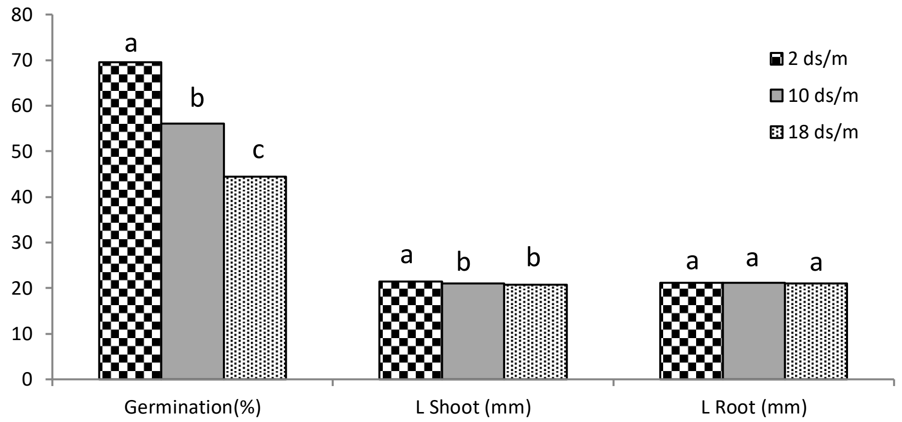

Figure 1. Effect of water quality on germination percentage, length of shoot and root

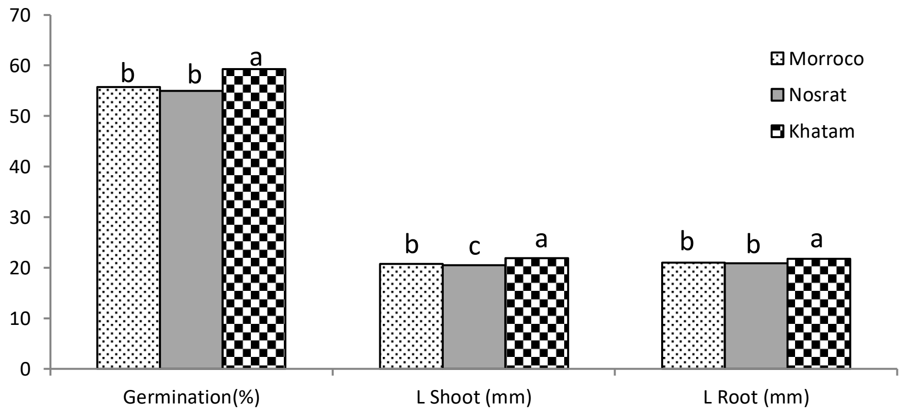

Figure 2. Effect of three barley genotypes on germination percentage, length of shoot and root

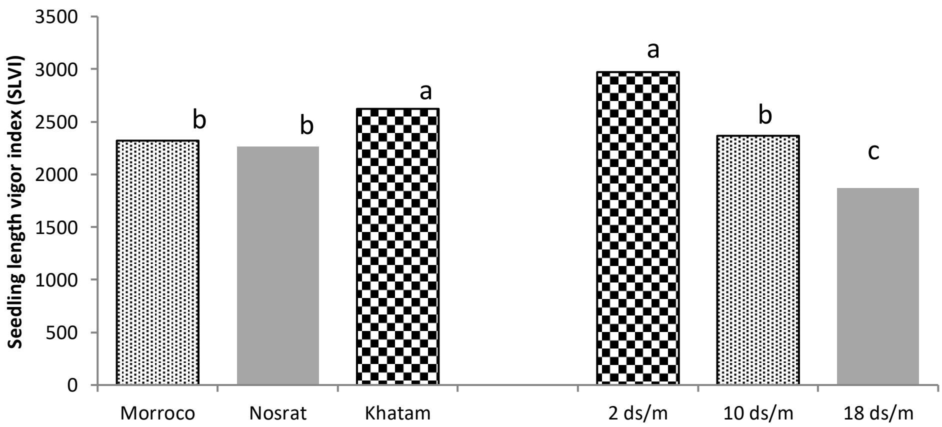

Figure 3. Effect of three barley genotypes and water quality on seedling length vigor index

With increasing salinity stress, all of the traits decreased sharply, so the highest obtained from control treatment and the lowest obtained from a salinity level of 18 dS m^{-1}. These results were generally consistent with findings by Naeem et al., (2017) and Khodarahmpour et al., (2012). The genotype differences also contributed to the observed differential response to the salinity treatments. It seems that the Khatam genotype has more tolerance to salinity conditions. Genotypes of Nosrat and Morocco were sensitive to salinity stress (Figure 2). Thus, salinity tolerance at the germination and seedling stages is an indicator for screening tolerant genotypes. This also agrees with the reports from Shahid et al., 2012; Ravelombola et al., 2017. The negative effects of salinity on germination may be due to reducing osmotic potential (Somani, 2007), increasing ionic concentration of ions on metabolism (Khodarahmpour et al., 2012), interfering with certain aspects of metabolism, such as changing the balance of growth regulators (Khan and Ungar, 2001), which results in limited water uptake by seeds (Chachar et al., 2008).

Table 1. Mean comparison for germination percentage, length of shoot and root, and seedling length vigor index of three barley genotypes in three concentrations of nanoscale ZnO and Chelated-Zinc.

Concentration (ppm)	Germination (%) (GE)		Shoot length (mm) (LS)		Root length (mm) (LR)		Seedling length vigor index (SLVI)	
	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO	Chelated Zinc	Nano ZnO
0.5	57.89 b	55.11 cde	20.91 b	20.89 b	21.22 b	20.92 bc	2425.41 b	2311.58 bcd
1.0	57.11 bc	54.22 de	20.51 bc	20.62 bc	21.19 b	20.86 bc	2353.74 bc	2252.22 cd
1.5	55.89 bcd	53.44 e	20.33 c	20.72 bc	21.11 b	20.58 c	2291.78 bcd	2215.06 d
Control	63.28 a		23.19 a		22.33 a		2968.27 a	
LSD 5%	2.11		0.48		0.45		134.36	

Different lowercase letters indicate significant differences between treatment based on LSD test at p<0.05.

Table 2. Correlation coefficients between traits of three barley genotypes grown under different salinity levels.

Traits	Germination	Length shoot spike	Length root	Seedling length vigor index (SLVI)
Germination	1			
Length shoot	0.61 **	1		
Length root	0.39 **	0.65 **	1	
SLVI	0.95 **	0.81 **	0.58**	1

**Significant at 0.01 level of probability.

3.2. Grain filling

Grain filling rate (GFR) and maximum grain weight (MGW) were significantly affected by salinity levels of irrigation water quality, zinc foliar applications, and genotypes (Table 3). The Result of the mean comparison of irrigation quality showed that the highest GFR (0.00148 g /day^{-1}) and MGW (0.0405 g) were at minimum saline water. In comparison to the control (2 dS m–^{1}), GFR decreased by about 16 and 23% in salinity levels of 10 and 18 dS m–1, respectively, and MGW decreased by about 15% and 20% in salinity levels of 10 and 18 dS m^{-1}, respectively. According to the mean comparison of zinc foliar applications, the highest GFR (0.00138 g day^{-1}) and the MGW (0.0356 g) belonged to the Zn-EDTA spraying solution and the lowest one was related to the control. Application of Zn-EDTA in GFR and MGW increased about 15 and 20% in comparison with check, respectively. A Comparison of averages showed that the maximum GFR (0.00148 g day^{-1}) and MGW (0.0367 g) belonged to the Khatam genotype, but Morocco (sensitive genotype) had the lowest GFR and MGW. In comparison with the Khatam genotype, GFR declined by about 5.4 and 33% in Nosrat and Morocco, and MGW was reduced by approximately 10.4 and 11% in Nosrat and Morocco, respectively. In addition, a significant and positive correlation was found between the grain yield and GFR (r = 0.72, p = 0.01) and MGW (r = 0.63, p = 0.01), which can be attributed to the importance and effective role of these two physiological traits in tolerance to salinity (Table 4).

Table 3. Effects of saline water quality and fertilizer application on agrophysiological parameters of barley genotypes

Treatments	Grain filling rate (GFR) (g.day^{-1})	Maximum grain weight (MGW) (gr)	Saturation water deficit (SWD) (%)	Proline (μg.g^{-1})	Number of spike (NS)	Kernel number per spike (KNS)	Thousand-kernel weight (gr)(TKW)	Grain yield (GY) (kg.ha^{-1})
S (Quality(dS m^{-1})								
S_1=2	0.00148 a	0.0405 a	14.17 a	196.87 b	333.72 a	27.38 a	33.00 a	3123.26 a
S_2=10	0.00124 b	0.0331 b	14.98 a	212.43 b	286.37 b	23.36 b	28.69 b	1737.85 b
S_3=18	0.00114 b	0.0281 c	15.23 a	255.12 a	259.81 c	20.57 c	22.85 c	1524.03 c
LSD 5%	0.0002	0.003	4.36	15.84	19.69	2.02	2.09	132.32
F (Fertilizer)								
F_N=Nano-ZnO	0.00113 a	0.0352 a	12.97 a	241.37 a	297.04 a	22.59 b	27.2 a	2069.81 b
F_Z=Zn-EDTA	0.00138 a	0.0356 a	12.63 a	183.52 b	318.37 a	24.15 a	29.13 a	2365.46 a
F_C=Check	0.00115 b	0.0309 b	17.77 a	239.54 a	264.50 b	24.56 a	27.59 a	1818.52 b
LSD 5%	0.0002	0.002	5.18	15.14	22.29	1.31	2.57	292.87
G (Genotype)								
G_1=Moroco	0.00099 b	0.0327 b	18.90 a	170.76 c	317.37 a	12.91 c	29.62 a	1381.94 c
G_2=Nosrat	0.00140 a	0.0329 b	11.89 b	230.86 b	294.22 b	27.74 b	26.08 b	2232.57 b
G_3=Khatam	0.00148 a	0.0367 a	11.57 b	262.80 a	268.31 c	30.66 a	29.84 a	2770.63 a
LSD 5%	0.0001	0.002	2.85	12.60	12.45	1.55	0.94	124.16
S	*	**	NS	**	**	**	**	**
F	*	**	NS	**	**	*	NS	*
S*F	NS	*	**	**	NS	NS	**	**
G	**	**	**	**	**	**	**	**
G*S	NS	NS	**	NS	NS	NS	**	**
G*F	NS	NS	NS	**	**	NS	**	**
G*S*F	NS	NS	NS	**	**	**	**	**
CV%	14.44	10.69	22.97	10.31	7.68	11.79	6.05	8.82

Means within similar letters in each column are not significantly different (LSD 5%). NS, non-significant, * p<0.05, ** p<0.01

Table 4. Coefficient correlations between traits of three barley genotypes grown under different salinity levels

Traits	Grain yield (GY)	Number of spike (NS)	Kernel number per spike(KNS)	Thousand-kernel weight (TKW)	Saturation Water deficit (SWD)	Proline	Grain filling rate(Slope)	Maximum grain weight (MGW)
GY	1							
NS	0.38 **	1						
KNS	0.74 **	- 0.12 NS	1					
TKW	0.46 **	0.53 **	0.12 NS	1				
SWD	- 0.26 *	0.01 NS	- 0.28 *	- 0.01 NS	1			
Proline	- 0.01 NS	- 0.43 **	0.24 *	- 0.34 NS	- 0.05 NS	1		
Slope	0.72 **	0.17 NS	0.69 **	0.24 *	- 0.31 **	0.16 NS	1	
MGW	0.63 **	0.41 **	0.38 **	0.61 **	- 0.14 NS	- 0.08 NS	0.71 **	1

NS, non significant. ** and *, significant at 0.01 and 0.05 probability levels, respectively.

Our results indicated that the salinity declined at the rate of grain filling. Likewise, grain filling is the main stage in production, and the longer this period allows the transfer of photosynthetic production from source to sink, resulting in increased grain yield. Therefore, we can conclude that the cause can be disrupted by the process of photosynthesis due to ionic toxicity, reduction of GFR, and MGW. Reducing the MGW reduces the final yield by shortening the grain filling period and accelerating grain maturation and grain prematurity. Reducing the GFR could be due to destroying the chloroplasts, decreasing carbon dioxide, chlorophyll content, fluorescence, leaf area, and photosynthesis caused by ionic and osmotic stress. These results are in accordance with those reported by Azizipour et al. (2010), and Munns and James (2003).

Due to the essential role of the element zinc in the plant, which is directly involved in the biosynthesis of growth materials such as auxin, and the supply of nutrients, the period of grain filling is prolonged. Although plants need little zinc, if they do not have enough of this element, they will be affected by the physiological stresses caused by the dysfunction of various enzymatic systems and other zinc-related metabolic functions. It seems that consumption of zinc increases the total amount of carbohydrates, starches, and proteins made by the plant. The more carbohydrates, the faster and longer the filling period, and as a result, the kernel-grain-weight improves. Many researchers have suggested that in salinity conditions, the growth periods of vegetative and reproductive wheat genotypes decrease, which leads to their premature maturation (Munns and James, 2003). There are some reports that confirm the consumption of nutrients, increased growth hormone production, and the period of wheat grain filling in salinity stress (Hagh Bahari and Seyed Sharifi, 2014).

3.3. Saturation water deficit (SWD)

Despite the non-significant effects of quality water irrigation and Zn fertilizer on SWD, the effects of genotype were highly significant (p0.01). In addition, the lowest SWD of minimum saline water (2 dS m^{-1}) and Zn-EDTA were produced (Table 3). Compared to control, SWD (12.63%) of flag leaf decreased with Zn-EDTA. Furthermore, the Khatam genotype had the least SWD (11.57%). The results demonstrated that the tolerant genotype, rather than the sensitive genotype (Morocco), showed a lower SWD under salinity. SWD was decreased in both the Khatam (salt-tolerant) and Nosrat (semi-tolerant) genotypes. The results demonstrated a negative significant correlation between SWD (r = -0.26*) and grain yield (Table 4). Similarly, results have been reported by Kadkhodaei et al. (2014), Ram et al. (2015), and Maghsoodi and Razmjoo (2014).

Higher SWD was recorded when the temperature exceeded the normal range and was observed under water deficit stress. It seems that species having a low rate of water loss through their leaf cuticle are better adapted to abiotic stress. In line with our results, Kafi et al., (2011), Ullah et al., (2012), and Raza et al., (2017), reported that SWD gradually decreased under salt conditions. However, a greater reduction was observed in salinity tolerant varieties. Plants subjected to salinity stress showed signs of wilting. The leaf SWD indicates that the leaf water deficit is considered to be an important marker of tolerance in plants. It seems that the high Na^+ absorption under saline conditions was due to impaired water absorption and increased SWD. So, higher values of leaf SWD under tension may be due to the reduction in water, leaf area, leaf turgor, transpiration, stomatal conductance, absorption of radiation under leaf rolling, production of leaves, and yield. Moreover, leaf senescence, abscission, and plant canopy temperature were also higher in plants with high SWD. These results were generally consistent with the findings of Munns and Tester (2008), Ebrahimian and Bybordi (2011), Ardestani and Rad (2012), and Mahlooji et al. (2018).

3.4. Proline content

Data on proline measurement indicated that the differences in quality of water irrigation, zinc foliar applications, and genotype were significant (p0.01). In addition, the lowest proline content of minimum saline water (S_1: 2 dS m^{-1}) and Zn-EDTA were produced (Table 3). Salinity increased flag leaf proline content. In comparison with S_1, flag leaf proline content was inclined by about 8 and 30% in S_2 and S_3, respectively. Compared to control, the proline content (183.52 µg.g^{-1}) decreased with Zn-EDTA (approximately 24%). Furthermore, the Khatam genotype had the most proline content (262.8 µg.g^{-1}). Proline content increased in Nosrat (G_2 = semi-tolerant) and Khatam (G3 = salt-tolerant) by around 35 and 54%, respectively, as compared to G_1 (Table 3).

Additionally, the proline content increased with the rise of salinity. Through the accumulation of proline, barley plants may deal with cell dehydration and help to maintain survival. Under saline conditions, high concentrations of sodium and chloride ions reduced leaf area and may have deformed water absorption, anabolic enzymes, and -

glutamil kinase. These increased proline content. These findings agreed with those of Munns and Tester (2008); Ebrahimian and Bybordi (2011); Misra and Saxena (2009) and Aflaki Manjili et al., (2012). Also, a considerable increase in the proline content was observed in barley treated with Nano-ZnO and check (non-Zn fertilizer). It seems that the induction of proline accumulation in response to Nano may be due to an activation of proline synthesis through the glutamate pathway. In line with our results, Mohammadi et al. (2014, 2016); Karimi and Sepehri (2018) reported that proline content was promoted in plants treated with Nano. Notably, the results demonstrated that the tolerant genotype (Khatam) accumulates a markedly higher concentration of proline in leaves than the sensitive genotype (Morocco). It was observed that proline played a significant role in decreasing lipid peroxidation. These results are in accordance with those reported by Abbasi et al., (2016) and Ahmadi et al., (2009).

3.5. Yield and yield components

However, the effects of irrigation-water quality, zinc fertilizer and genotype were highly significant on yield and yield components, but no significant differences in thousand-kernel weight (TKW) were found among the zinc fertilizer. Number of spikes (NS) of approximately 15 and 22%, kernel number per spike (KNS) of approximately 15 and 25%, thousand-kernel weight (TKW) of approximately 13 and 31%, and grain yield (GY) of approximately 39 and 48% were reduced in S_2 (10 dS m^{-1}) and S_3 (18 dS m^{-1}), respectively, when compared to S_1 (2 dS m^{-1}).grain yield (GY) increased by about 30 and 13%, respectively, while kernel number per spike (KNS) decreased by about 2 and 8% in Zn-EDTA (F_2) and Nano-ZnO (F_1) compared to check (F3). Khatam (G_3 = tolerant) had the highest KNS (30.66), TKW (29.84 gr), GY (2770,63 kg/ha) and the lowest NS (268), but Morocco (G_1 = sensitive) had the lowest KNS (12.91), GY (1381.94 kg/ha) and the highest NS (317.37) and TKW (29.62 gr) (Table 3). Furthermore, Khatam had the highest GY, KNS, and TKW. This revealed that genotypic differences were markedly important. Moreover, a significant positive correlation (Table 4) was found between GY and NS (r = 38**), KNS (r = 0.74**) and TKW (r = 0.46**).

All yield components were adversely influenced by salt stress, among which KNS was the most sensitive component. This revealed that the pollen development processes are sensitive to salinity during the flowering stage, causing a lapse of viable pollen and floret abortion. These results, therefore, show that salinity inhibited grain filling, yield, and yield components. Similar consistent results were reported for barley (Steppuhn and Raney, 2005) and oats (Zhao et al., 2007).

It seems that low salinity conditions of irrigation water and zinc foliar application are advisable to have the most GY and all yield components. As a result of consuming zinc element the total amount of carbohydrates, starches, and proteins produced by the plantincreases, and with increasing carbohydrates, the speed and duration of the grain filling period increase, resulting in increased grain yield. Similar results were observed by Mahmood (2011); Hagh Bahari and Seyed Sharifi (2013) reported that applying zinc in various ways, especially by spraying, increases the yield compared to the control.

In line with our results, Ashrafi et al. (2014) and Pirasteh et al. (2016) reported that the grain yield and yield components of barley genotypes were reduced under salt stress, but the reduction was greater in the salt-sensitive cultivar compared to the salt-tolerant one. They concluded that this was due to the reduction in water absorption by plant tissues, cellular growth and development, and physiological and biochemical traits. Consistent with these results, many researchers have shown that the growth of plants declined under saline conditions, but the degree of reduction depended on environmental conditions, salt content, water consumption, type of genotype (Abdallah et al., 2017), and stage of plant growth (Mahmood, 2011; Shafaqat et al., 2012).

4. Discussion

The world population is constantly increasing and is estimated to reach 9 billion by 2050. Therefore, there is a need to cultivate salinized soils to improve food production globally. In addition, salinized soils require irrigation water, which ultimately can result in increased soil salinity. Because arable land has varying degrees of salinity, the use of saline water in salinity soils seems to be necessary due to limited freshwater resources and the degree of sensitivity of different genotypes, so managing water use and selecting the right genotype are important. Various factors, such as plant species, ambient temperature, plant growth stage, soil or water composition, environmental variables, and plant resistance, affect salinity. In addition, the tolerance of plants to such elevated salinity levels varies from species to species. One solution to the salinity problem is using salt - tolerant species and cultivation of resistant cultivars within species. Barley can be salted for cultivation due to its high genetic diversity. Cultivation of this plant is also limited due to the need to use special genotypes that are highly adaptable to the conditions of the region and also due to the presence of very saline water drains (Mahlooji, 2017; Naeem et al., 2017).

Understanding salt-tolerant mechanisms is imperative for crop improvement in salt-affected areas. Tolerance to salinity often depends on the physiological and structural complexity of the plant. Traditional screening techniques for salt tolerance are usually based on the grain yield and are expensive and time-consuming. In recent years, the focus of screening has shifted towards examining germination characteristics and specific physiological traits involved in salt tolerance. The germination and seedling growth process depend on saline water quality and osmotic potential. however, little is known about the germination responses of genotypes to salinity. Therefore, there is a need for the introduction of reliable germination and physiological markers for the selection of salt-tolerant genotypes to be planted directly or used in breeding programs. Salt stress decreased the germination characteristics of the genotypes. In addition, tolerant cultivars had germination indexes unchanged or less affected by salinity. Many studies report that elevated salinity levels become a limiting factor for seed germination and seedling development. However, salinity levels showed an inhibitory effect on germination with an increase in salt concentration (Ahmed et al., 2017; Louf et al., 2018; Naeem et al., 2017; Sonia et al., 2019).

Among different abiotic stresses, salinity stress crucially reduces plant growth and also causes nutrient imbalances. Depending on the conditions, the use of field methods such as optimal levels of chemical fertilizers can also help to increase the growth and yield of crops to some extent. One easy, low-cost, and cost-effective solution is to use spraying to improve salinity tolerance and increase crop production in saline conditions. Under intolerable salinity, plant growth and yield are not raised by increasing nutrient concentration in soils because, in a saline environment, the micronutrient uptake is terribly variable. In calcareous soils, zinc precipitates in unavailable forms to plants. Soil salinity is also associated with zinc efficiency in alkaline. The high pH and $CaCO_3$ content of these soils are usually considered the reasons for the low availability of Zn. Micronutrient foliar application plays an important role in alleviating and increasing the nutrient uptake under salinity. Zn is used for improving seed germination, seedling development in barley, seedling vigor, field establishment, protein synthesis, membrane function, cell elongation, and tolerance to environmental stresses. The Zn foliar application method is a very economical alternative to more expensive broadcast Zn fertilizer applications and soil application. As a quick solution to the Zn deficiency problem in human populations, fertilizer strategies should be applied nationwide (Alvarez and Gonzalez, 2006; Cakmak, 2000; Gonzalez et al., 2007; Prasad and Sinha, 1981; Mahlooji, 2017).

It is clearly seen from our results that treated barley seeds with water followed by chelated ZnO with a concentration of 0.5 ppm have shown a significant increment in germination, shoot length, root length and seedling length vigor index over other concentrations of the same material and varying concentrations of another material (Nano ZnO) tested. The results showed that different levels of salinity had significant effects on germination percent and these longitudinal traits of seed. However, the performance of the zinc material is less than the control. both plant growth and yield decreased at higher concentrations of nanoscale ZnO, and these results were in accordance with the reports by Prasad et al. (2012). Such inhibitory effects of nanoparticles were also reported by Lin and Xing (2007). Hence, it may be concluded that the effects of different salt concentrations on physiological traits (grain filling rate, maximum grain weight, saturation water deficit, and proline content) and the grain yield of barley cultivars with contrasting salt tolerance (tolerant, intermediate, and sensitive) and foliar applications under field conditions are more operational. Salinity increased flag leaf proline content, saturation water deficit in the plant and declined the rate of grain filling. It was also found that a lower SWD indicates a better plant water status. In line with our results, Ganji Arjenaki et al. (2012) showed similar results in wheat and reported that tolerant genotypes maintained lower SWD under stress than sensitive ones. Compared to control, the proline content and SWD of flag leaf decreased with Zn-EDTA. Proline amino acid is an organic molecule and one of the most commonly used solutions that protect membranes in addition to participating in osmotic regulation and can play a role in salinity stress, though proline is a positive factor for adaptation under salinity stress (Hong et al., 2005; Peng et al., 1996; Mansour, 1998). The supply of nutrients allows for a longer period of grain filling. Although the plant's need for micronutrients is low, if not enough of these elements are available, the plants will be affected by the physiological stresses caused by the dysfunction of various enzymatic systems and other zinc-related metabolic functions. Consistent with these results, many researchers have shown that the use of zinc fertilizer has increased the rate of grain filling and the maximum weight of the grain, and the reason may be the increase in assimilation and, ultimately, the increase in material transfer to the grain (Yamaguchi et al., 1995; Seyed Sharifi and Nazarli, 2016).

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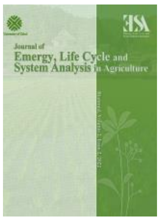

The impact of embryonic thermal manipulation on the intestinal microbiota, morphology, and long bone characteristics of male broiler chickens

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ARTICLE INFO

Article history:
Received: 11 July 2022
Accepted: 5 November 2022
Available online: 12 November 2022

Keywords:
Chronic heat stress
Global warming
Ross strain
Thermal manipulation
Thermotolerance

ABSTRACT

Climate change is caused by global warming, which is our most pressing environmental concern. However, some of these modifications will have negative effects on animal welfare and the quality and quantity of poultry products. We examined the effects of different periods of thermal manipulation (TM) during embryogenesis on the European production efficiency index (EPEI), intestinal microbiota and morphology, and long bone characteristics of Ross (308) broilers strain exposed to Chronic Heat Stress (CHS). Consequently, 608 fertile eggs were utilized in a completely randomized design comprising four treatments and four replicates. 7 to 16 days were spent incubating experimental groups with different TM (for control (0 h), 6, 12, and 18 hours). Humidity and temperature were maintained at 65% and 39.5°C. After hatching, male chicks were chosen, housed under standard conditions, and then subjected to chronic heat stress (CHS) between 28 and 42 days later. Mortality in the TM-treated groups was significantly ($P \leq 0.05$) lower than in the control group during CHS, and mortality was lowest after 12 hours of treatment. The EPEI was greater in treated chickens at 12 and 18 hours compared to untreated chickens ($P \leq 0.015$). The treatments have no effect on the intestinal microbiota ($P \geq 0.05$). The tibial length ($P \leq 0.05$) and width ($P \leq 0.048$) of birds given 12- and 18-hour treatments increased significantly. ≤ TM caused significant changes in the villus's height and area of the villus ($P \leq 0.05$). TM-treated birds had higher villus height than control. It can be concluded that TM may increase the height of villus and long bone characteristics and decrease the mortality rate in broilers exposed to CHS due to adaptation and thermotolerance.

Highlights

- The study investigates the effects of thermal manipulation (TM) during embryogenesis on broiler chickens under chronic heat stress (CHS).
- TM reduced mortality and increased production efficiency index (EPEI) of chickens exposed to CHS compared to control.
- TM did not affect the intestinal microbiota but enhanced the intestinal morphology and long bone characteristics of chickens.
- The paper demonstrates that TM is a potential strategy to improve the welfare and performance of broiler chickens under global warming.

1. Introduction

The global warming phenomenon is a serious challenge facing poultry production in tropical and subtropical regions. Heat stress begins when the ambient temperature rises above the thermoneutral zone, which ranges between 16 and 25 ˚C for poultry species. Heat stress is one of the most challenging environmental conditions influencing commercial poultry. Feed consumption, growth rates, feed efficiencies, and survival abilities all decrease as environmental temperature rises (Mashaly et al., 2004;

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https://doi.org/10.22034/aes.2022.350864.1041

Zaboli et al., 2019). The Broilers are very sensitive to heat stress due to feathering and the absence of sweat glands (Yahav et al., 2009). On the other hand, genetic selection has improved the relative weight of breast muscle, body weight gain, and feed efficiency. However, this is not accompanied by an adequate increase in cardiovascular and respiratory system function, which consequently causes the birds to be incapable of regulating their body temperature properly (Havenstein et al., 2003). Heat stress is common in tropical and subtropical areas like Iran, where spring and summer temperatures range from 35 °C to 45 °C. According to Akbarian et al. (2013), global warming makes the effects of heat stress worse.

Overall, the combined complications of intensive genetic selection, global warming, and expanding poultry production in the tropical climate caused economic losses. Therefore, various techniques have been tested to overcome this problem, such as nutritional and genetic methods, but most are costly and have low efficiency. Recently, thermal manipulation (TM) (postnatal and prenatal) has been developed and proposed as a new technique. It can improve performance and decrease mortality by reducing metabolic rate, body temperature, and thyroid activity (Loyau et al., 2015; Zaboli et al., 2016; 2022). Some studies (Yahav and Mcmurtry, 2001; De Basilio et al., 2001; Zaboli et al., 2022; 2016) explain that postnatal exposure of 3- or 5-day-old chicks (for 24 h at 37.5-38 °C) enables chickens to regulate body temperatures efficiently during heat challenges later and can diminish the mortality rate by about 50%. Furthermore, TM during embryogenesis has been tested in many studies (Moraes et al., 2004; Collin et al., 2007). The treatment combining 39.5 °C with 65% RH for 12 h/d between days E7 and E16 of embryogenesis appears to enhance the thermotolerance of broiler chickens without negative effects (Loyau et al., 2015; Zaboli et al.,2022; 2016).

Heat stress adversely impacts the intestinal epithelial structures, such as alteration in the digestibility and metabolism of various nutrients; disruption in the structure and function of the intestinal epithelium (Burkholder et al, 2008); and alteration of the normal and protective microbiota (Bailey et al, 2004). Burkholder et al (2008) noted that heat stress significantly decreased the intestinal bacterial populations of birds (Burkholder et al., 2008). In addition, the TM improved intestinal morphology (Temim et al., 2000; Uni et al., 2001). Bone weakness and skeletal disorders associated with rapid growth in the new genotype lead to economic losses and animal welfare issues (Kim et al., 2011). Also, heat stress intensifies the disorders and decreases long bone length and width (Bruno et al., 2007; Zaboli et al., 2017). So, we hypothesized that TM might improve long -bone-related traits and intestinal microbiota.

The profitable role of TM in broilers under acute heat stress has already been described. However, little is known about how TM affects performance under chronic heat stress (CHS) and how it affects the long bone characteristics, the shape of the intestine, and the microbiota in Ross strain broilers.

Therefore, the present investigation aimed to evaluate the effect of TM on performance, long bone traits, intestinal morphology, and microbiota of male broiler chickens exposed to CHS from 28 to 42 days of age.

2. Materials and Methods

2.1. Experimental procedures

The experimental protocol was approved by the Tarbiat Modares University Animal Care Protocol. Hence, 608 fertile eggs were used in a completely randomized design of four treatments with four replicates. Experimental groups with different TM (for control (0 h), 6, 12, and 18 hours) were incubated at 65% humidity and 39.5°C from 7 to 16 days of incubation for 0, 6, 12, and 18 hours. After hatching, the male broiler was selected, housed in standard conditions, and then exposed to CHS from day 28 to day 42 (Zaboli et al., 2016). To induce CHS, all groups in experimental pens were exposed from 10:00 AM to 4:00 PM to 32-36 °C and 55% RH, with overnight temperatures of 28 2°C and 46 5% RH. The average time it takes for the ambient temperature to rise from 27 to 32 °C is about 30 minutes. To avoid potential incubator influences, all eggs were incubated in the same incubator at 37.8 °C, 56% RH, and were turned once per hour from E0 to E7 (Zaboli et al., 2022). At 7 days of incubation, infertile and embryo mortalities were removed after candling. On day 19 of incubation, all eggs were relocated to a hatcher with 37.8 °C and 56% RH.

Newly hatched chicks were recorded every hour. Chicks with dry feathers, at almost 180 minutes after hatching, were taken out of the incubator for evaluating and sexing. Male broiler chickens of the same weight were carefully chosen, and 12 male broiler chickens were randomly distributed into the experimental cages. This experiment was performed only on male birds because of their better sensitivity to heat stress than females (Piestun et al., 2008).

All Ross Management Guide recommendations were used to feed all the chicks. Water and feed were provided *ad libitum* under 23-hour lighting. The temperature was 32 °C in the first week and then decreased to 24 °C. At the end of the experiment, the birds were euthanized by CO2 asphyxiation, and cervical dislocation was accomplished for dissection and then sampled.

2.2. The European production efficiency index

The European Production Efficiency Index (EPEI) was calculated using the equation as follows (Hajati et al., 2015):

$$EPEI = \frac{BW\,(kg) \times \,survived\,percentage}{PP \times FCR} \times 100$$

where PP is the production period length (days).

2.3. Intestinal Microbial Population

The contents of the ileum and cecum were used to look at the total number of Lactobacillus, Coliforms, and aerobic bacteria that were still alive. Therefore, 1 g of Ileal and Cecal contents of two birds in each replicate were separately collected into the sterile tubes for serial dilution. The culture medium for microbial enumeration was de

Man, Rogosa, and Sharpe agar for Lactobacillus and Mac Conkey agar for Coliforms. Plate count agar was used for the total count of bacteria under aerobic conditions. The bacterial numbers were expressed as log10 CFU per gram of DM.

2.4. Intestinal Morphometric Parameters

The region from Meckel′s diverticulum to a point 40 mm proximal to the Ileocecal junction was defined as an intestinal ileum segment. Two birds in each replicate were randomly selected for measuring after slaughter as described by Burkhulder et al. (2008) with a little modification (Burkhulder et al., 2008). In brief, two-centimeter tissue samples were taken from the midpoint of the aforementioned section, then rinsed with saline and immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine by microtome (Sakura SRM 200, Tokyo, Japan) and embedded in paraffin wax. Transverse sections were cut (3 µm), stained with hematoxylin-eosin, and analyzed under a light microscope to determine morphometric indices, according to Burkholder et al. (2008). The morphometric parameters (all expressed in micrometer) measured included villus height and area, crypt depth, and villus width at the top and base. The ten longest and straightest villus and associated crypts were measured from each segment. Measurements for the villus height were taken from the tip of the villus to the villus-crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villus, and the villus width was measured at the top and bottom of the villi. The mean of 10 measurements per sample was used as the average value for further analysis.

2.5. Long Bone Measurement

Tibia, femur, and humerus were evaluated at 42 d of age. Also, 12 birds per group were individually weighed, and after slaughtering, the bones were removed and frozen. For analysis, the bones were boiled in water, and the adhered muscle was scraped off with scissors and blades. After cleaning, the bones were defatted in ether for 24 h and then dried in an oven with forced ventilation at 105 °C for 75 h. After 12 h at room temperature, the bones were weighed, and length and width (in the medial portion) were measured with a caliper.

2.6. Statistical Analyses

Data were subjected to statistical analysis using SAS (SAS Institute, 2002), and means were separated by Duncan's multiple range tests ($P < 0.05$). Mortality data was subjected to chi-square ($\chi 2$) analysis. Statistical significance is considered as ($P < 0.05$).

3. Resalts

3.1. European Production Efficiency Index (EPEI)

The performance data showed no difference between the TM-treated groups and those with control (no published). EPEI was affected by treatments significantly ($P \leq 0.05$), and EPEI was higher in chickens treated for 6, 12, and 18 hours than in control. The lowest EPEI was found in the control group. The treatments statistically decreased mortality in the treated birds compared to the control ($P \leq 0.05$). The lowest mortality was observed in 6 and 12 hour treated groups (Table 1).

Table 1. The effect of TM on mortality percent and EPEI of male broiler under CHS

Treatments	Mortality (%)			EPEI
	1-28 days	28-42 days	1-42 days	
18	7	7	13	355[a]
12	4	5	9	350[a]
6	4	5	9	350[a]
control	3	19	20	320[b]
SEM	--	--	--	72.1
p-value	0.562	0.067	0.056	0.015

[a,b] Means within columns with different superscript letters are significantly different ($P < 0.05$). Treatments: In the control group, eggs were incubated at 37.8°C and 56% RH; other treatments (6, 12, and 18 hours) incubated at 65% humidity and 39.5°C from 7 to 16 days of incubation for 6, 12, and 18 hours a day. After hatching, housed in standard conditions, all treatments were subjected to CHS from day 28 to 42.

3.2. Intestinal Microbiota

The results concerning the effect of the TM on ileal and cecal microbiota in the main parts of the digestive tract on day 42 of the experimentation are given in Table 2. statistical analysis showed that Total Anaerobes, *Lactobacilli*, and *coliforms* were not significantly influenced by treatment ($P > 0.005$).

3.3. Intestinal Morphology

The results of intestinal morphology are listed in Table 3. There was a significant impact on villus height ($P<0.05$). CHS significantly decreased villus height in the control group, while the TM improved it. The villus area was significantly affected among the groups due to the TM. The lowest villus area was observed in the PRE and PO3 groups, respectively. Villus width and crypt depth of the ileum did not differ among groups ($P>0.05$).

3.4. Long -Bone Parameters

The results of tibia, femur, and humerus length, width, and weight are shown in Table 4. Tibia length was affected and improved significantly by treatments ($P \leq 0.05$). Birds experienced TM exhibiting higher value than the control group. The 12 hours group also showed the highest value. There was a significant improvement in tibia width in the 18 groups ($P \leq 0.05$).

Furthermore, TM affected Femur length statistically. The highest and lowest value was observed in 18 hours of treatment and control, respectively. No changes were found in the tibia, femur weight, and humerus parameters.

Table 2. The effect of TM on Ileal and Cecal microbial population (log10 cfu/g of DM) of male broiler under CHS

	Ileum			Cecum		
Treatments	***Lactobacillus***	***Coliforms***	**Aerobic bacteria**	***Lactobacillus***	**Coliforms**	**Aerobic bacteria**
18	90.7	7.022	60.9	21.10	10.13	70.10
12	88.7	6.99	71.9	31.10	10.14	74.10
6	90.7	7.05	81.9	35.10	10.10	76.10
Control	87.7	7.02	67.9	20.10	10.13	73.10
SEM	0.006	0.015	0.022	0.012	0.012	0.066
P-value	0.15	0.099	0.101	0.19	0.22	0.25

[a,b] Means within columns with different superscript letters are significantly different ($P < 0.05$). Treatments: In the control group, eggs were incubated at 37.8°C and 56% RH; other treatments (6, 12, and 18 hours) incubated at 65% humidity and 39.5°C from 7 to 16 days of incubation for 6, 12, and 18 hours a day receptivity. After hatching, housed in standard conditions, all treatments were subjected to CHS from day 28 to 42.

Table 3. The effect of TM on ileal morphology of male broiler under CHS

Treatments	**Villus height (μM)**	**Villus area (μM^2)**	**Villus width (μM)**	**Crypt depth (μM)**
18	950.11±9^a	0.086±0.002^c	95.60±8	120.25±2.3
12	946.41±69^b	0.092±0.002^b	95.93±8	122±3.9
6	956.92±7^a	0.096±0.004^a	96.8±7	122.60±2.1
Control	916.11±11^c	0.095±0.003^a	97.65±6	122.44±5.5
SEM	9.29	0.003	1.8	1.9

[a,b] Means within columns with different superscript letters are significantly different ($P < 0.05$). Treatments: In the control group, eggs were incubated at 37.8°C and 56% RH; other treatments (6, 12, and 18 hours) incubated at 65% humidity and 39.5°C from 7 to 16 days of incubation for 6, 12, and 18 hours a day receptivity. After hatching, housed in standard conditions, all treatments were subjected to CHS from day 28 to 42.

Table 4. The effect of TM on the long bone characteristics of male broiler under CHS

	treatments					
Variables	**6**	**12**	**18**	**Control**	**SEM**	***p-value***
			Tibia			
Length	100.2^b	102.91^a	101.68^{ab}	99.21^c	1.51	0.051
Width	10.98^b	11.25^b	11.78^a	10.95^b	0.15	0.052
Weight	8.85	8.85	8.75	8.8	0.13	0.87
			Femur			
Length	72.01^{ab}	71.9^{ab}	73.8^a	70.59^b	0.93	0.028
Width	7.61	7.61	7.43	7.51	0.096	0.059
Weight	7.3	7.25	7.15	7.25	0.054	0.054
			Humerus			
Length	67	66.5	68.05	67.22	0.69	0.21
Width	7.08	6.00	7.85	7.52	0.098	0.22
Weight	4.45	4.75	4.65	4.58	0.046	0.33

[a,b] Means within columns with different superscript letters are significantly different ($P < 0.05$). Treatments: In the control group, eggs were incubated at 37.8°C and 56% RH; other treatments (6, 12, and 18 hours) incubated at 65% humidity and 39.5°C from 7 to 16 days of incubation for 6, 12, and 18 hours a day receptivity. After hatching, housed in standard conditions, all treatments were subjected to CHS from day 28 to 42.

4. Discussion

4.1. European Production Efficiency Index (EPEI)

Results showed that treated groups exhibited higher EPEI. Despite no significant difference in mortality from 0 to 28, 28 to 42, and 0 to 42 days of age. However, mortality was decreased statistically in treated chicks compared to control. Some studies also reported that treated birds performed better (Piestun et al., 2011,2009, 2008). Moreover, decreased mortality in TM-treated chicks agreed with Yahav and Hurwitz (1996) and Zaboli et al. (2016) findings. Thermotolerance is a physiological response in TM-treated birds. Tzschentke and Basta (2002) reported that exposure to a warmer prenatal incubation temperature resulted in an elevated neuronal hypothalamic warm sensitivity through an increased proportion of cold-sensitive neurons and a reduced proportion of warm-sensitive neurons in comparison with the control group (Tzschentke et al., 2002). So, a significant increase in EPEI and a lower mortality rate could indicate the greater resistance of TM-treated chicks to CHS due to the epigenetic temperature adaptation. Furthermore, this trial might reduce metabolic rate, thyroid hormone, and body temperature (Zaboli et al., 2016). Probably these changes positively affect EPEI.

4.2. Intestinal microbiota

In the gastrointestinal tract (GIT), the microbiota plays a major role in elevating nutrient absorption and amplifying the immune system, so the microbiota affects both the growth and health of the chicken (Choi et al., 2015). We hypothesized that TM could improve performance and intestinal morphology (Uni et al., 2001) and thereby positively change microflora in chickens exposed to CHS. Uni et al. and Temmim et al. (2000) reported that the birds treated by the TM pattern exhibited better brush border characteristics than the control (Uni et al., 2001; Temim et al., 2000). Bailey et al. noted that heat stress could alter the normal microbiota in the GIT (Bailey et al., 2004). Thus, it reinforced our hypotheses that TM could improve microflora. On the contrary, our results showed no significant changes in the Ileal and Cecal microbial populations. The mortality of sensitive birds can explain in the control group, and CHS for two weeks may create adaptation in the control group.

4.3. Intestinal morphology

Intestinal morphology condition could be considered as a proper response for TM experiments. The small intestine is the main site of the digestive system that is affected by environmental conditions. Moreover, it gives information about the health and performance of birds. The most important part of the brush border is the enterocytes located on top of the villus and is responsible for observation and disability (Yahav et al., 2007). Burkholder et al. (2008) showed that heat stress for 24 hours did not affect the villus height. They suggested that the short duration of the stressor, and the resistance of the ileum to structural change, could be possible responses to remain unchanged. We can conclude that improved villus height in treated birds caused increased performance during CHS. TM could induce thermotolerance and prevent the adverse effects of CHS on intestinal morphology. Likewise, Uni et al. (2001) reported an improvement in the enterocyte proliferation and the activity of brush border enzymes 48 hours after thermal conditioning. Meanwhile, TM reduces body temperature, and as a result, the treated bird could help the improvement of intestinal morphology (Zhu et al., 2002; Piestun et al., 2009).

4.4. Long Bone Parameters

performance improvements in poultry production are associated with skeletal disorders and are the major negative factor affecting poultry production. Furthermore, heat stress intensifies skeletal problems (Yalçin et al., 1996). Burno et al. (2007) reported that heat stress caused a decrease in both the tibia and humerus length and width. As a result, alterations in development caused by pre- and post-hatch treatment affected bone development. Small differences in incubation temperature applied throughout incubation have been shown to influence the growth of the long bone in the birds (Brookes et al., 1972). Raising the temperature of the eggs by 1°C, from 37.5 to 38.5°C, during ED 4 to 7 could increase the length of tibia and tarsus bones in Leghorns (Hammond et al., 2007). Moreover, our result showed that TM could lead to the development and improvement of a long bone, but the mechanisms are unclear (Maltby et al., 2006; Brookes et al., 1972; Zaboli et al., 2017). Otherwise, the results from this trial showed that TM decreased mortality and improved performance and also decreased the metabolic rate and body temperature (Zaboli et al., 2016). These changes may help improve long bone development (Collin et al., 2012). So that the negative impact of CHS is reduced.

5. Conclusion

In summary, the application of TM during the development and maturation of the thermal regulation system of the male broilers may induce positive effects on the intestinal morphology, long bone development, and EPEI, thus preventing the negative effects of CHS on broiler chickens in the first week of CHS to reduce mortality and improve EPEI. Overall, TM can affect intestinal microbiota and improve long bone characteristics. Notwithstanding, the underlying mechanisms of TM's long-lasting effect on broiler chickens' physiological responses remain to be elucidated in the future.

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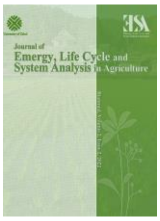

Estimating the relative performance of rainfed crops using Fraction of Absorbed Photosynthetically Active Radiation index, land, and field data

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ARTICLE INFO

Article history:
Received: 12 October 2022
Accepted: 1 January 2023
Available online: 11 January 2023

Keywords:
Absorbed photosynthetically active radiation index
Crop yield
Drought
Lorestan province

ABSTRACT

In most regions of Iran, including the province of Lorestan, the majority of agricultural activities are conducted in the open air. Climate risks have a significant impact on agricultural productivity. Drought and its effects are among the most significant natural threats to the agriculture industry in that area. The purpose of this study is to investigate the effects of drought on the yield of rain-fed crops in Aleshtar county, Lorestan province. To achieve this goal, a combination of field methods, remote sensing, and statistical methods was employed. During ground surveys, data required for laboratory operations (direct method) and measurements using AccuPAR and MODIS sensor images were collected (indirect method). In addition, precipitation data from synoptic stations in the province of Lorestan over the course of 27 years (1991-2017) were utilized to calculate the drought and its impact on yield. According to the calculated drought indices, in the province of Lorestan and the county of Aleshtar, the trend of increasing drought and the recurrence of long-term cycles of wet and drought are evident. The study of phenology characteristics of rainfed crops (barley) in relation to climate conditions revealed that an increase in thermal and water stress has a direct effect on the performance of rainfed crops. Therefore, an increase of 2.5 °C in the average temperature, combined with a lack of moisture supply during flowering, results in a decrease in the number of seeds per spike (16 seeds per spike) and, consequently, a decrease in the plant's yield. At various growth stages of rain-fed plants, the correlation index between LAI harvested by direct methods and remote sensing methods ranges between 0.57 and 0.96. This value represents the precision of remote sensing techniques. From 1991 to 2017, the correlation index values between the yield of rainfed plants, especially wheat and barley, and the values of various drought indices indicate a positive and direct relationship between yield and drought index values. The correlation index between yield and drought index values reaches its maximum value during 1-6 months, and its value decreases as time scales become longer. The physiological properties of various products are one of the primary causes of this circumstance. On the basis of the obtained results, it can be concluded that the increase in drought and heat stress in the province of Lorestan and the county of Aleshtar has caused a decrease in yield at various stages of plant growth and an increase in water demand for a variety of rainfed crops.

Highlights

- According to drought indices, Aleshtar are experiencing increasing drought and long-term cycles of wet and dry.
- A temperature increase of 2.5 °C combined with a lack of moisture during flowering reduces the number of seeds per spike and the plant's yield.

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https://doi.org/10.22034/aes.2023.365450.1045

- From 1991 to 2017, correlation index values between rainfed plant yields, especially wheat and barley, and drought indices indicate a positive and direct relationship.
- Based on the results, it can be concluded that drought and heat stress in Aleshtar have decreased yield at various growth stages and increased water demand for rainfed crops.

1. Introduction

Despite the high risks of drought occurrence and its impact on agriculture, there have been few studies on the impact of drought on agricultural products in Iran. This is despite the fact that the occurrence of this phenomenon in any part of Iran has the possibility of happening for several years. On the other hand, in some areas, such as the southwest of the country, agriculture is the main source of people's income. Lorestan province, located in the southwest of Iran, is one of the provinces where agriculture is one of the main activities of its residents. In Lorestan province, agricultural activity is both rainfed and irrigated. Agriculture encompasses the cultivation of grains, legumes, industrial, and fodder plants, as well as the raising of livestock and aquatic animals. Therefore, the occurrence of drought in this region will have many adverse effects on the agricultural sector (Zand, 2018). Yang et al. (2006) analyzed the leaf area index using a combination of Terra and Aqua satellite data. They stated that there is no significant difference in the leaf area index obtained from these two satellites on a large scale. Deng et al. (2006) presented a new method to retrieve the leaf area index using satellite images. Garrigues et al. (2008) studied the sensitivity of leaf area recovery estimated by Li-Cor LAI-2000 optical instruments, AccuPAR, and hemispherical imaging methods in the above fields. The results showed that the hemispherical photography method has the least sensitivity to light conditions and is better than other tools for estimating the lower vegetation canopy. Yingbin et al. (2010) estimated the yield of rice crops in cold climatic conditions by using MADIS and Landsat images based on the leaf area index.

Sepulcre-Canto et al. (2012) developed a moisture index to detect agricultural drought in Europe. In this research, the standard precipitation index (SPI), soil moisture anomaly, and photosynthetic active radiation anomaly (FAPAR) absorbed by plants were used. The calculation of this composite index at the European level showed that this index provides a general and synoptic view of the drought situation in the form of a specific classification. Pérez-Blanco and Gómez (2014), in a study entitled "drought management programs and available water in the agricultural sector," examined a risk assessment model for a basin in southern Europe and concluded that if drought management programs are successfully implemented, available water will meet an average of 62.2% of current demand, and this figure may decrease to 50.2% by the end of the century as a result of climate change. Meroni Dutta et al. (2015) monitored agricultural drought through NOAA-AVHRR Sanjand NDVI data for a long-term period. They calculated the index (VCI) for the whole of Rajasthan and succeeded in obtaining a significant relationship between the rainfall anomaly index and the crop anomaly index. Meroni et al. (2017) conducted research titled "evaluation of the standard precipitation index" in order to provide an initial forecast of the state of seasonal vegetation in the Sahel region. By examining the relationship between the standard precipitation index and standardized cumulative measurements of active photosynthetic radiation (zCFAPAR[†]), they concluded that there is a significant linear relationship between these two variables in 32-66% of the studied area.

Ahmadi et al. (2010) estimated the cultivated area of soybean and corn with satellite images and stated that the LAI index could be used to identify vegetation and cultivated products, including soybean and corn. Bakhsandeh et al. (2014) measured the leaf area index for wheat plants using the AccuPAR device using two direct (destructive sampling) and indirect methods. The results showed that there is no significant difference between the figures and the two conditions in terms of the coefficients of the equation. Fatehi Marj et al. (2011) evaluated the drought of pasture and rain for three years, from 2007 to 2009, using Modis -measured images. In this research, it was found that the reduction of vegetation cover in the country in 2018 was significant. The comparison of the meteorological drought with the agricultural drought shows the adaptation of both droughts this year. At the same time, in 2019, despite the lower-than-average rainfall, the condition of the pasture cover was the wettest of the year.

Mirmusoi and Karimi (2013) investigated the effect of drought on the vegetation of Kurdistan province during the period 2000-2009 using the images of the Modis sensor. The results showed that there is a high correlation between the average SPI and NDVI indices at a significance level of 1%, and with a decrease of approximately -0.20 of the SPI index, an average of 1.2% of the weak vegetation area increases. Nowrozi and Mohammadi (2016) studied the effect of hydrological drought on agriculture in the Lanjan region using the SWSI index and the Mann-Kendall test. Their results showed that the production, cultivated area, and yield of crops in the region are decreasing. Among crops, only the amount of production and cultivated area of rice are increasing. Zand (2018) investigated the economic effects of drought on the income of dryland farmers (wheat and barley) in Khorramabad city over 15 years using SPI, PN, and DI indices. The results of this research showed that the effect of the SPI, PN, and DI indices on the yield and net value of wheat production was significant at levels of 0.01 and 0.05, respectively, and for the barley product at levels of 0.05 and 0.01, respectively.

According to the estimates made by the disaster headquarters of Lorestan governorate in the crop year

[†] z-score of the cumulative value of the Fraction of Absorbed Photosynthetically Active Radiation

2014–2015, the drought caused 1300 billion riyals of damage to the gardens and lands of the province. Drought has affected the amount of demand for agricultural inputs, such as fertilizers, poisons, machinery, credits, etc. In addition, the drought has had a negative impact on water resources, forests, pastures, and other natural resources in the province. In Lorestan province, agricultural activity is carried out in both rainfed and irrigated forms, in the groups of cereals, legumes, industrial, and fodder plants, livestock, and aquatic animals. The most damage caused by drought in the last few years has been directed at the agricultural sector. Precipitation and its characteristics are very important in all aspects of agriculture, and the amount of the crop is strongly influenced by the spring rainfall, especially in May. In the meantime, the agricultural drought causes problems, such as the reduction of household income (which has caused the amount of investment in this sector to also degrade), increasing unemployment among rural communities (migration), decreasing the number of livestock, and decreasing the production of crops, horticulture, livestock, and aquatic products. In this research, an attempt has been made to investigate the effect of drought on the production of rainfed agricultural products in Aleshtar County and its effects using a combination of field, modeling, remote sensing, and statistical methods.

2. Materials and Methods

2.1. Geographical location of Lorestan province

The studied area includes Lorestan province in the west of Iran. This province, with an area of 28559 square kilometers in the west of Iran, covers 1.7% of the total area of the country. Lorestan province is located in the west of Iran, with longitudes ranging from 32.37° to 34.22° E and latitudes ranging from 46.51° to 50.3° N . Lorestan province is bordered by Markazi and Hamadan provinces from the north, Khuzestan province from the south, Isfahan province from the east, and Kermanshah and Ilam provinces from the west. Figure 1 shows the location of Lorestan province by city division in Iran. (Climatic Atlas of Lorestan Province, 2016).

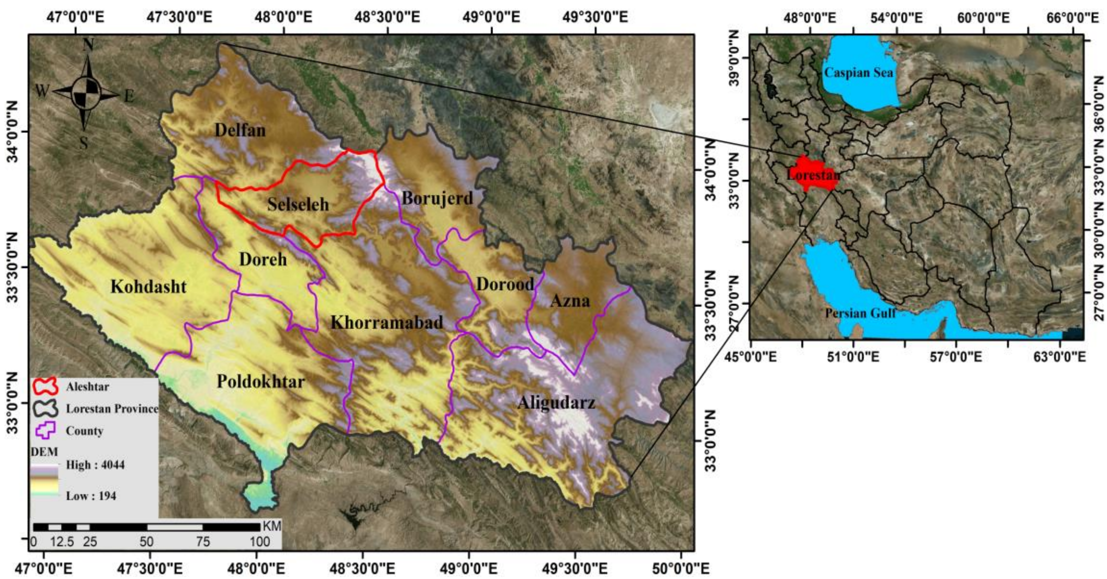

Figure 1. The location of Lorestan province

2.2. Data and methodology

2.2.1. Data

The data used to conduct this research includes field data, satellite information, and station data from the Meteorological Organization and the regional water company.

1. Land use map of Aleshtar county obtained from satellite images
2. Moody's satellite images on a daily scale and on an 8-day time scale
3. Field information collected using an AccuPAR device
4. Drought indicators calculated in the meteorological drought assessment project of Lorestan province
5. Information on the phenology of the rainfed barley crop at the Silakhor Agricultural Climate Research Station

2.2.2. Method

In this research, first, library studies and a review of sources related to the project were carried out to determine the general framework of the research. In the following, using field studies and interviews with provincial and Aleshtar county experts, sample fields were selected to harvest leaf area index and other plant characteristics during the growth period until harvest. In the following, the images of the Modis sensor during the growth period until the harvest of the selected fields in the water year of 2020 were determined. The images from this sensor were received at the specified times, and the leaf area index was calculated. Also, by using the AccuPAR Model LP-80 device, the characteristics of the products of the selected rainfed farms (leaf level, amount of radiation available at the top and bottom of the plant community, amount of active photosynthesis, peak angle of the sun's radiation) were collected. These two processes (taking pictures and harvesting with the AccuPAR device) were continued until the harvest stage. After conducting library and field studies, the drought conditions of the province were investigated based on the drought indicators calculated in the meteorological drought assessment project of Lorestan province. In this regard, SMI[‡], SPI[§], SPEI[**], RDI[††], NDVI[‡‡], VCI[§§], VHI[***], and TCI[†††] indexes were investigated based on the data of the synoptic station of Lorestan province and neighboring provinces from the period 1990-2017. Using their results, the spatial and temporal changes in drought occurrence on time scales of 3, 6, 9, 12, 18, and 24 months were investigated at the province level. Finally, by using the principal components method (PCA[‡‡‡]), the aforementioned indices were combined, and a composite index was extracted. The combination of indicators was performed in the MATLAB 2019a software. The combined results of drought indicators were prepared using the capabilities of GIS and MATLAB software in the form of maps, charts, and necessary tables.

After the temporal and spatial investigation of the meteorological drought that occurred in the province, in the continuation of leaf surface indices using satellite images and ground collection, the index of absorbed photosynthetically active radiation (FAPAR[§§§]) was calculated. Finally, the relationship between meteorological drought and the yield of rainfed crops was investigated with the use of regression relationships.

3. Results and Discussion

After examining the general conditions of drought in Lorestan province for different regions, drought values for rainfed crops were also calculated and analyzed separately for each city. It should be mentioned that the calculation of different drought indicators was done on different time scales. However, to avoid lengthening the report, we refrained from presenting graphs of all time scales, and only 12-month scale graphs were displayed. Figures 2–4 show the values of different drought indices in the rainfed areas for Aleshtar, Khorram Abad, and Borujerd counties. According to the results obtained on the graph in the studied area, the occurrence of droughts from the late 2001s to the middle of the 2011s is significant. According to the obtained results, the drought that occurred during the 2017–2018 water year is one of the most severe droughts in terms of rainfall recorded in the region. As can be seen on the graphs, during recent years, the water year 2017–2018 had drought conditions, the water year 2016–2017 had normal conditions, and the water year 2015–2016 had drought conditions.

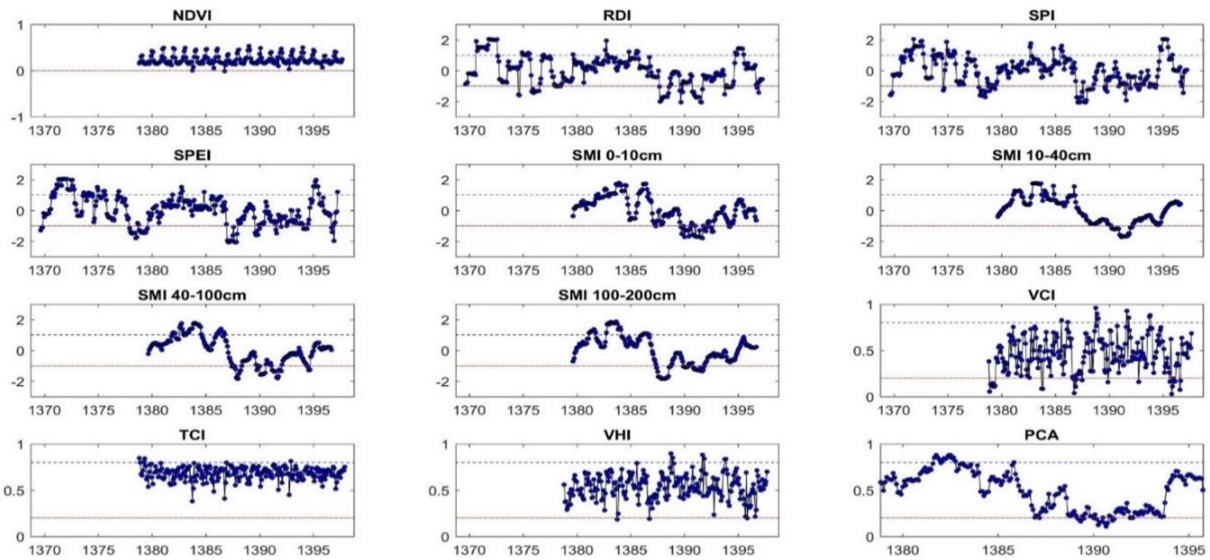

Figure 2. Time series of different drought indicators on a 12-month time scale (SPI, RDI, NDVI, SPEI, SMI, VCI, TCI, VHI, PCA) for the cultivation area of rainfed plants in Aleshtar county

3.1. Investigating the phenology characteristics of rainfed crops using laboratory operations

In order to monitor the relative performance of rainfed crops and investigate the phenology characteristics of rainfed crops in the study area in different stages of growth, information on the phenology of rainfed crops was collected and analyzed at the only agricultural climate research station in Lorestan province, namely Silakhor station, from the General Meteorological Department of Lorestan province. The phenology characteristics of rainfed barley during different stages of growth from January 2021 to June 2021 are explained here, and the yield

‡ Soil Moisture Index
§ Standardized Precipitation Index
** Standardised Precipitation-Evapotranspiration Index
†† Reconnaissance Drought Index
‡‡ Normalized Different Vegetation Index
§§ Vegetation Condition Index
*** Vegetation Health Index
††† Temperature Condition Index
‡‡‡ Principal component analysis
§§§ Fraction of absorbed photosynthetically active radiation

and other agricultural traits of this rainfed crop at Silakhor station are presented in Table 1. In the crop year 2020-2021, in most of the temperate regions, such as the Silakhor plain (located in Borujerd, the city of Lorestan province), we saw a relatively mild winter, which resulted in an increase in daily temperatures compared to long-term statistics. In addition, there was no effective rainfall from March 20 to April 16, which coincided with the time of sprouting. The lack of rainfall, along with the relative increase in temperature, led to a decrease in the length of the growing season, and finally, cereals with a low plant height (35 cm for rainfed barley) entered the reproductive stage. The results of the percentage of plants in the stemming stage also show the same thing. According to the results, the plants entered the pregnancy stage with 75% of the stems. This indicated that there was a competition for resources between spike growth and stem elongation.

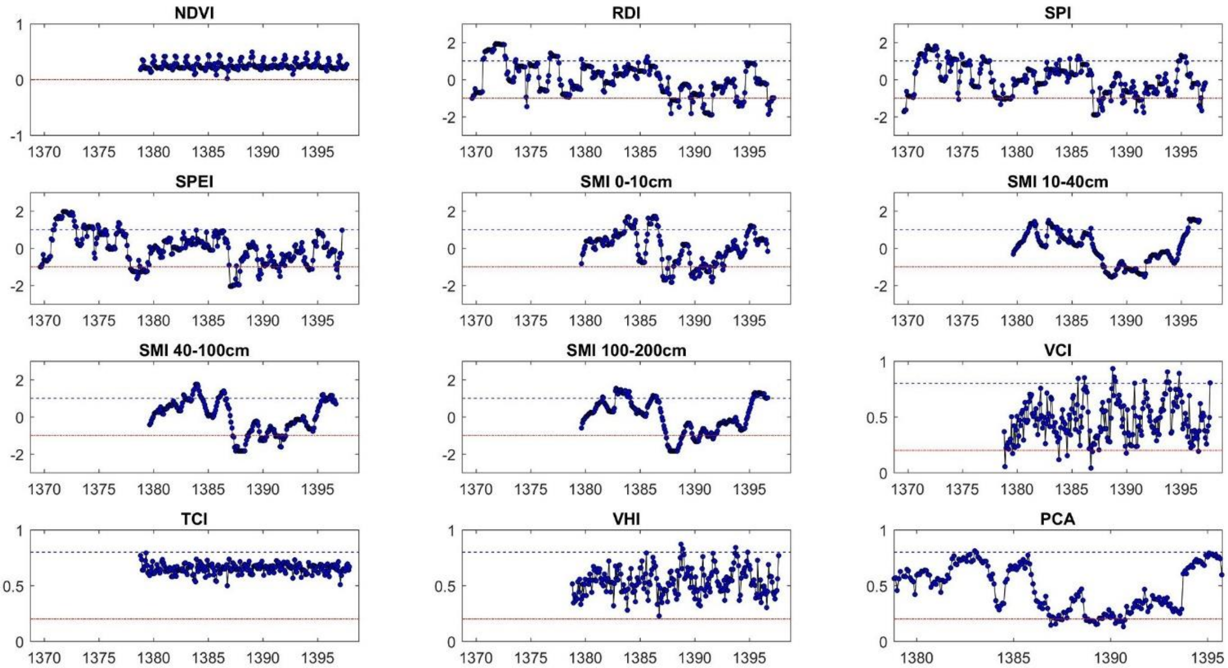

Figure 3. Time series of different drought indicators on a 12-month time scale (SPI, RDI, NDVI, SPEI, SMI, VCI, TCI, VHI, PCA) for the cultivation area of rainfed plants in Khorramabad County

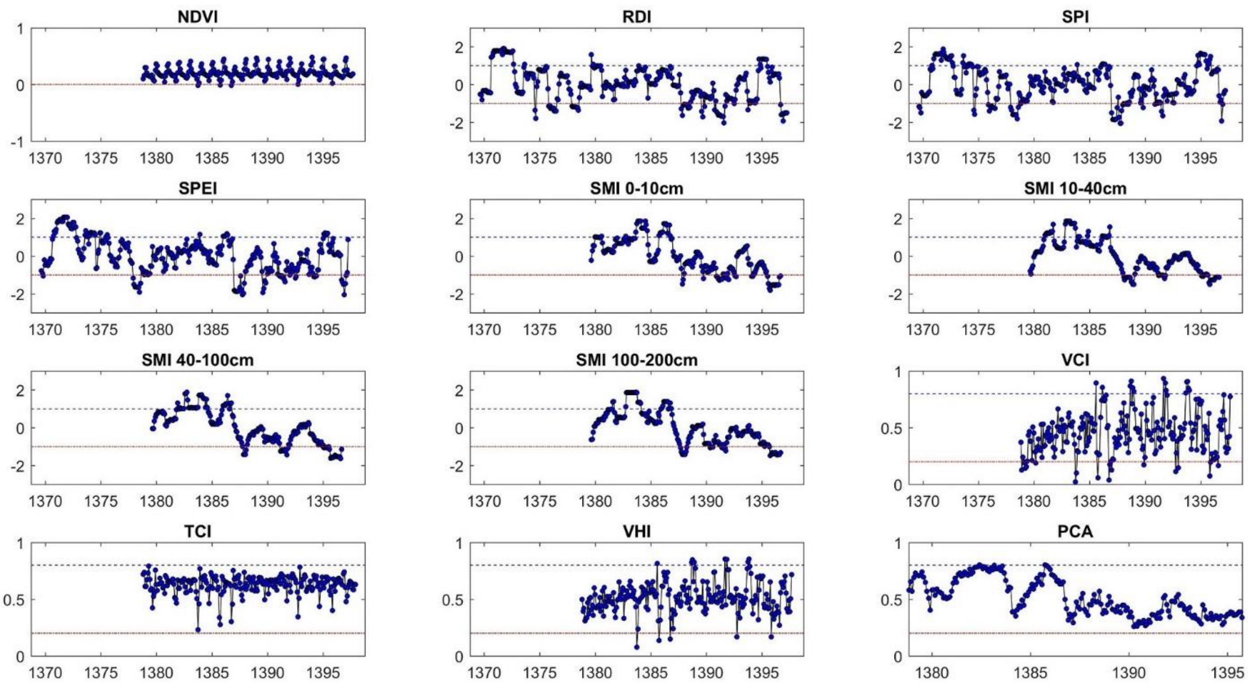

Figure 4. Time series of different drought indicators on a 12-month time scale (SPI, RDI, NDVI, SPEI, SMI, VCI, TCI, VHI, PCA) for the cultivation area of rainfed plants in Borujerd County

The number of spikes per unit area is influenced by the number of claws. Pinching is done from the stage of 3–4 leaves to the beginning of the stem elongation period. With the lengthening of the stem and the formation of the canopy, the competition between the claws begins, which eventually leads to the death of some claws. Due to the lack of rain during the stemming stage, which was from May 22 to April 16, and the average temperature increase of 1.7 degrees Celsius compared to the long-term average temperature, the number of fertile paws decreased from 370 paws per square meter to 360 paws per square meter (ten Sterile paws per square meter).

Ten days before the spike, which coincided with April 14 to 24, and during the spike, which coincided with April 24 to May 10, moisture stress led to a decrease in the number of spikes, especially in the tips of the spikes, which in turn resulted in a decrease in the number of seeds per spike. During the 30 days before flowering, for every one-degree increase in temperature above 14°C, the number of seeds decreases by 4%. The average temperature in the period from April 10 to May 10, which coincides with the 30-day period before flowering, was above 14°C and between 15-20°C on most days.

In the period from May 12 to 18, which coincided with the flowering time, the increase in wind speed (especially on May 15) led to an increase in evaporation from the soil surface, as well as an increase in temperature by 2.5 degrees Celsius compared to the long-term average temperature in this region. The period of drought stress caused damage to grain fields and subsequently reduced grain yields in dry lands. Thus, the drought stress conditions in the flowering stage led to a decrease in the number of seeds per spike (16 seeds per spike). In other words, the decrease in yield due to drought stress in the flowering stage was caused by the decrease in the number of seeds in the spike. In addition, it is important to create favorable soil moisture conditions, especially in the tillering, stemming, and spike emergence stages, for the formation of the number of seeds per unit area. According to the rainfall statistics from February 27 to April 16, which coincided with the aforementioned growth stages, only one effective rainfall (27 mm on May 13) occurred.

The weight of 1000 seeds is determined as one of the important yield components from the time interval of pollination to maturity. The seed filling time is mainly affected by temperature. As it is clear from the results, from May 18 to June 20, which coincides with the seed-filling period, the average temperature increased by 1.6 degrees Celsius. Also, due to the fact that there was no rainfall during this period, the moisture stress at this stage (when the seeds are filled) reduces the photosynthetic capacity through premature aging of the leaves. In this way, the length of time that carbohydrates can be transferred to the grain is reduced, which is equal to the reduction of the duration of grain filling and, ultimately, the weight of a thousand seeds.

Table 1. the yield and other agricultural traits of rainfed barley at Silakhor station

Traits	Yield
Plant height (cm)	62
Number of spikes per square meter	370
The number of fertile spikes	360
The number of unproductive spikes	10
Number of seeds per spike	16
Thousand seed weight (grams)	21
Seed yield (kg/ha)	2500
Straw yield (kg/ha)	1900

3.2. AccuPAR leaf surface index

Table 2 shows the index values of plant leaf area of selected farms (in the crop year 2020-2021) in the study area. Field harvesting from selected fields has been done on four dates at different stages of wheat plant growth. Based on the values presented in Table 2, the wheat plant leaf area index values during a logical process from the early stages of growth to the peak growth stage in May increased. Simultaneously with the ripening of the crop and harvest, LAI index values decreased; in other words, simultaneously with the increase in the number of leaves and the area of wheat and barley plants in the region, the index values also increased. Among other notable points on the graph below (Figure 5), we can mention the difference in the amount of leaf surface obtained in each period at different stages of plant growth for sample farms. So in one stage, for example, spike formation, the amount of leaf surface is different in the fields under study. One of the reasons for this can be the difference in the types of planted species and the effect of microclimatic factors on different farms.

Table 2. the index values of plant leaf area of selected farms

	2021-04-13	2021-04-28	2021-05-18	2021-06-01
Sarab-e Honam	0.02	0.14	1.11	0.31
Chahar Takhteh	0.01	0.08	0.36	0.28
Nurullahi	0.05	0.21	0.31	0.055
Deh-e Kadkhoda	0.07	0.05	0.62	0.43

Figure 6 shows the index values of wheat plant leaf area in selected fields of Aleshtar county. Based on the values obtained from MODIS satellite images on the days of harvesting with the AccuPAR device, the maximum value of the leaf area index of the dominant crop in the region occurs in the villages of Nurolahi and Chahartakhteh in the middle of May and the villages of Sarab Hanam and Deh Kodkhoda in early may Among the causes of this difference is the maximum time of the leaf surface index. Accordingly, the timing of processing and harvesting can be affected by the type of product variety and the effect of microclimatic factors on different farms.

Examining the relationship between the values of the leaf area index harvested using the AccuPAR device and the images of the Modis sensor showed that, although there is a difference between the values harvested at different stages of plant growth in the region, despite this behavior, the values of the leaf area index harvested by the Modis sensor and the AccuPAR device are similar. Tables 2–3 show the R index values between the data taken by the AccuPAR device and the Modis sensor at different stages of growth. As it is clear, there is a direct relationship between these two tools when harvesting the plant leaf surface index. In our stage, the maximum relationship was obtained before the maximum greenness. The difference in the types of planted varieties can be one of the reasons for the lower values of the correlation index in the stage of maximum greenness.

Table 3. Value of correlation indices and coefficient of determination between LAI data collected by Acupar and MODIS satellite

Time	R	R2
2021-04-13	0.78	0.61
2021-04-28	0.96	0.94
2021-05-18	0.57	0.32
2021-06-01	0.6	0.36

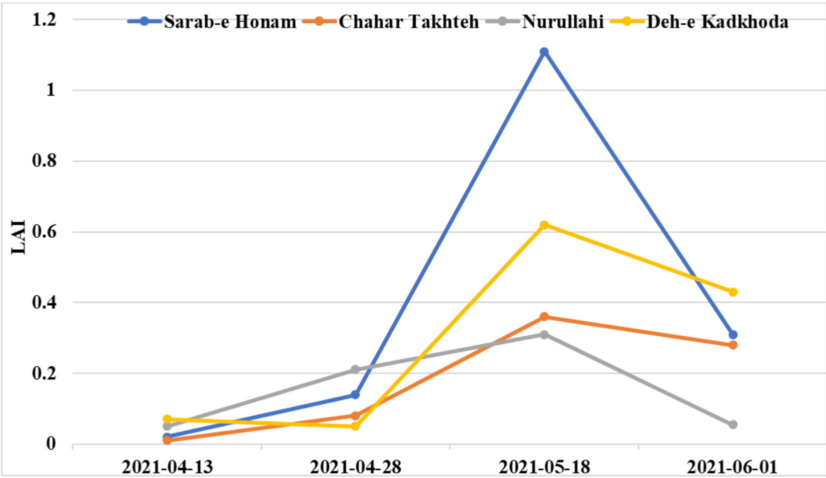

Figure 5. Leaf area index values of rainfed wheat for different farms based on the estimate of AccuPAR

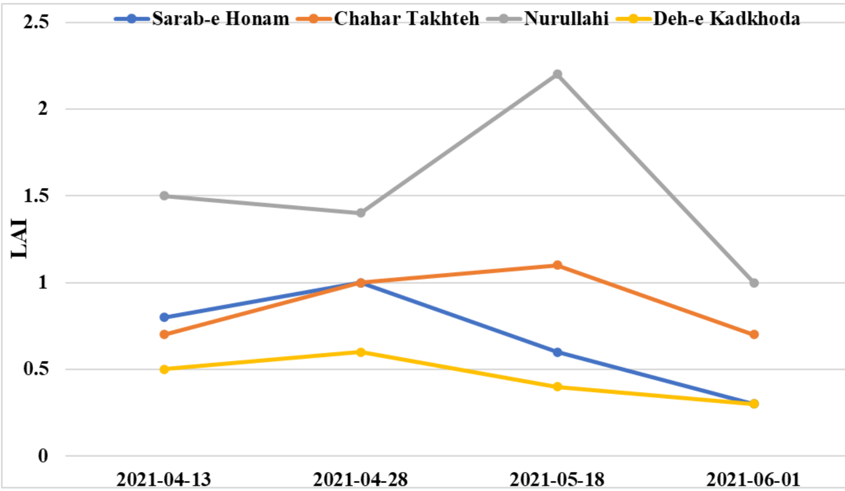

Figure 6. Leaf area index values of rainfed wheat for different farms based on the estimate of MODIS satellite images

3.3. Relationship between the performance of rainfed plants and drought in the long term

In this part, the relationship between the yield of rainfed plants and the values of drought indicators in Lorestan province has been investigated. Figure 7 shows the correlation between the yield of rainfed atmosphere in Lorestan province and various drought indicators used in this research. Indices such as NDVI, Standardized NDVI, or SNDVI, SPI, RDI, SPEI, SMI1 (at a depth of 0 to 10 cm), SMI2 (at a depth of 10 to 40 cm), SMI3 (at a depth of

40 to 100 cm), and SMI4 (at a depth of 100 to 200 cm): all these indicators are in time series of 1, 3, 6, 9, 12, 18, and 24 months. VCI, TCI, and VHI indices, and finally the combined drought index based on PCA, which is also in the time scales of 1, 3, 6, 9, 12, 18, and 24 months. The red dashed lines that appear horizontally on the chart show the range of each indicator. Since some indicators have different time scales (such as SPI), they have also been assigned a larger range. In the range of indicators with a time scale, the column charts belong to the time series of 1, 3, 6, 9, 12, 18, and 24 months, respectively. Within the polygon of each county and in each water year, the average drought indices were adapted and then correlated with the yield of rainfed wheat and barley in the cities. Figure 7 shows the correlation between different drought indicators and rainfed performance in different cities of Lorestan province. About rainfed barley, as with rainfed wheat, the short-term time scales of drought show a greater correlation with the yield of rainfed barley. However, on long-term time scales, even this correlation is reversed. Although the analysis of these results needs more investigation, it seems that the vegetative period of barley is shorter than that of wheat. Therefore, it shows a stronger correlation than drought indicators with a smaller time scale.

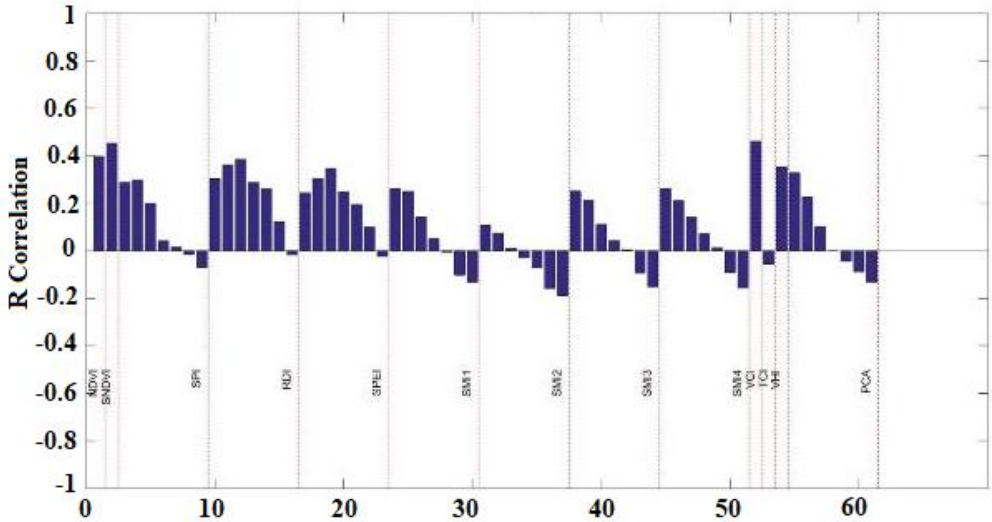

Figure 7. Value of Correlation index between drought indices and rainfed barley yield in Lorestan province

4. Conclusion

Water shortages and droughts are two of the biggest challenges the country's agricultural development will face now and in the future. Estimates of food needs show that if a suitable strategy for water management and preparation to reduce the effects of drought is not adopted in the agricultural sector, the water and land potentials of the country will not provide the food needs the country. The main goal of this research is to estimate the values of the leaf surface index using direct and indirect methods and to finally reach a suitable method for estimating the yield of the product. To achieve this goal, a combination of telemetry, statistical, and field-laboratory methods were used. The information used includes the daily images of the Modis sensor and samples taken from fields during different stages of wheat plant growth in Aleshtar county.

Calculation and analysis of drought indices (especially the meteorological drought indices RDI and SPI) in order to monitor the drought conditions of Lorestan province during 1991–2017 showed that the increasing trend of drought and the repetition of long-term cycles of drought and wet are evident in Lorestan province. In fact, based on the results related to drought indicators (Figures 2–4), three cycles of drought and wet were observed during the period under review. The first cycle is before 2011, which ended in a significant drought around 2011. In the next stage, this cycle peaked again and ended in a significant wet period in the years around 2017. However, this period of wetness is less severe than the previous one that happened around 1994. The occurrence of long-term droughts during the years 2008 to 2015 is evident, and we witnessed the creation of a wet period again in 2017. The decrease in the intensity of rain and the increase in the duration of droughts, as well as their severity, can indicate climate changes in recent years.

Processing and the results of field and laboratory harvests showed that the amount of leaf surface during the growth period shows an upward trend until a certain stage and then a downward trend. Thus, the maximum amount of leaf area is in the flowering period. As the harvest stage approaches and most of the plant's energy is focused on its performance, the increase in the plant's leaf area decreases, and over time and near the time of harvest, the trend of greenness and the plant's leaf area decrease. The results obtained from processing the data recorded at the Silakhor agricultural station and in the laboratory environment showed that it is important to create favorable thermal and soil moisture conditions, especially in the tillering, stemming, and spike emergence stages, for the formation of the number of seeds per unit area. By increasing the temperature at the wrong time, the barley plant will face stress, so that in the stemming stage, with an increase of 1.7 degrees Celsius in the average temperature compared to the long-term average temperature, the number of fertile claws decreased from 370 claws per square meter to 360 claws per square meter. Also, in the period from May 12 to 18, which coincided with the flowering time, the increase in wind speed (especially on May 15) and the increase in temperature by 2.5 degrees Celsius compared to the long-term average temperature caused drought stress conditions and reduced the number of seeds per spike. Based on the results obtained in the laboratory environment, the barley yield per hectare is estimated to be 2500 kg.

The results of investigating the relationship between the yield of rainfed plants and various drought indices in Lorestan province during the period 1991–2017 showed that the values of the correlation index between the yield of rainfed plants and most of the drought indices are positive, although the correlation index is different at different time scales. Is. The positive correlation values between the performance of rainfed plants and different drought indicators indicate a direct relationship and influence between the performance of rainfed plants and the amounts of rainfall and moisture in the soil. As a result, with the increase of rainfall and soil moisture, the amount of yield increases, and with the decrease of rainfall and moisture in the soil, the amount of yield also decreases. It is also necessary to mention that although the correlation index values between the yield of rainfed plants and the values of drought indices are less than 0.5 in many cases and are not very high, it should be considered that crop yield is not only a function of drought and the indices The other factors, especially the weather conditions and especially the temperature conditions, farm management, the timely availability of inputs, etc., also affect the yield of crops. Also, in a fixed cultivation area in a region or province, the occurrence of drought, wetness, or other factors can lead farmers to produce another crop. Therefore, the process of analyzing the available information is very complicated. In addition to the mentioned cases, since there is still no up-to-date location system related to the collection of agricultural statistics for the country, the accuracy of the collected statistics can also be another source of uncertainty in the correlation calculations between actual and estimated performance. However, high correlations are not expected here, and it seems that the correlations are more reasonable at the current level.

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Journal of Emergy, Life Cycle and System Analysis in Agriculture (2022) Volume 2, Issue 2, P. 161-172

Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir

Effect of vacuum packaging on body chemical composition, peroxide, and TVB-N of phytophagous fish during frozen storage

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ARTICLE INFO

Article history:
Received: 28 May 2022
Accepted: 3 January 2023
Available online: 10 January 2023

Keywords:
Ash, Protein
Fat
Moisture
Peroxide (PV)
TVB-N

ABSTRACT

This study aimed to investigate the effects of vacuum packaging on body chemical composition and changes in peroxide and TVB-N of phytophagous fish and determine the optimal storage time in freezing conditions (-18 ° C). In this study, 36 samples of 100 g fillets were prepared for both vacuum and non-vacuum conditions in completely hygienic conditions. Then half of the samples were vacuum-packed using an EBOR vacuum packing machine, and the other half were packaged under conventional conditions. These fillets were stored for five months at freezing temperature (-18 °C). After packing, all factors were analyzed in 6 replications by standard methods. Peroxide content increased significantly from 1.96 meq/kg to 44.44 meq/kg in vacuum conditions and to 47.77 meq/kg in non-vacuum conditions. Also, the amount of TVB-N increased significantly from 6.53 mg/100g to 18.66 mg/100g in vacuum conditions and from 6.53 mg/100g to 38.26 mg/100g in non-vacuum conditions. The result of the analysis shows the positive effect of packaging in vacuum conditions on the process of changes in the body's chemical composition. The samples in these conditions had less change range than in conventional conditions, which has reduced the spoilage rate of these samples.

Highlights

- This study aimed to determine the optimal freezing storage time and the effects of vacuum packaging on the chemical composition of phytophagous fish.
- Half of the samples were then vacuum-packed with an EBOR vacuum packing machine.
- Peroxide concentration rose from 1.96 meq/kg in vacuum to 44.44 meq/kg in non-vacuum.
- TVB-N concentration rose from 6.53 mg/100g to 18.66 mg/100g in vacuum and to 38.26 mg/100g in non-vacuum.

1. Introduction

Undoubtedly, food and nutrition are the most crucial subjects on a global scale at this time. The imperative to address the nutritional requirements of future generations and the expansion of the human population highlight the criticality of research in numerous domains, including agriculture, animal husbandry, technology, and related sciences. In this situation, it is crucial to consider not only the matter of food preparation, but also the provision of nutritious food that is chemically and health-friendly (Safi Yari et al., 2005). Fisheries and their byproducts are vital to human nutrition because they can supply the desired quantity of nutrients that are essential to human health (Piri et al., 1998). Fish and seafood consumption has increased in recent years. The preference for fish and aquatic products over alternative foods, rising income, and population expansion are all contributing factors to the escalating demand for aquatic products (Alasalvar, 2002). Aquatic fish are regarded as a nutritious food source due to their abundance of unsaturated fats, vitamins, minerals, and high-quality proteins (Venugopal l, 2006). Aquatic animals constitute an essential and substantial proportion (16%) of the protein consumed by humans (FAO, 2004).

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https://doi.org/10.22034/aes.2023.340914.1036

Environmental factors render fishery products exceedingly perishable, notwithstanding their considerable nutritional value, because the muscle tissue of fish undergoes numerous changes subsequent to the fishing process. These alterations manifest in a biochemical manner, causing numerous microbial, chemical, and enzymatic decay processes to commence simultaneously (Huss, 1994). Therefore, greater care must be taken in its preparation and maintenance. The approximate nutritional components of fish meat, namely protein, fat, and ash, are the most significant in terms of nutritional value (Ali et al., 2005). Presently, a critical component of the technological, scientific, and economic capabilities of human society is devoted to the study, evaluation, and implementation of projects that offer superior quality and sustainability. Notwithstanding the advancements achieved in the manufacturing and distribution of aquatic products and their byproducts within our nation, the assortment of novel seafood products produced and supplied by processing machines and plants has fallen short of expectations. As a consequence of this circumstance, a multitude of products that can be manufactured and prepared from aquatic organisms are currently unavailable. Presently, scientific and nutritional experts concur that consuming fish as a nutritious food item serves as a preventative measure against numerous illnesses and a viable treatment for certain conditions; thus, those responsible for nutrition and production are concerned with ensuring that it is accessible and included in household food baskets (Safi Yari et al., 2005). The silver carp is referred to as Phytophagous or Tolstolobic, and the market is erroneously acquainted with the brand name for farmed salmon. This fish has a spherical head adorned with minute scales (Adeli, 2005). These are lengthy gill blades. Its diet consists of 1.5 centimeters of algae (Vosoughi and Mostajir, 2002). It initially consumes zooplankton and phytoplankton, but eventually transitions to phytoplankton. It possesses a long, silver body and a lower mouth. Asgari (2009) reports that within our nation, one-row pharyngeal teeth with the formula 4-4 are observed in animals weighing up to 14 kg. Its maximum feeding capacity is 17% of its total weight, which is 20 kg. Spawning occurs in water that is stagnant. The mean quantity of eggs falls within the range of 467 to 542 thousand. Their optimal laying temperature is between 21 and 23 degrees. The incubation period for the 0.7-1 mm in diameter eggs is 1.5 days. Approximately 50 to 85 percent of co-cultivation consists of this fish. Differentiating it from the stubborn carp are its small head, full keel, and lack of connection between the pectoral fin and ventral fin. The public considers this fish to be the finest-farmed due to its delectable, fatty, and skeletal flesh (Adeli, 2005).

Understanding the chemical makeup of fish flesh is currently critical. Because, based on the information at hand (including the proportions of water, protein, fat, starch, minerals, and vitamins in fish meat), the optimal processing method can be determined. Without a doubt, the primary distinction in the chemical composition of fish ought to be associated with the nutrition or food sources they consume. As a result, once the fish have adequate access to food, the relative increase in muscle protein is followed by a rapid increase in fat. On the contrary, when fish encounter food scarcity during spawning or migration, protein, and fat reductions result in gradual alterations to the chemical composition of muscles (Moeini, 2001).

Although the typical shelf life of fish is one to two days, long-term preservation of the product's quality is possible in the refrigerator. According to Vidya et al. (1996), freezing is the most critical method for storing seafood. The quality and shelf life of frozen fish will vary significantly by the end of the storage period due to biological differences, methods of capture, and pre-freezing preparation. Both the final quality and the shelf life of the product in cold storage will undoubtedly fall short of expectations if the initial quality of the fish is unsaturated. Furthermore, the level of attention and precision that is maintained while the product is stored in the refrigerator will significantly influence its ultimate quality. By forming ice crystals, for instance, the concentration of salt and organic compounds in the liquid phase is increased when fish is frozen. Consequently, the destruction of denatured and dehydrated muscle proteins or cell membranes may occur (Aubourge and Medina, 1999).

Nonetheless, the ongoing oxidation and hydrolysis of fish fat result in undesired alterations during the freezing phase, consequently diminishing the product's quality. The best shelf life was determined and the qualitative and chemical index, as well as the measurement and comparison of body chemical compounds, in fresh and vacuumed tissue of phytophagous fish (Hypophthalmichthys molitrix), were assessed in this study.

2. Materials and Methods

2.1. Preparation and processing of phytophagous fish

Under vacuum conditions, fish were frozen at -18 °C for five months using the vacuum packaging method in this study. A total of eighteen phytophagous fish samples, each weighing 1.5 kg, were acquired. When selecting, every effort is made to ensure that the fish have a consistent length and weight range and lack any abnormal appearance signs. The heads and fins were removed from the fish subsequent to their weighing, while their stomachs were emptied. From the captured samples, 100 g fillets were prepared under ultra-hygienic conditions; 33 packages of 100 g fish fillets were produced.

Subsequently, under vacuum conditions and in the presence of ice, these fillets were transferred to the packing center of Kaleh Amol Company for packaging. These fillets were assessed at three different time points throughout the day and at -18 °C for 1, 2, 3, and 4.5 months in three replicates. Initially, the chemical composition of fresh phytophagous fish was assessed on day zero. Subsequently, fillet packages were examined on day zero and throughout months 1, 2, 3, 4, and 5 in order to determine the peroxide and TVB-N spoilage indices.

2.2. Vacuum packaging (VP)

A vacuum pump-equipped EBOR vacuum packing machine was utilized for this objective. Following the establishment of a steady-state condition in the apparatus using the control panel and the provided program, the pressure of the adjusting gas within the package was evacuated for a duration of 1.5 seconds, resulting in the creation of a vacuum within the package. The plastic bag lid was subsequently sealed using heat stitching at a stitching temperature of 250 °C and coated with polyamide.

2.3. Protein measurement

Protein extraction is done in three steps.

2.3.1. Digestion stage

Pour 1 gram of the weighed sample into the digestion tube to begin. Subsequently, incorporate 18 ccs of 95% sulfuric acid, followed by 10 g of potassium sulfate, 0.3 g of titanium dioxide, and 0.3 g of copper sulfate. The digestion step was then initiated by placing the tube in the digester and setting its temperature to between 300 and 100 degrees Celsius for three to five hours.

2.3.2. Distillation step

Subsequently, the digested sample is combined with 20 cc of distilled water and positioned on the left side of the Kajeldal distillation apparatus. On the right side of the Kajeldal distiller, combine 3-5 drops of methylene red as a reagent with 40 cc of 2% boric acid containing a human subject. Activate the device and configure the settings to set the gain to 80 cc; the amount is then appended automatically to the pipe on the left. After a five-minute period of operation, this device will shut down automatically. As the distillation process nears its culmination, nitrogen is introduced into the beaker via the tube.

2.3.3. Titration stage

As a titrant at this stage, 0.1 N sulfuric acid is utilized. The aforementioned substance is introduced into the distillation material to induce a color transformation from yellow to purple. For the percentage of protein in the carcass, multiply the quantity of sulfuric acid consumed by 0.875.

2.4. Measurement of fat and peroxide

A. Determination and measurement of fat by Kinsella et al. (1971), which is expressed in grams per hundred grams of muscle.

B. Determination and measurement of peroxide number (PV) by Malaysian palm oil method (Johnson et al., 1995).

2.5. Chemical experiments

Following the meat grinder's grinding of the sample, 50 g of it was simultaneously mixed with 50 cc chloroform and 100 cc methanol at high speed for three minutes using an electric mixer. Following the addition of an additional 50 cc of chloroform, the mixture was stirred for 30 to 60 seconds. Following this, 50 cc of distilled water was added and the mixture was stirred at high speed for an additional 30 to 60 seconds.

After transferring the mixture to a decanter and observing the oil separate from the meat, the water was positioned at the top of the pan, the meat was positioned in the middle, and the oil containing the solvent was located at the bottom. By agitating a metal or glass rod subtly within the mixture, one can accelerate the oil's accumulation at the container's base. The oil and solvent can be introduced into the glass balloon for the Rotavipur device using a funnel lined with filter paper, albeit with a degree of perseverance. Notably, the glass balloon ought to have undergone prior weighing.

Following this, the balloon is linked to the Rotavipur device. The temperature of the water is adjusted between 50 and 60 °C. Once the vacuum pump is activated, the solvent and oil are separated. The amount of oil that remains in the balloon is determined by reweighing it and calculating the difference in weight from its empty weight. The quantity of fat that was extracted was measured in grams per gram of moist muscle.

2.6. Measurement of peroxide (PV) number

Utilizing 3 grams of extracted oil in a 250 ml laboratory container, this procedure was carried out. In this container, 10 ml of a chloroform and acetic acid solution was added while stirring. Following the addition of one milliliter of saturated potassium iodide solution, the mixture was left in the dark for five minutes. Following this, 20 ml of distilled water was added while stirring the mixture. 1% normal sodium thiosulfate was then used to titrate the mixture until the yellow hue disappeared. Following the addition of 1 ml of a 1.5% starch solution to the mixture, titration was maintained until the absence of the dark blue hue was observed. The control sample was conducted in a comparable manner, with the exception that the mixture did not contain any oil. Using the following formula, the quantity of peroxide in mill equivalents per 1000 g of fat (fat kg / meqO2) was determined:

$$100 (V1\text{-}V2) N/W$$

V1 represents the quantity of sodium thiosulfate, while N denotes the standard deviation in milliliters. The volume of sodium thiosulfate utilized for the control test is denoted by V2. The sample's weight (g) is denoted by W. The normality of sodium thiosulfate intake is denoted by N.

2.7. TVB-N Assessment

In order to quantify TVB-N, 5 g of the sample must be separated, followed by the addition of 1.5 g of copper oxide. We subjected it to five minutes in the distillation machine. The quantity of Caustic soda is currently zero. Along with a few drops of Methyl Red (MR) Reagent, incorporate 40 cc. Titration is conducted using a 0.1 N sodium thiosulfate solution at this juncture. The quantity of TVB-N present in 5 g of sample meat is determined by multiplying the volume of acid consumed by 28 by the color change of the aforementioned solution.

2.8. Humidity measurement

After preparing a watch glass that is both clean and dry, 5 grams of minced fish meat are inserted into the watch glass after being separated with a scalpel. As soon as the watch glass containing the meat is weighed and placed in the oven, preheat it to 100 °C for three to five hours. Once the fish meat has dried for the designated duration, each container containing the samples is assessed for weight. Subsequently, the secondary weight is subtracted from the initial weight. By performing this operation, the moisture content of 5 grams of fish meat is determined. The moisture content of the fish meat can be determined by dividing the weight of the moisture obtained by the weight of the raw sample, which is 5 grams.

2.9. Ash Assessment

The crucible is initially weighed, and the resultant value is documented. Following this, 2 grams of fish mince are weighed and added to the crucible. Following the completion of the initial smoking or burning process, the specimen is subsequently heated in an electric oven. The sample is heated to 550 degrees Celsius for two to five hours, or until it turns gray. Following the completion of the crucible's cooling process and the completion of the machine time, the sample was re-weighed. The value derived from the empty crucible's weight was subtracted, thereby yielding the quantity of ash. The quantity of ash present in the fish meat can be determined by dividing the weight of the resulting ash by the weight of the raw sample, which is 2 grams.

2.10. Statistical analysis

SPSS16 and Excel 2007 were utilized for the generation of graphs and statistical analyses, respectively. Furthermore, the acquired data are subjected to statistical analysis using factorial method 2.6 (GLM) and Duncan's mean comparison test at the 0.05 significance level.

3. Results

3.1. Fat tests

Table 1 presents the fat content of phytophagous fish fillets that were stored at -18 °C under standard packing conditions on various days of the experiment. The mean fat contents of phytophagous fish fillets, which were stored at -18 °C for various days of the experiment, were as follows: 3.90 g, 3.54 g, 3.15 g, 2.80 g, 2.26 g, and 2.00 g. Table 2 presents the fat content of phytophagous fish fillets that were vacuum-packed and stored at -18 °C on various days throughout the experiment. On various days of the experiment, the mean fat content of rainbow trout fillets stored at -18 °C was as follows: 3.91 grams, 3.71 grams, 3.47 grams, 3.12 grams, 2.88 grams, and 2.79 grams, respectively.

Table 1. Measurement of fat content in phytophage fish fillets kept under usual packaging at –18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average	3.903	3.545	3.151	2.802	2.260	2.008
Standard deviation	0.044	0.131	0.088	0.181	0.235	0.097
Minimum	3.869	3.415	3.051	2.615	2.219	1.899
Maximum	3.954	3.677	3.218	2.978	2.514	2.040
Number of repetitions	3	3	3	3	3	3

Table 2. The amount of fat measured in phytophage fish fillets stored in vacuum packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (g)	3.918	3.713	3.474	3.120	2.888	2.79
Standard deviation	0.047	0.062	0.109	0.145	0.121	0.229
Minimum (g)	3.864	3.641	3.389	2.999	2.745	2.541
Maximum (g)	3.951	3.750	3.598	3.282	2.969	2.986
Number of repetitions	3	3	3	3	3	3

Table 3. Comparison of the average fat content of phytophagous fish fillets in different treatments

Package type	Day	Number	Average cStandard daviation
Vacuum	0	3	3.9183± 0.04737 a
	30	3	3.7130 ±0.06236 ab
	60	3	3.4743 ±0.010965 b
	90	3	3.1207± 0.14561 cd
	120	3	2.8880± 0.12421 de
	150	3	2.8880± 0.12421 de
No Vacuum	0	3	3.9030 ± 0.04498 a
	30	3	3.5457± 0.13100 b
	60	3	3.1510 ±0.08826 c
	90	3	2.8023± 0.18178 e
	120	3	2.2603 ±0.23573 f
	150	3	2.0083± 0.09744 g

* Similar letters do not differ significantly (P = 0.05)

Using Duncan's multiple amplitude method, it was possible to ascertain which day of the experiment exhibited a significantly different fat content compared to the others. A statistical analysis of means revealed that the treatments assigned to distinct groups differed significantly from one another, whereas the treatments assigned to the same group did not differ significantly. Table 4 presents the outcomes of the average fat comparison test conducted at -18 °C. The data presented in Tables 3 and 4 indicates that there are notable variations in fat quantities across different days. Furthermore, as shown in the tables, the positive impact of packaging on these values is quite apparent

Figure 1 illustrates a comparison of the mean fat contents of phytophagous fish fillets stored at -18 °C. The process of reducing the fat content of vacuum-packed fillets and conventionally packaged fillets is illustrated in this diagram. Furthermore, it was observed that the fat content of phytophagous fish fillets diminishes with time in both packaging types. However, with the exception of the initial day of the experiment, the vacuum-packed samples exhibited a higher fat content than the control samples on all other days. As time progresses, the rate of fat reduction in samples packaged conventionally is greater than that of samples packed vacuum-style.

Table 5 presents the protein content of phytophagous fish fillets that were stored at -18 °C under standard packaging conditions on various days throughout the experiment. On various days of the experiment, the mean protein content of phytophage fish fillets stored at -18 °C was as follows: 20.1, 18.4, 16.27, 15.14, 14.14, and 13.87%, respectively. Additionally, the protein content of phytophagous fish fillets preserved in vacuum packaging is illustrated in Figure 2. On various days of the experiment, the mean protein content of phytophage fish fillets stored at -18 °C was calculated to be 20.76, 20.03, 18.59, 17.69, 16.84, and 15.47%, respectively.

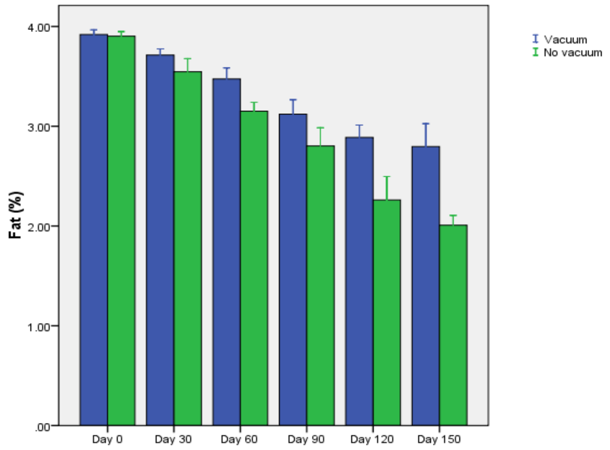

Figure 1. Comparison of the amount of fat in phytophagous fish fillets kept at -18 degrees on different days

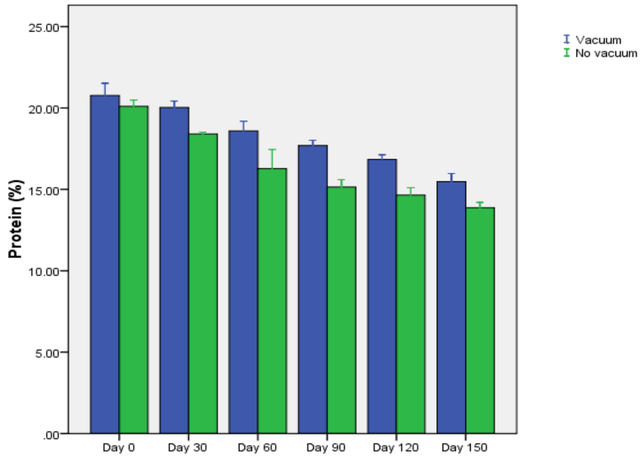

Figure 2. Amount of protein measured in phytophagous fish fillets stored in vacuum packaging

Table 4. The result of the comparison test of the average fat content of phytophagous fish fillets kept at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	2.008						
120 Normal	3		2.260					
150 Vacum	3			2.796				
90 Normal	3			2.802				
120 Vacum	3			2.888	2.888			
90 Vacum	3				3.120	3.120		
60 Normal	3					3.151		
60 Vacum	3						3.474	
30 Normal	3						3.545	
30 Vacum	3						3.713	3.713
0 Normal	3							3.903
0 Vacum	3							3.918

The results of a substantial analysis of protein content across various days and packaging conditions revealed that the treatment means of the various groups differed significantly. There is minimal variation among the means comprising a group with respect to the degree of significance. (0.05 P-value) (Table 6). Significant variations in protein concentrations across different days are evident in Tables 6 and 7, suggesting that vacuum packing has contributed positively to these fluctuations. Figure 2 further illustrates the trend of this disparity. As time has passed, the protein content of phytophagous fish fillets has decreased, as indicated by data on both types of packaging. Additionally, with the exception of the initial day of the experiment, the vacuum-packed samples contain a greater quantity of protein than the control samples on all other days. The rate of protein reduction in conventionally packaged samples is greater than that in vacuum-packed samples over time.

Table 5. The amount of protein measured in phytophagous fish fillets kept under conventional packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	20.103	18.408	16.274	15.146	14.646	13.877
Standard deviation	0.381	0.104	1.77	0.452	0.460	0.319
Minimum (%)	19.718	18.475	13.312	14.814	14.125	13.540
Maximum (%)	20.481	18.175	17.587	15.622	15.001	14.175
Number of repetitions	3	3	3	3	3	3

Table 6. The amount of protein measured in fish fillets stored under vacuum packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	20.764	20.033	18.592	17.692	16.842	15.475
Standard deviation	0.753	0.395	0.596	0.333	0.284	0.499
Minimum (%)	19.955	19.712	17.914	17.325	16.514	15.008
Maximum (%)	21.446	20.475	19.037	17.937	17.011	16.001
Number of repetitions	3	3	3	3	3	3

Table 7. Comparison of mean values of phytophagous fish fillet protein in different treatments

Package type	Day	Number	Average ±Standard daviation
Vacuum	0	3	20.7643 ±0.75365 [a]
	30	3	20.0330 ± 0.39563 [a]
	60	3	18.5920 ± 0.59666 [b]
	90	3	17.6920 ± 0.32372 [be]
	120	3	16.8420 ± 0.28410 [cd]
	150	3	15.4757 ± 0.49901 [ef]
No Vacuum	0	3	20.1037 ± 0.38157 [a]
	30	3	18.4080 ± 0.10499 [b]
	60	3	16.27476 ±1.17712 [de]
	90	3	15.460 ± 0.45295 [f]
	120	3	14.6460 ± 0.46099 [fg]
	150	3	13.8773 ± 0.31329 [g]

* Similar letters do not differ significantly (P <0.05)

Table 8. The result of the comparison test of the average phytophagous fish fillet protein stored at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	13.877						
120 Normal	3	14.646	14.646					
150Vacum	3		15.146					
90 Normal	3		15.475	15.475				
120Vacum	3			16.276	16.274			
90 Vacum	3				16.842	16.842		
60Normal	3					17.692	17.692	
60Vacum	3						18.408	
30Normal	3						18.592	
30Vacum	3							20.033
0Normal	3							20.103
0Vacum	3							20.764

Tables 9 and 10 detail the quantities of ash that were quantified in phytophagous fish fillets that were vacuum-sealed and stored at -18 °C under standard conditions on various days of the experiment. An examination of the mean ash values across various treatments (Table 11) revealed that there is a statistically significant difference in the means of the treatments in the different groups. A further observation was that there was no significant difference in the means of the groups within a given group (P = 0.05).

Table 9. The amount of ash measured in fish fillets kept under normal packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average	2.366	1.683	1.433	1.150	0.816	0.041
Standard deviation	0.057	0.028	0.028	0.132	0.104	0.076
Minimum	2.21	1.65	1.40	1	0.7	0.45
Maximum	2.4	1.7	1.45	1.25	0.9	0.5
Number of repetitions	3	3	3	3	3	3

Table 10. Measure ash content in phytophage fish fillets stored in vacuum packing conditions at -18 ° C on different experiment days

Day	0	30	60	90	120	150
Average (%)	2.266	1.966	1.733	1.300	0.950	0.583
Standard deviation	0.189	0.076	0.246	0.132	1.00	0.104
Minimum (%)	2.05	2.05	1.45	1.2	0.85	0.5
Maximum (%)	2.4	1.9	1.9	1.45	1.05	0.7
Number of repetitions	3	3	3	3	3	3

As indicated by the data presented in Tables 11 and 12, as well as the explanations provided regarding the method for determining whether a particular factor's influence on the number of factor changes (ash) is significant and evident, the quantity of ash has decreased considerably. As indicated by data from both packaging types, the ash content of fillets of phytophagous fish diminishes with time. With the exception of the initial day of the experiment, the vacuum-packed specimens exhibit a greater concentration of ash on all other days compared to the conventional specimens. Ash reduction in vacuum-packed samples is slower than the rate of ash reduction in conventional packing ash over time.

3.3. Humidity

Tables 13 and 14 detail the moisture content of phytophagous fish fillets that were vacuum-sealed and routinely packaged at -18 °C on various days of the experiment. An examination of the mean humidity values across various treatments (Tables 15 and 16) reveals a statistically significant difference in the means of treatments within distinct groups. A further observation was that there was no significant difference in the means of the groups within a given group (P = 0.05). The moisture content of phytophagous fish fillets diminishes with time in both packaging types. With the exception of the initial day of the experiment, the humidity level of the vacuum-packed specimens is consistently higher than that of the conventional specimens on all other days. The rate of moisture depletion is greater for samples packaged vacuum-packed than for those packaged in conventional packaging over time.

Table 11. Comparison of mean values of phytophagous fish fillet ash in different treatments

Package type	Day	Number	Average ±Standard daviation
Vacuum	0	3	2.2667 ± 0.18930 [a]
	30	3	1.9667 ± 0.07638 [b]
	60	3	1.7333 ± 0.24664 [c]
	90	3	1.3000 ± 0.13229 [de]
	120	3	0.9500 ± 0.1000 [fg]
	150	3	0.5833 ± 0.10408 [h]
No Vacuum	0	3	1.3000 ± 0.13229 [de]
	30	3	1.6833 ± 0.02887 [c]
	60	3	1.4333 ± 0.02887 [d]
	90	3	1.1500 ± 0.13229 [ef]
	120	3	0.8167 ± 0.10407 [g]
	150	3	0.4167 ± 0.07638 [h]

* Similar letters do not differ significantly (P <0.05).

Table 12. The result of the comparison test of the average phytophagous fish fillet ash kept at –18° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
150 Normal	3	0.416							
120 Normal	3	0.583							
150Vacum	3		0.816						
90 Normal	3		0.950	0.950					
120Vacum	3			1.150	1.150				
90 Vacum	3			1.300		1.300			
60Normal	3					1.433			
60Vacum	3						1.683		
30Normal	3						1.733		
30Vacum	3							1.966	
0Normal	3								2.266
0Vacum	3								2.366

Table 13. The amount of moisture measured in phytophagous fish fillets kept under normal packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	77.686	66.333	58.640	54.993	52.306	46.233
Standard deviation	2.200	1.990	2.053	2.518	6.984	2.366
Minimum (%)	75.48	46.36	56.28	52.72	45.08	43.72
Maximum (%)	79.88	68.34	60.02	57.7	52.82	48.42
Number of repetitions	3	3	3	3	3	3

Table 14- The amount of moisture measured in phytophagous fish fillets stored in vacuum packing conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	77.593	69.460	62.993	58.833	56.753	51.353
Standard deviation	0.650	2.862	1.678	2.198	2.078	2.839
Minimum (%)	76.94	66.88	61.12	56.30	54.90	48.26
Maximum (%)	78.24	72.54	64.36	60.24	59.00	51.96
Number of repetitions	3	3	3	3	3	3

3.4. TVB-N Assessment Results

The quantities of TVB-N ascertained in phytophagous fish fillets that were preserved under standard vacuum conditions at -18 °C on various days of the investigation are detailed in Tables 17 and 18.

A statistically significant distinction was observed in the mean values of TVB-N across various treatments, as indicated by Tables 19 and 20. These treatments were categorized into distinct groups. Over time, the quantity of TVB-N phytophagous fish fillets increased in both packaging types. On all other days, with the exception of the initial day of testing, vacuum-packed samples contain a reduced quantity of TVB-N compared to standard TVB-N. The rate of increase in TVB-N for samples packaged conventionally is greater than that for samples packed vacuum-packed

Table 15. Comparison of average moisture values of phytophagous fish fillets in different treatments

Package type	Day	Number	Average ±Standard daviation
	0	3	77.5933± 0.65003 ^{a}
	30	3	69.4600± 2.8694 ^{b}
vacuum	60	3	62.933± 1.67837 ^{cd}
	90	3	58.8333± 2.19839 ^{de}
	120	3	56.7533± 2.07811 ^{ef}
	150	3	51.3533± 2.83904 ^{g}
	0	3	77.6867± 2.2003 ^{a}
	30	3	66.3333± 1.99021 ^{bc}
	60	3	58.6400± 2.05358 ^{de}
No vacuum	90	3	54.9933± 2.5181 ^{efg}
	120	3	52.3067± 6.98416 ^{fg}
	150	3	46.2333± 2.36697 ^{h}

* Similar letters do not differ significantly (P<0.05)

Table 16. The result of the comparison test of the average moisture content of phytophagous fish fillets kept at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
150 Normal	3	46.233							
120 Normal	3		51.353						
150Vacum	3		52.306	52.306					
90 Normal	3		54.993	54.993	54.993				
120Vacum	3			56.753	56.753				
90 Vacum	3				58.640	58.640			
60Normal	3				58.833	58.833			
60Vacum	3					62.993	62.993		
30Normal	3						66.333	66.333	
30Vacum	3							69.460	
0Normal	3								77.593
0Vacum	3								77.686

Table 17. The amount of TVB-N measured in phytophagous fish fillets kept under normal packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	6.533	26.133	27.066	30.800	33.600	38.266
Standard deviation	1.616	1.616	4.277	2.800	2.800	1.616
Minimum (%)	5.60	25.20	22.40	28.00	30.80	39.20
Maximum (%)	8.40	28.00	30.80	33.60	36.40	36.40
Number of repetitions	3	3	3	3	3	3

Table 18- The amount of TVB-N measured in phytophagous fish fillets kept under normal packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	6.533	13.066	15.866	14.00	15.866	18.666
Standard deviation	1.616	1.616	1.616	28.00	1.616	1.616
Minimum (%)	5.60	11.20	14.00	11.20	14.00	16.8
Maximum (%)	19.60	16.80	16.80	16.80	14.00	8.40
Number of repetitions	3	3	3	3	3	3

Table 19. Comparison of average TVB-N values of phytophagous fish fillets

Package type	Day	Number	Average ±Standard daviation
	0	3	6.5333±1.61658 ^{g}
	30	3	13.0667± 1.61658 ^{f}
vacuum	60	3	15.8667± 1.61658 ^{ef}
	90	3	14.000± 2.8000 ^{f}
	120	3	15.8667± 1.61658 ^{ef}
	150	3	18.6667± 1.61658 ^{e}
	0	3	6.5333±1.61658 ^{g}
	30	3	26.1333± 1.61658 ^{d}
	60	3	27.0667± 4.27707 ^{cd}
No vacuum	90	3	30.800± 2.8000 ^{bc}
	120	3	33.6000± 2.8000 ^{b}
	150	3	38.2667±1.61658 ^{a}

* Similar letters do not differ significantly (P <0.05).

Table 20. The result of the comparison test of the average TVB-N of phytophagous fish fillets kept at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	6.533						
120 Normal	3	6.533						
150Vacum	3		13.066					
90 Normal	3		14.00					
120Vacum	3		15.566	15.566				
90 Vacum	3		15.866	15.866				
60Normal	3			18.666				
60Vacum	3				26.133			
30Normal	3				27.066	27.066		
30Vacum	3					30.800	30.800	
0Normal	3						33.600	
0Vacum	3							38.266

3.5. Peroxide assay results

The quantities of peroxide ascertained in phytophagous fish fillets that were vacuum-sealed and packaged as usual at -18 °C on various days of the experiment are detailed in Tables 21 and 22. An examination of the average peroxide levels across various treatments revealed a statistically significant difference in the means of treatments between groups (Table 23). The peroxide content of phytophagous fish fillets increases with time in both packaging types. With the exception of the initial day of testing, the peroxide concentration in the vacuum-packed samples is comparatively lower than that of the standard samples on all other days. The rate of increase in peroxide concentration in conventional packaging is found to be greater than that in vacuum packaging over time (Table 24).

Table 21. Measurement of peroxide measured in phytophagous fish fillets kept under usual packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	1.966	33.330	28.886	39.996	38.883	47.773
Standard deviation	0.611	3.330	1.928	3.335	3.850	1.928
Minimum (%)	1.66	30.00	26.66	36.66	36.66	46.66
Maximum (%)	2.06	36.66	30.00	40.00	43.33	50.00
Number of repetitions	3	3	3	3	3	3

Table 22. Measurement of peroxide measured in phytophagous fish fillets stored in vacuum packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	1.693	33.220	24.440	34.440	32.220	44.440
Standard deviation	0.542	3.845	1.922	1.922	3.845	1.922
Minimum (%)	1.45	30.00	23.33	33.33	30.00	43.33
Maximum (%)	1.86	36.66	26.66	36.66	36.66	46.66
Number of repetitions	3	3	3	3	3	3

Table 23. Comparison of average values of phytophage fish fillet peroxide in different treatments

Package type	Day	Number	Average ±Standard daviation
Vacuum	0	3	1.6933± 0.54271 ^{h}
	30	3	32.2200± 3.84515 ^{ef}
	60	3	24.4400± 1.92258 ^{g}
	90	3	34.4400± 1.92258 ^{de}
	120	3	32.2200± 3.84515 ^{ef}
	150	3	44.4400± 1.92258 ^{ab}
No Vacuum	0	3	1.9667± 0.61199 ^{h}
	30	3	33.3300± 3.3300 ^{ef}
	60	3	28.8867± 3.33000 ^{fg}
	90	3	39.3367± 3.33500 ^{c}
	120	3	38.8333± 3.85093 ^{cd}
	150	3	47.7733± 1.92835 ^{a}

* Similar letters do not differ significantly (P <0.05).

Table 24. The result of the comparison test of the average phytophage fish fillet peroxide stored at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	group5	Group 6	Group 7	Repetitions
150 Normal	3	1.693							
120 Normal	3	1.966							
150Vacum	3		24.440						
90 Normal	3		28.886	28.886					
120Vacum	3			32.220	32.220				
90 Vacum	3			32.220	32.220				
60Normal	3			33.330	33.330				
60Vacum	3				34.440	34.440			
30Normal	3					38.883	38.883		
30Vacum	3						39.996	39.996	
0Normal	3							44.440	44.440
0Vacum	3								47.773

4. Discussion

Different results were obtained when body chemical compounds, peroxide, and TVB-N in phytophage fish fillets were subjected to vacuum packaging for five months or 150 days at freezing temperature (-18 °C). We discuss the results of various studies on other compounds and species in order to facilitate comparisons with the present study's findings.

All the variables assessed in this investigation exhibited substantial variations, with a discernible contrast in the rate of change between the two packaging types (refer to the results). Significantly slower trend changes were observed in the packing factors of samples packed under vacuum conditions compared to the packing factors under standard operating conditions. Furthermore, the majority of the assessed factors were determined for samples that were vacuum-packed during the higher months, whereas the initial month's values were derived from samples that were packed according to standard procedure.

The aforementioned data indicates that there was a decline in the outcomes of chemical compound measurements, including those for fat, protein, moisture, and ash, throughout the specified time period. It is worth mentioning that vacuum packaging played a substantial and beneficial role in decelerating the progression of these alterations, while also ensuring the samples remained intact in terms of quality for an extended duration.

Storage time is dependent on variables including freezing status, type of fish, and storage conditions, as demonstrated by Ben et al. (2009). Additionally, these variables have a substantial impact on the chemical composition of the body and spoilage samples. The aforementioned findings align with the results reported in this study. They indicate that the quality of the product degrades as its shelf life increases, and that all other factors undergo substantial changes. Additionally, it is worth noting that under vacuum conditions, the trend of these changes will be diminished. This article alludes to the identical freezing storage method utilized in the aforementioned research; consequently, the outcomes of the two investigations are congruent and aligned.

According to a study conducted by Jorkesh in 2004 regarding the shelf life of Caspian white fish (Rutilus frisii kutum) packaged under vacuum conditions at 4 °C, all indicators exhibited viability for a maximum of nine days; thereafter, they progressively deviated from their intended standards. Furthermore, during the course of this study's analyses, all the measured indicators deviated from their standard ranges gradually. However, this deviation was more pronounced in vacuum packages and corresponded to the outcome reported by Jorkesh (2004).

The effects of temperature, duration, and freezing storage on fat changes in Clupeonella engrauliformis were investigated by Rezaei (2003). The findings of his investigation revealed that the values of spoilage and peroxide characteristics are significantly influenced by temperature and storage time in the frozen state. Vacuum packaging and freezing significantly slowed the ascent of these characteristics in the current investigation; thus, the quantity of peroxide determined in the second month of vacuum-packed samples was equivalent to the quantity of peroxide determined in the first month of closed samples classified under standard operating conditions. These results indicate that vacuum packaging is more effective, and the sample exhibits a slight oscillation pattern that persists for a longer period of time.

The vacuum packaging technique was also applicable to peroxide and TVB-N. As indicated by the results, there was an increase in the values of these two factors. Once more, vacuum packaging played a significant role in retarding the rate of this increase, allowing the samples that were sealed using this method to reach their unauthorized limit for a longer duration. Consequently, they can be utilized for a longer period of time.

In his study, Falaki Moghadam (2012) examined the impact of vacuum packaging on fat peroxide alterations in fillets of Caspian white fish stored at temperatures of -4 and -18 degrees Celsius. The findings of this research demonstrated that the quantity of peroxide, as measured at temperatures of -4 and -18 degrees Celsius, exhibited a significant decrease over time when compared to the fresh fillet of Caspian whitefish. On day zero, the peroxide content was found to be zero. Undoubtedly, what was noteworthy was the favorable impact that vacuum packaging had on the augmentation of said factors. As a result, at -18 degrees Celsius, the samples that were vacuum-packed increased at a slower rate than the control samples and required a more extended period of time to reach the critical level of these attributes; this allowed for an extended storage period. Consistent with the findings of the present study are these results. The current investigation revealed a marginal increase in the quantity of this index in the vacuum, resulting in a rise in its value from 6.53 to 18.66. Consequently, the current study reveals a discernible upward trajectory in this quantity; under typical packaging and conditions, this growth has escalated from 6.53 to 38.26.

The current investigation revealed that the range of changes in vacuum packaging during the 150-day duration was significantly reduced compared to the range of changes observed under typical circumstances. This amounted to $26.13 within a single month, which would normally transpire. Additionally, the current investigation demonstrates the impact of vacuum packaging on said value.

Stamatis et al. (2007) conducted a quality assessment of Scombercolias japonicas fish using two methods: packaging in a vacuum at temperatures of 6 and 3 °C and exposure to a modified atmosphere (MAP). The findings indicated that samples subjected to the altered atmosphere exhibited a more rapid deterioration in quality when compared to samples maintained under vacuum conditions. The aforementioned study yielded findings pertaining to the impact of vacuum packaging on chemical compounds.They showed that the rate of change in samples packed in vacuum conditions was less than that of packaged samples under normal conditions, that the samples with longer shelf life have better quality, and that vacuum packaging has a positive role in the shelf life of samples. The findings presented here align with those documented in the study conducted by Stamatis et al. (2007).

5. Conclusion and final remarks

As indicated by the findings of this study and comparisons with other researchers, the storage duration of aquatic products is significantly impacted by the implementation of non-oxygen or vacuum conditions. In contrast, samples preserved under vacuum conditions exhibit reduced fluctuations across all factors when compared to samples preserved under standard conditions with oxygen present. Consequently, the quality of the products remains consistent for a more prolonged duration.

Given the findings of the research, the following recommendations may be put forth:

1. Given that augmenting the population, diversifying consumption patterns, and increasing the proportion of aquatic protein will effectively increase demand and consumption, it is imperative to prioritize the processing of various farmed and marine fish species.

2. Following production, various products derived from different fish (particularly phytophagous fish) should be stored at a low temperature and in suitable packaging (vacuum, vacuum packaging) to preserve their quality, value, and marketability. It is possible to produce a product of superior quality and durability by employing these procedures.

3. An investigation should be conducted into the impact of various packaging materials on alterations in body chemical compounds, peroxide, and TVB-N.

4. Prolonged exposure to environmental conditions for the storage of aquatic products should be avoided, and special provisions should be implemented to ensure the safekeeping of fish for extended periods of time.

Additionally, it is recommended that investigations concerning fluctuations in the chemical composition of the body, peroxide levels, and TVB-N be conducted at more frequent time intervals.

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Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir

Effect of endophytic fungus *Serendipita indica* and vermicompost water extraction plant growth and development of *Stevia rebaudiana* Bertoni

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ARTICLE INFO

Article history:
Received: 12 December 2022
Accepted: 25 January 2023
Available online: 30 January 2023

Keywords:
Inoculation
In vitro culture
Stevia rebaudiana
Steviol *Glycosides*

ABSTRACT

Because it generates the zero-calorie sweeteners steviol glycosides, *Stevia rebaudiana* is a valuable medicinal plant that offers herbal care for diabetic patients. The effects of applying vermicompost water extract and inoculating *S. rebaudiana* with *Serendipita indica* on the growth of the plant were investigated in this study using an in vitro experimental design that was completely randomized. The application of vermicompost water extract and inoculation with S. indica had a significant impact on the majority of the plant's growth parameters, as indicated by the results. *S. rebaudiana* inoculated with S. indica exhibited the greatest root length, chlorophyll content, and dry weight of aerial parts. Additionally, when inoculated with vermicompost water extract, *S. rebaudiana* demonstrated the maximum chlorophyll content and the ratio of fresh weight of roots to fresh weight of aerial parts. Large-scale application of the findings from this study is possible in the tissue culture of medicinal plants. Conversely, in sustainable agriculture, the utilization of water extract vermicompost and S. indica fungus may lead to enhanced vegetative products and, consequently, secondary metabolites in medicinal plants.

Highlights

- The study employed a completely randomized in vitro experimental design to investigate the effects of vermicompost water extract and inoculation with *Serendipita indica*.
- Results showed a significant improvement in most growth parameters of *S. rebaudiana* when treated with vermicompost water extract and inoculated with S. indica.
- Plants inoculated with S. indica had increased root length, higher chlorophyll content, and greater dry weight of aerial parts.
- The maximum chlorophyll content and an improved ratio of fresh weight of roots to aerial parts were observed in plants treated with vermicompost water extract.
- The study suggests that these treatments could be scaled for large-scale tissue culture of medicinal plants and could enhance vegetative growth and secondary metabolites in sustainable agriculture.

1. Introduction

Stevia rebaudiana Bertoni is a perennial sweet herb that belongs to the Asteraceae family. It produces zero- calories of diterpene glycoside in its leaves, which can be used as a substitute for sucrose around the world (Kalpana et al., 2009). It has been demonstrated that the compounds obtained from the leaves of *S. rebaudiana* have no side effects on humans and can be used by both healthy people and diabetic patients (Kalpana et al., 2009). The major limiting factor for large -scale cultivation of *S. rebaudiana* is its low seed germination percentage. Nowadays, the use of *in vitro* culture techniques is an appropriate method for increasing plant production in a shorter timeframe (Debnath, 2008). The micropropagation method can mediate rapid multiplication of plants, and it may overcome many of the limitations associated with conventional

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https://doi.org/10.22034/jelsa.2024.381189.1050

methods, such as the instability of the produced plantlets through stem cutting plantation (Kumar et al., 2014).

Serendipita indica is an endophytic fungus that belongs to the order Sebacinales (Varma et al., 2014). The trait of being axenically cultivable with a wide range of hosts opened a new vision of a symbiotic relationship between *S. indica* and plants. *S. indica* can be cultivated *in vitro* under controlled conditions for studying its potential effects on the plant's morphogenesis and on the production of secondary metabolites. The biotechnological application of *S. indica,* by using cultivable mycelium, stimulated the host plant's growth (Kari Dolatabadi et al., 2011b). In a study, inoculation of barley roots (*Hordeum vulgare* L.) with *S. indica* enhanced biomass (Deshmukh et al., 2006). Furthermore, it has been demonstrated that inoculation of *Coleus forskohlii*'s roots with *S. indica* induced more aerial biomass, chlorophyll contents, and phosphorus absorption (Das et al., 2012). Organic fertilizers, especially vermicompost, can improve soil properties and essential oil yield (Heidarzadeh et al., 2021). Vermicompost is a process of degradation and detoxification of organic matter through the interactions of earthworms with other microorganisms, which eventually becomes the required product for agricultural purposes (Sartaj et al., 2019). As an organic fertilizer, it contains nutrients in the available forms and has some characteristics such as higher porosity, water holding capacity, aeration, and drainage, which may affect plants's growth (Arancon et al., 2004; Nadi et al., 2011).

Considering the medicinal importance of *S. rebaudiana*, the present study was designed to investigate the effects of *S. indica* and vermicompost water extracts on the morphological traits of *S. rebaudiana,* which were not well investigated previously, especially in *in vitro* culture.

2. Materials and methods

The effects of vermicompost water extract and *Serendipita indica* on *the* morphological traits of *Stevia rebaudiana* were investigated in an *in vitro* culture experiment in a completely randomized design with three replications. The experiment was conducted at the Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, in 2018.

2.1. Plant material

Tissue cultures of plants were bought from the Sari Khazar Abad institute, Mazandaran, Iran, and placed in a growth chamber at 25 ºC under cool white fluorescent lamps in a 16L/8D photoperiod regime.

2.2. Fungal culture (*Serendipita indica*)

For preparing the fungal culture, 48 g of agar malt extract was brought to a volume of 1 liter, autoclaved at 121 ºC for 20 minutes, and poured into the sterile Petri Dishes. After 48 h, the dishes were inoculated with 5mm mycelia disks of *S. indica* from 10 days old agar under quite sterile conditions. Plates were incubated at 25±1 ºC for 10 days for the multiplying and re-growing of spores, so dense mycelia suspensions were generated. Then, the fungal culture was used in the *in vitro* culture experiment.

2.3. Vermicompost water extract preparation

Vermicompost was prepared by Professor Mohammadi Goltapeh on the campus of Tarbiat Modares University from poultry manure and *Eisenia fetida,* the earthworm species. In order to prepare the vermicompost water extract, 1 kg of vermicompost was dissolved in 1 liter of water and kept in stable condition for 24 hours. Then, the solution was filtered with muslin fabric.

2.4. *In vitro* plant inoculation

In this study, three different treatments, including vermicompost water extract, *S. indica*, and control, were used. The vermicompost water extract was injected near the plants' roots using a syringe. For the treatments of *S. indica,* 30 days -old established micropropagated plants in transparent glass culture were inoculated at the center with a 5 mm -diameter mycelia disc of 10 days -old *S. indica* culture. An effort was made to keep the root system in direct contact with the inoculum materials. The control plants were not treated. Sixty days after inoculation, the micropropagated plants grown in glass bottles were removed from the bed and analyzed for the growth parameters of *S. rebaudiana.*

2.5. Statistical analysis

Data analysis was performed by SAS 9.4 software. The Least Significant Differences (LSD) test was applied for the means comparison at $P< 0.05$. Two orthogonal contrasts were determined, including contrast 1: control versus treatments (*S. indica* inoculation and vermicompost water extract application) and contrast 2: *S. indica* inoculation versus vermicompost water extract application.

3. Results

The influence of vermicompost water extract application and *S. indica* inoculation on the morphology of the micropropagated plants in the *in vitro* cultures were assessed 60 days after inoculation. Analysis of variance showed that except plant height, other traits: root length, chlorophyll content (a+b), aerial parts' fresh weight (APFW), aerial parts' dry weight (APDW), root fresh weight (RFW), root dry weight (RDW), and the ratio of root fresh weight/aerial parts' fresh weight (RFW/APFW) were significantly affected by the tested treatments (Table 1).

The orthogonal contrasts showed that all indices except plant height, RFW, and RDW were significantly different in control versus treatments (*S. indica* inoculation and vermicompost water extract application) (Table 1). In addition, comparison between *S. indica* and vermicompost water extract treatments showed significant differences in all indices except plant height and the ratio of RFW/APFW (Table 1).

Based on the results, the highest root length (7.10 cm) was recorded in plants inoculated with *S. indica*. The chlorophyll contents were highest in both vermicompost water extract and *S. indica* treatments and lowest in control plants (Table 2). The aerial parts fresh weight was highest in control. Furthermore, the highest aerial parts' dry weights was obtained in *S.indica* treatment, The root fresh and dry weights ranged from 1.27 to 1.69 g and 0.07 to 0.11 g, respectively on different treatments, which the highest values of these parameters were obtained in control plants (Table 2).The results of this study showed that application of vermicompost water extract induced the highest ratio of RFW/APFW (Table 2).

Table 1. Analysis of variance and orthogonal contrasts for plant growth parameters in *Stevia rebaudiana* in the in vitro culture experiment

S.O.V	df	Mean square							
		Plant height (cm)	Root length (cm)	Chlorophyll content (a+b) (μg.ml^{-1})	APFW*** (g)	APDW*** (g)	RFW***(g)	RDW*** (g)	RFW / APFW (g)
Treatments	2	3.29 ^{ns}	4.82**	74.39**	1.43**	0.09**	0.13 **	0.0015 **	0.026 **
Contrast 1	1	1.32 ^{ns}	5.15 **	27.07 **	2.03 **	0.016 **	0.004 ^{ns}	0.00002 ^{ns}	0.05 **
Contrast 2	1	5.26 ^{ns}	4.49 **	121.72 **	0.84 **	0.16 **	0.26 **	0.002 **	0.001 ^{ns}
Error	6	1.09	0.055	1.76	0.01	0.0003	0.004	0.00003	0.0004
Total	8								
CV		4.88	4.14	4.90	4.56	3.96	4.70	6.40	4.47

^{ns}: non significance,* : significant at *P*< 0.05 and,** : significant at *P*< 0.01
***APFW: Aerial Parts' Fresh Weight; APDW: Aerial Parts' Dry Weight; RFW: Root Fresh Weight and RDW: Root Dry Weight

Table 2. Growth parameters (mean ± SE) of *Stevia rebaudiana* treated with vermicompost water extract and *Serendipita indica*

Treatments	Plant height (cm)	Root length (cm)	Chlorophyll content (a+b) (μg.ml^{-1})	APFW* (g)	APDW* (g)	RFW*(g)	RDW* (g)	RFW / APFW (g)
Vermicompost water extract	22.00 ± 0.76^{a}	4.63 ± 0.07^{c}	29.50 ± 0.88^{a}	2.39 ± 0.03^{c}	0.41 ± 0.008^{b}	1.43 ± 0.03 ^{b}	0.09 ± 0.005^{b}	0.59 ± 0.02^{a}
S. indica	20.25 ± 0.43^{a}	7.10 ± 0.06^{a}	30.32 ± 0.30^{a}	3.02 ± 0.09^{b}	0.66 ± 0.01^{a}	1.27 ± 0.03 ^{c}	0.07 ± 0.0008^{c}	0.41 ± 0.002^{b}
Control	22.12 ± 0.57^{a}	5.37 ± 0.21^{b}	21.31 ± 0.94^{b}	3.77 ± 0.09^{a}	0.33 ± 0.008^{c}	1.69 ± 0.04 ^{a}	0.11 ± 0.003^{a}	0.44 ± 0.004^{b}

Means followed by different letters in each column are significantly different (*P*< 0.05, LSD Test).
*APFW: Aerial Parts Fresh Weight; APDW: Aerial Parts Dry Weight; RFW: Root Fresh Weight and RDW: Root Dry Weight

4. Discussion

In the current study, the highest root length, chlorophyll content and aerial parts' dry weight were observed in plants inoculated with *S. indica*. Similar to our results, *S. indica* stimulated growth of other medicinal plants like *Artemisia annua, Curcuma longa*, *Stevia rebaudiana,* and *Bacopamonniera* in the *in vitro* experiments (Varma et al., 2014). Barley root growth was also stimulated by inoculation of *S. indica* 2 or 3 weeks after inoculation (Achatz et al., 2010). Numerous factors are reported to have influence on the success of *in vitro* propagation of different medicinal plants. The effect of plant growth regulators and their interaction on micropropagation of different plant species have been discussed in detail by Rout and colleagues (Rout et al., 2000).

The heavy root proliferation in inoculated plants has been attributed to the synthesis of phytohormones. It has been reported that *S. indica* produces auxins and cytokinin (Kari Dolatabadi et al., 2012). Several researches reported that auxin and cytokinin influence micropropagation of medicinal and aromatic plants (Al-Sulaiman and Barakat, 2010; Meena et al., 2010; Kari Dolatabadi et al., 2011b).Chen et al. showed that cytokinin play the most critical role in the micropropagation of many medicinal plants (Chen et al., 1995). It has been demonstrated that *S. indica* produces the low amounts of auxins, but relatively high levels of cytokinins. So, cytokinins may be higher in treated roots compared to untreated plants (control) (Vadassery et al., 2008). *S. indica* was also reported to produce IAA (indole acetic acid) in liquid culture that promote root growth in plants (Sirrenberg et al., 2007).

The increased growth parameters of *S. rebaudiana* inoculated with *S. indica* were probably due to greater water and nutrients absorption due to extensive colonization of roots by S. *indica.* It seems that the stimulation of *S. rebaudiana*'s root system extension by *S. indica* almost caused the promotion of above ground growth in our experiment.

Some other advantageous of Arbuscular mycorrhizal fungi (AMF) inoculation are enhancing plant growth by increasing the availability of nutrients, water uptake and exploring soil volume nearly 100 times greater, preventing pathogenic infection, and improving soil structure (Kari Dolatabadi et al., 2011a). It has been also reported that *S. indica* not only stimulated faster growth of aerial parts of plant, but also induced higher chlorophyll a content (mg. g^{-1} fresh weight) in *Coleus forskohlii* than in non-colonized plants (Das et al., 2012). It could be confirmed by the hypothesis that plants beneficially affected from the mutualistic interaction with fungi and delivery of phosphorus to their roots by the fungal hyphae through a phosphate transporter (PiPT).Varma et al. reported the increased chlorophyll content in plants treated with *S. indica* (Varma et al., 2014). Increase in chlorophyll levels, photosynthetic potential and leaf area could stimulate higher carbon absorption in plant colonized with *S. indica*, which consequently induce more biomass production (Jurkiewicz et al., 2010; Varma et al., 2014).

Based on the results of present study, the beneficial effect of *S. indica* was confined to the aerial parts of plants whereas the underground biomass was decreased by fungal inoculation. Plants colonized by AMF have shown lower root/ shoot ratios. The reason for this may be related to taking the absorbed nutrients over by hyphae (Tsang and Maun, 1999). Inoculation of Arabidopsis with *Trichoderma* induced more lateral root which were attributed to auxin production, even though there were no significant effects on primary root growth with the inoculation of *T. atroviride* or *T. virens* (Contreras-Cornejo et al., 2009). However, reduction in growth of hairy roots of the medicinal plant *Linum album* was observed soon after inoculation with live fungal cells (Varma et al., 2014).The increased number of secondary roots and root length may be associated with auxin, since it plays a pivotal role in plant and fungal symbiosis (Das et al., 2012). The pattern of root morphogenesis and development is also modified in mycorrhizal plants. Plants forming mycorrhizae tend to have a greater above ground biomass, since less energy is directed to root formation so their root/shoot (R/S) ratio would be lower (Lovato et al., 1996).

Root thickness was reduced in *C. forskohlii* plants treated with *S. indica* as they became fibrous, but more lateral roots were recorded (Das et al., 2012). The symbiotic interaction of *C. forskohlii* (*Plectranthus barbatus*) with *S. indica* under field conditions prompted more aerial part biomass production including flower development. The plant aerial parts are important source for metabolites which have medicinal characteristics. Therefore, the usage of the root endophyte fungus (*S. indica*) in sustainable agriculture would be beneficial in higher production of active in gredients (Das et al., 2012).

The results of this study showed that application of vermicopost water extract induced the highest chlorophyll content and ratio of RFW/APFW in treated plants. Bijeh keshavarzi et al. showed that among different kind of bio-fertilizers and chemical fertilizers, the highest chlorophyll content was obtained in vermicompost treated plants (Bijeh Keshavarzi et al., 2012). Several studies have stated that vermicompost contain hormones such as gibberellins, auxins and cytokinins and plant-growth regulating materials like humic acid which could increase plant's growth and production (Nadi et al., 2011; Tomati et al., 1990). In the present study, application of vermicompost water extract only affected the above-mentioned parameters of *S. rebaudiana.*

5. Conclusion

The results of this research showed inoculation of micropropagated *S. rebaudiana* plants with *S. indica* positively influenced some growth parameters of *S. rebaudiana*, which could assure the success of tissue culture technique for propagation of this medicinal plant. Also, the apply water extract vermicompost and *S. indica* fungus in sustainable agriculture may be improved vegetative items and, as a result, secondary metabolites in medicinal plants.

Acknowledgments

This study was supported by the Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University. The authors are thankful to Mrs. Zangi Setare (Sari Khazar Abad Institute, Mazandaran, Iran) for providing the plant material used in this study.

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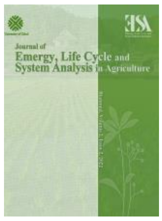
Journal of Emergy, Life Cycle and System Analysis in Agriculture (2022) Volume 2, Issue 2, P. 179-186

Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir



Scaling and corrosion quality zoning of groundwater in the aquifer of the ghorove–dehgolan plain

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ARTICLE INFO

Article history:
Received: 1 October 2022
Accepted: 18 December 2022
Available online: 16 January 2023

Keywords:
Aquifer
Corrosion index
Ghorove– dehgolan plain
Scaling
Water quality

ABSTRACT

Chemical quality is a significant and determining factor in a variety of water applications. Understanding the characteristics of subterranean water is regarded as a viable instrument for assessing water resource management. o determine the chemical quality of the groundwater in the Ghorove– Dehgolan aquifer, as well as to examine changes over three statistical periods (2001–2018), the basin was sampled for the following chemical parameters: electrical conductivity, total dissolved solids, sodium absorption ratio, bicarbonate, carbonate, chlorine, sulfate, calcium, magnesium, sodium, and potassium. Information from 276 exploitation sources on an annual scale pertaining to an 18-year statistical period was utilized to accomplish the objectives. The geographic information system (GIS) and the geostatistical interpolation method were utilized to determine the distribution of effective variables in the quality of industrial consumption in order to generate quality zoning maps of water consumption utilizing the Langelier index and the available data for the study area. The analysis of water quality variables revealed that the aquifer exhibited the highest values of electrical conductivity (669 µS/cm), total dissolved solids (430 mg/l), and sodium absorption ratio (0.95%) for groundwater quality during the period 2013–2018. In comparison to the other courses, they are lower. The assessment of industrial water quality revealed that scaling affected 22% of the water samples, while corrosion affected 78%. The examination of qualitative zoning maps intended for industrial applications revealed that the aquifers in the southern portion of the Ghorove–Dehgolan plain, along with a restricted region in the aquifer's northern section, exhibit sedimentation characteristics. Conversely, the majority of the aquifers in the area demonstrate corrosive attributes. Thus, it is imperative to exercise utmost caution when utilizing these resources in pressurized irrigation systems and industrial, urban, and agricultural water supply systems in order to mitigate potential harm to pipelines and metal connections.

Highlights

- The study provides a comprehensive assessment of groundwater chemical quality over an 18-year period for industrial use in the Ghorove–Dehgolan aquifer.
- The study found the highest levels of electrical conductivity, total dissolved solids, and sodium absorption ratio in the aquifer during 2013–2018.
- The finding revealed that 22% of water samples are affected by scaling, while 78% are prone to corrosion, with qualitative zoning maps indicating sedimentation in the southern and a portion of the northern aquifer.
- The study highlights the need for caution in using these water resources to prevent damage to pipelines and metal connections in various systems

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https://doi.org/10.22034/jelsa.2024.416742.1053

1. Introduction

As one of the most vital elements, water plays a critical role in all aspects of human life, including human well-being, social economic development, and ecosystem life (An et al., 2014). In addition to being essential for human life, water is also one of the determining factors in the quality of human life (Raju et al., 2011; Raju et al., 2014 and Toumi et al., 2014). One of the important sources of fresh water needed by humans is groundwater, which is the largest fresh water reserve on earth after glaciers (Yousefi Mobarhan and Peyrowan, 2022 and Todd and Mays, 2005). Groundwater is a renewable, limited, and vital resource for human life, social, and economic development, a valuable component of the ecosystem, and vulnerable to natural and human impacts (Singh et al., 2011). In recent years, because of the sharp decrease in the volume of groundwater, it has been important to check its quality and protect it accordingly. Water quality is a function of physical, chemical, and biological variables and is mainly controlled by two natural and human factors.

Natural processes such as the geology of the region, the speed of groundwater movement, recharge of water quality, the interaction of water with rock and soil, the reaction with other aquifers, and activities related to human intervention, including agricultural, industrial, and urban development activities and increasing the use of water resources (Chan, 2001) Currently, understanding the quality of groundwater is one of the most important and vulnerable sources of water supply (Shokuhi et al., 2011), and a better understanding of the spatial and temporal relationships between water quality and variables Geography and environment can play an important role in the effective and efficient planning of resources. Many industries use water for various purposes, such as cooling devices, producing materials, and using steam boilers and hydroelectric power plant.

Waters can undergo corrosion or scaling, both of which have adverse effects on the industry (Lotfinasabasl et al., 2020). The effects of corrosion and scaling in water supply systems, transmission, and distribution can increase operating costs and create negative effects on human health (Bamdad Machiani et al., 2014; Ehsani et al., 2013). Therefore, the science of water quality will remain an important issue for engineers and scientists in the coming years (Arand et al., 2008). Several methods have been invented and developed to investigate the chemical quality of water (Kelly et al., 1940; Najafzadeh and Tafaroj, 2016; Najafzadeh and Zahiri, 2015; and Nouri et al., 2012).

The hydrogeochemistry of the Sahand watershed using the Piper diagram by Naseri et al. (2010) was evaluated. They introduced the facies and groundwater type of this basin as calcium/sodium bicarbonate. They also assessed water quality as suitable for drinking and agriculture. In the study of the quality of water resources in the Koh-Zar mineral area in the west of Torbat Heydarieh, Khmer et al.(2011), after measuring the cations and anions of the water samples taken from the groundwater sources, determined the type of water in the area as Cl– Na and HCO3–Na and the water quality based on Schuler and Wilcox diagrams in terms of inappropriate drinking and agriculture.

Sahbaei Lotfi (2013) investigated the condition of the Baba Aman River from the headwaters of the Etrak River for drinking, industrial, and agricultural purposes, as well as its long-term quality changes. The results of the study were drawn on the basis of the Wilcox and Schuler charts. The results showed that the water in this river at the site of the station is suitable for agricultural and drinking purposes and that the water salts are increasing at the site of this station. Hoseinsarbazy and Esmaili (2014), by examining the indicators of corrosion and scaling of groundwater quality, concluded that all the samples in the Neyshabur Plain have scaling properties. Investigating the hydrogeochemical quality of groundwater in the Siyahu Basin by Gholamdokht Bandariet al.(2018), they showed that according to the quality index of Wilcox and Schuler, the groundwater for agricultural purposes was at the average level, and in terms of potability and Langelier saturation coefficient, they evaluated the available water sources as Corrosion to Scaling. Aghdam et al. (2019) investigated the qualitative zoning of groundwater resources in the Naghade plain for drinking, agriculture, and industry. The evaluation of industrial water quality showed that 61% of the water samples in the study area were scaled and 39% were corrosive.

The water quality assessment of the Kopal River by Lotfinasabasl et al. (2020) was investigated, and the results showed that the quality of water for three purposes (drinking, agriculture, and industry) has been greatly reduced by moving toward the final years, especially the last five years under study, and industrially, the water of this river has sedimentation effects. Jiang et al. (2020) investigated the hydrochemical characteristics and water quality assessment of rivers in different areas of cities. The results of the irrigation water quality evaluation showed that the Tuo River samples have high salt and low alkalinity and can be used for irrigation under suitable soil washing conditions, whereas the Bian River water samples have high salt content and are suitable for watering saline plants.

Chai et al. (2020) investigated hydrogeochemical properties and assessed the quality of groundwater in Dahui city, China, using multivariate statistical analysis. The results will be useful for the development and management of groundwater resources. The quality of water resources in the Ruin Esfrain karst aquifer in North Khorasan province was reviewed by Motamedi Rad. The water quality of springs in the region in terms of industrial use also showed that all water samples in the region have scaling properties, except for the Sengua spring, which has scaling water. Also, in research by Yousefi et al. (2022), they evaluated the water quality of aqueducts for different uses in Nain, drew the charts of Shuler, Wilcox, and Piper, and determined the quality of aqueducts for drinking, agriculture, and industry. most of the Qanats under study were part of the Scaling group, and only two Qanats from Hyderabad and Arend were in the corrosive category in terms of water quality for industrial use.

By reviewing the sources, it was found that the Langelier index is one of the most useful and reliable indicators in determining the quality of groundwater for industrial purposes, and the researchers' use of this index confirms this. Since excessive harvesting, meteorological droughts, and the climate change trend have become more intense in recent decades and have changed the quality of groundwater, a comprehensive study of the quality is still needed. Groundwater for various uses, especially for industrial purposes, has not been used in the Ghorveh–Dehgolan plain. Therefore, this research tries to investigate the trend of variables affecting the quality of groundwater in the time periods and spatial areas of the Ghorveh–Dehgolan aquifer. Using GIS to zone the spatial changes in groundwater quality in the study area for industrial uses with the Langelier index, the results can be used to plan for the proper exploitation of groundwater resources.

2. Materials and Methods

2.1 The study area

The Ghorveh-Dehgolan is one of the 11 areas or plains of the Sefidroud basin, which has an average annual rainfall of 352 mm and a semi-arid and cold climate. It is located to the east of Sanandaj city and northwest of Hamedan. The geology of the Ghorveh-Dehgolan plain is part of the Sanandaj-Sirjan construction zone, which is considered to be one of the most active construction zones in Iran (Rahmati et al., 2016). The southern elevations of the Ghorveh plain consist of metamorphic rocks such as Schist, Marble, Amphibolite, and Gneiss, along with igneous masses with different compositions. The intensity of tectonic forces in the northern parts of the region is less than that in the southern parts. In the northern half of the plain, from the Miocene to the beginning of the Quaternary, magmatic activities have caused the formation of basalt and andrite volcanic formations in the region.

This plain is bounded from the west by red sandstone heights and early dolomitic limestones, from the east by dolomitic limestones, from the north and northeast by Plio-Pleistocene formations, and from the south by internal igneous and metamorphic formations. the highlands of Bi-Khair are separated from the Ghorve plain (Abassi et al., 2015). Its minimum and maximum temperatures are -23 and 41 °C, respectively; the average annual relative humidity is 45%; and the maximum evaporation in July is more than 350 ml. The soil of these lands has great talent and ability in terms of irrigation and agriculture, and the cultivation of all kinds of agricultural and native plants has good performance at low cost. These lands have deep - surface soils with medium- to heavy texture and high water retention capacity. The study area is shown in Figure 1.

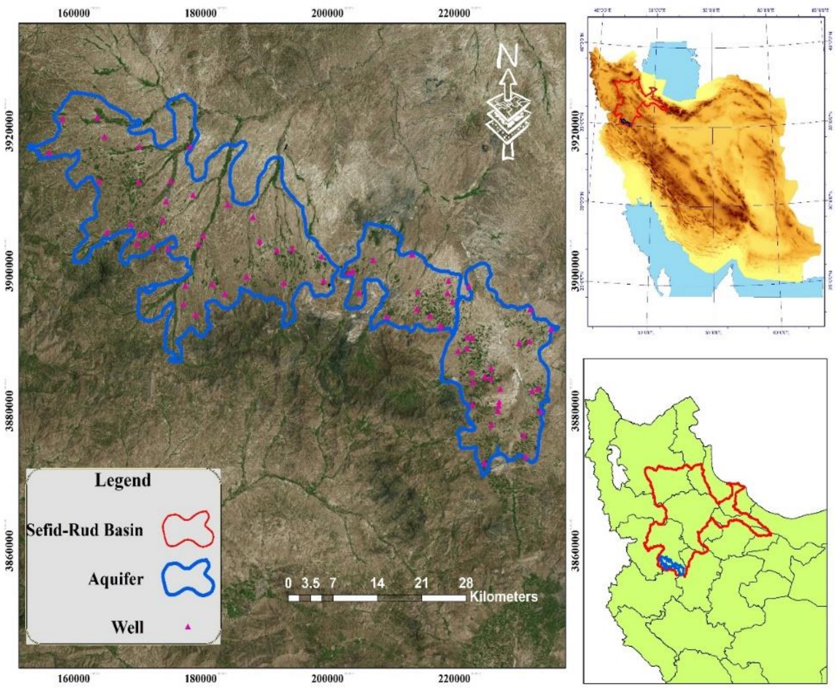

Figure 1. Geographical location of the study area

2.2 Statistics and qualitative information

The first step in using water quality data is to check the accuracy of the collected data. In the investigation of the quality data of the underground water, after preliminary checks of the data from the last sampling period and elimination of the existing errors, samples with an ion error percentage higher than 5% of the total anions and cations in the analyses were not used. Note that in the analysis of the water quality parameters of the Ghorveh-Dehgolan plain, 276 water samples were taken to measure the water quality in the Ghorveh-Dehgolan aquifer. In the current research, the chemical quality of groundwater sources in the plains has been investigated using the results of qualitative analysis of water samples in deep and semi-deep wells from three periods (periods 2001–2008, 2008–2013, and 2013–2018), and it has been analyzed according to the common statistical period of 18 years (2001–2018). The analyzed statistics and information include the results of a complete chemical analysis of water and variables such as electrical conductivity (EC) values, total dissolved substances (TDS), pH, cations (calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}), anions (chloride (Cl^{-}), sulfate ($SO4^{2-}$), bicarbonate (HCO_3–), and carbonate (CO_3^{2-}), sodium percentage (%Na), and sodium absorption ratio (SAR).

2.3 Water quality index based on industrial use

The Langelier index was used to measure water quality for industry based on equations 1 and 2 (Llyod and Heathcote, 1985; Rahimi et al., 2016; Sadeghi Aghdam et al., 2019).

$$LI = PH_a - PH_s \quad (1)$$

$$PH_s = A + B + C \quad (2)$$

In the above equations, pH is the measured acidity of water, pH is the modified acidity, A is the negative logarithm of the total concentration of Ca, Mg, Na, and K ions, B is the negative logarithm of the total concentration of Ca and Mg ions, and C is the negative logarithm of HCO_3^- and CO_3^{2-} ions. In this regard, the concentration of the ions is expressed in terms of equivalents per liter. In this regard, LI is the Langelier index, which is an index for corrosion and sedimentation. Table 1 shows the classification of water quality for industrial uses based on the Langelier Index (LI). Water with a negative saturation index is corrosive and can adversely affect wells and water supply facilities. Waters with a positive saturation index are sedimented, and their use in steam boilers and low-pressure heaters is not allowed. Waters with a zero-saturation index are in a state of equilibrium and do not have corrosive or sedimentation properties (Gholamdokht Bandari et al., 2018).

Table 1. Quality of groundwater in the Sefid– Rud Basin based on Langelier indices (Lotfinasabasl et al., 2020)

(LI)	Quality (Langelier)
LI<0	Water is supersaturated and tends to scale $CaCO_3$.
LI=0	Water is saturated with CaCO3 and does not tend to form or decompose $CaCO_3$.
LI>0	The water is undersaturated, and the decomposition of solid $CaCO_3$ is not expected.

Zoning is used to display water quality data and the trend of changes in parameters affecting the quality of underground water for industrial purposes (Yousefi Mobarhan and Karimi Sangchini, 2021; Yang et al., 2004). The distribution map of each of the effective parameters in the classification for 65 sampled points was prepared in the ArcGIS 10.5 software environment using the Kriging method. The Kriging interpolation method is calculated on the basis of the weighted moving average and is the best unbiased linear estimator with the minimum estimation variance (Nadiri et al., 2015). Each of the effective parameters in the water quality classification according to the type of use for drinking, agriculture, and industry was prepared by the normal Kriging interpolation method and the Gaussian model. Each layer was classified based on the Langelier (industrial) index.

3. Results and discussion

3.1 Statistics and qualitative information

In the statistical analysis of groundwater in the Ghorveh-Dehgolan aquifer plain, while determining the maximum, minimum, and average values of the qualitative variables at the level of the study areas and the trend of qualitative changes in the aquifer level according to the water electrical conductivity map, groundwater was investigated and analyzed.

According to Figure 2, it can be seen that the salinity value in the Ghorveh-Dehgolan aquifer in all three-time scales is lower than the maximum value given by Wilcox (2250), and it can be said that the plain is in a good state in terms of salinity. The average values of EC and TDS in the border of the Ghorveh-Dehgolan plain during the 3 time periods under study have had an increasing trend, which indicates that the plain has a trend toward salinity and its quality has decreased over time (Figure 3).

Note that the increasing trend of the electrical conductivity of the Ghorveh-Dehgolan aquifer in all three statistical periods is consistent with the findings of Abbasi et al. (2016), who stated that the quality of underground water in the Ghorveh-Dehgolan plain is decreasing, and if the current trend continues in the near future, it can cause many crises in terms of various uses.

The maximum electrical conductivity (EC) and total dissolved solids (TDS) were observed in the period of 2013–2018 in the Ghorveh– Dehgolan aquifer (669 µS/cm and 430 mg/l), respectively. In addition, the results showed that the increase in TDS had a significant effect on the increase in electrical conductivity, especially in the last years of the study. Figure 3 shows that the sodium absorption ratio (SAR) in the plain has an upward trend, and the maximum sodium absorption ratio in the third period (2018–2013) in the plain is 0.95%. According to Figure 4, among the cations, the highest amount is related to the calcium ion, followed by the sodium ion. It is possible that the presence of calcium ions is due to the dissolution of carbonate minerals in the geology of the region. In addition, the dissolution of carbonate minerals such as dolomite and calcite and minerals containing magnesium ions may be the reason for the increase in magnesium in water. It has been a study that has had a relatively constant trend in the years under review (Figure 5).

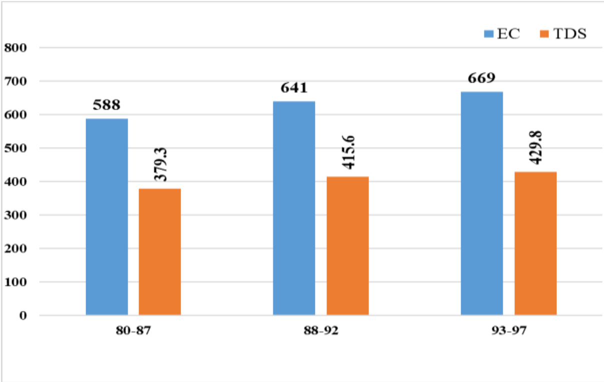

Figure 2. EC and TDS values in the groundwater of the Ghorveh– Dehgolan aquifer

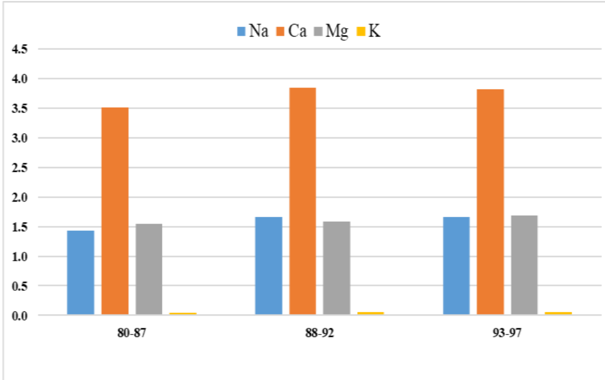

Figure 4. Values of cations (Na, Ca, Ma, and K) in the groundwater of the Ghorveh– Dehgolan aquifer

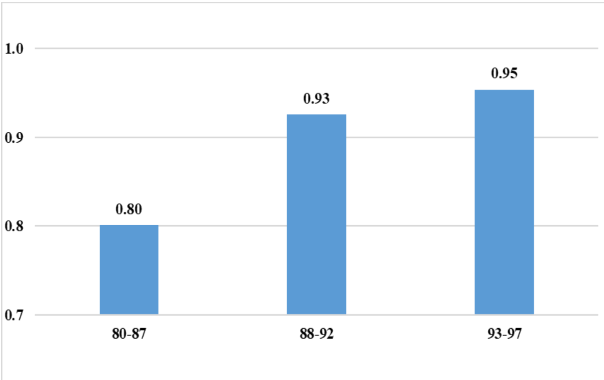

Figure 3. SAR values in the groundwater of the Ghorveh– Dehgolan aquifer

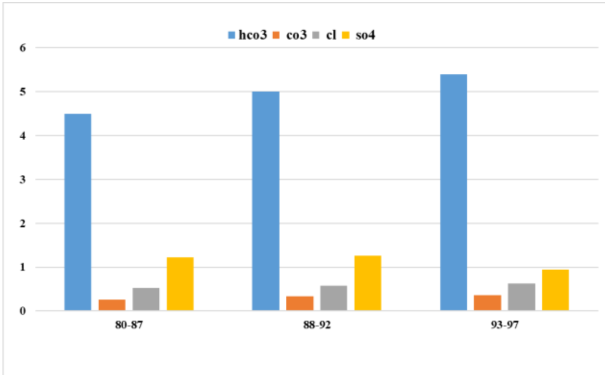

Figure 5. Values of anions (Hco3, Co3, Cl, and So4) in the groundwater of the Ghorveh– Dehgolan aquifer

3.2 Analysis of water quality based on industrial use

In the classification of water for industrial use, salinity, number of solutes, degree of hardness (permanent and temporary), and its reaction environment are essential. Therefore, first, after calculating the hardness of alkalinity, TDS, pH, and temperature, the pH value of water in the saturated state of calcium carbonate (pHs) was calculated from equation (2), and finally, using equation (1), the values The Langelier Index (LI) was determined to determine the corrosion and scaling properties of the Ghorveh-Dehgolan aquifer for the statistical periods under study. Figure 6 shows the zoning of the industrial uses of the Ghorveh-Dehgolan aquifer in three periods (2001–2008, 2008–2013, and 2018–2013).

Table 2. Quality classification of industrial water based on the Langelier index (LI)

Water quality for industrial use	Langelier index	Percentage of samples
Corrosion	LI<0	78
Scaling	LI>0	22

As Figure 6 shows, in all three time periods investigated based on the Langelier index, the spatial distribution of parameters affecting the quality of corrosion and scaling in the plain's groundwater is such that the zoning maps divide the quality of the plain into two scaling groups in the southern and some northern parts and corrosion in most of the other areas. The zoning results presented in Figure 6 show that the scaling property of the plain's groundwater has decreased, and in the third period (2013–2018), the quality of the groundwater in most of the plain's area has become corrosive. In general, the quality of underground water and its corrosion and sedimentation properties depend on the geological context of each region. In addition, according to Langelier classification, 78% of the samples are corrosion and 22% are scaling (Table 2), and the findings of this study are in agreement with the results of Motamedi Rad et al. (2021), Rahimi et al. (2016), and Gholamdokht Bandari et al. (2018).

In their study, they found that the quality of the groundwater in the studied areas, in terms of the industrial uses of the area, often has corrosive properties.

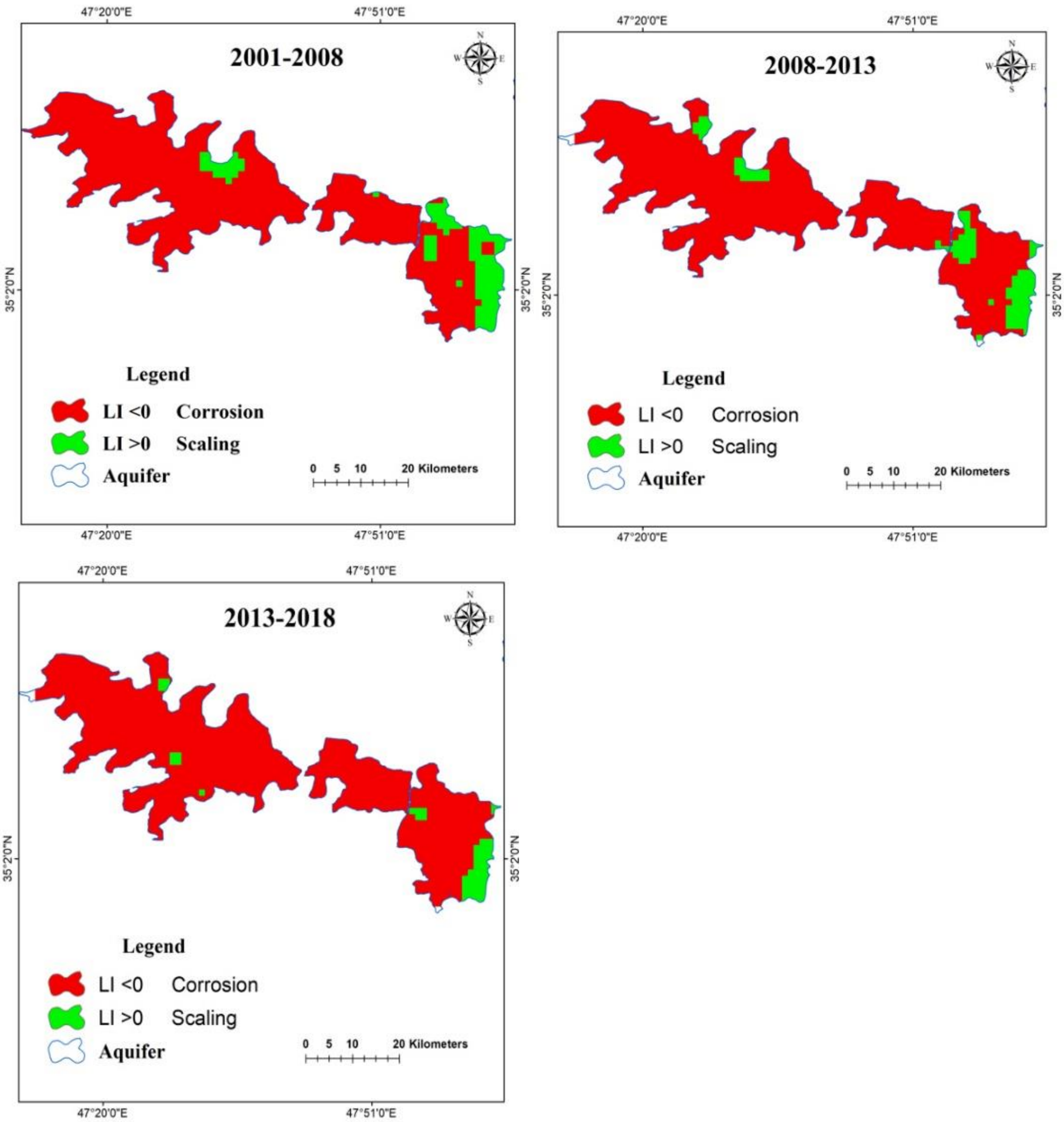

Figure 6. Zoning industrial uses the Ghorveh– Dehgolan aquifer, separated by 3 statistical periods

4. Conclusion

Surface waters, especially rivers, are one of the most important water sources that are used to resolve the needs of human societies and for various purposes, including drinking, industry, and agriculture. Therefore, water quality, like its quantity, is one of the most important determining factors. It is considered effective for its use for various purposes. The chemical variables of water play an important role in the classification and evaluation of water quality; therefore, measuring and examining these variables is one of the necessities of their study. In this study, to investigate the water quality of the Ghorveh-Dehgolan aquifer and the change process, the chemical variables of the water were investigated. The results of the study showed that, in the 18-year statistical period, the average values of EC, TDS, and sodium absorption ratio (SAR) in three statistical periods limited to the plain had an increasing trend, and the increase in TDS had a significant effect on the increase in electrical conductivity, especially in the last years of the study. It shows the highest values of cations, respectively, for Ca^{2+}, Na^{+}, Mg^{2}+, and K^{+} ions in the boundary of the aquifer. The highest numbers of anions included HCo_3^-, SO_4^{2}-, and Cl^-. The qualitative evaluation of the groundwater resources of the Ghorveh-Dehgolan plain for industrial purposes based on the Langelier index showed that the change trend of this index was toward corrosiveness, and out of 276 sources in the basin, 214 sources had corrosion characteristics and 62 sources had scaling characteristics. The results also showed that the groundwater in most of the aquifer area of the Ghorveh-Dehgolan plain has corrosive properties, and its use in urban water supply systems will cause disease in humans and various problems caused by the corrosion of pipes. Therefore, in the use of these resources in industrial, urban, and agricultural water supply systems, especially in pressurized irrigation systems, the necessary measures should be considered to minimize damage to pipes and metal connections. In addition, by reducing the extraction of underground water and reducing the area of cultivation in areas where the required water is supplied only through aquifers, the quality of the underground water can be managed even in dry years.

Acknowledgements

This article is taken from a part of the results of a research project with the approved code 99025-99025-030-09-09-01 in the Agricultural Research, Education, and Extension Organization, and from the respected officials of the SCWMRI, Research Institute of Forests and Rangelands, and Semnan Agricultural and Natural Resources Research and Education Center, Dr. Samira Zandifar, Maryam Naeimi, and Dr. Adel Jalili.

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Journal of Emergy, Life Cycle and System Analysis in Agriculture

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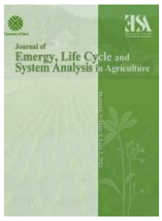

Biofertilizers and Superabsorbent polymers enhance cumin yield under water limitation

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ARTICLE INFO

Article history:
Received: 1 September 2022
Accepted: 13 November 2022
Available online: 1 December 2022

Keywords:
Cumin
Drought stress
Nitroxin
Phosphate Barvar2
Superabsorbent polymer

ABSTRACT

Biological fertilizers are regarded as a viable substitute for chemical fertilizers in sustainable agriculture. Conversely, drought stress constitutes a significant impediment to the development and growth of crops. Large quantities of rainwater or irrigation water can be absorbed by superabsorbent polymers, preventing their deep infiltration and resupplying the plant in dry conditions. The present study investigated the impact of superabsorbent polymer and fertile biofertilizers-2, in addition to Nitroxin, on the quantitative and qualitative yield of cumin when subjected to drought stress conditions. To examine the impact of superabsorbent polymers Barvar2 and bio-fertilizers Nitroxin on cumin seed yield and yield components during drought stress, a factorial split experiment was conducted in a randomized complete block design with three replications at the field during the 2014-2015 crop year. This study focused primarily on drought stress, including two levels of stress and non-stress. The experimental setup consisted of two levels of non-consumption and consumption of superabsorbent polymers (Nixin and Barvar2), as well as biofertilizers Nitroxin and Barvar2. The subfactors were represented in the subplots using a 2×2×2 factorial design. Under conditions of drought stress, the integrated application of bio-fertilizers Nitroxin and Barvar2, along with the utilization of a superabsorbent polymer, produced the highest seed weight (3.133 gr), seed yield (46.09 grm^{-2}), and biological yield (106.8 grm^{-2}). The optimal treatment combination, which consists of Barvar2 with superabsorbent polymer and Nitroxin integrated treatments, has been identified as the most effective method for increasing grain yield and yield components under wet conditions. Overall, the outcomes of these experiments indicated that the application of bio-fertilizers and superabsorbent polymers might be viable options for enhancing the productivity of cumin manufacturing. In drought-stressed conditions, such as dry farming, the application of biofertilizers Barvar2 and Nitroxin in conjunction with superabsorbent polymer consumption reduced the effects of drought stress on yield components, grain yield, and biological yield in comparison to the control treatment. When moisture limitation is present, the application of bio-fertilizers containing superabsorbent polymer can significantly enhance the productivity of rainfed cumin cultivation.

Highlights

- Application of superabsorbent is effective to resist drought stress.
- Biological fertilizers such as Nitroxin and fertilizing phosphate can help meet the nutritional needs of the cumin plant and increase its yield.
- Application of superabsorbent along with bio-fertilizers in cumin rainfed cultivation improved yield under drought stress conditions

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https://doi.org/10.22034/jelsa.2024.360109.1043

1. Introduction

Cumin cientifically known as *Cuminum cyminum*, is classified as a member of the umbel family. Aside from its fruit, it is aromatic and hairless. Its stem is herbaceous in nature and may consist of two or three branches. This plant has a grooved stem, peripheral collapsible tissue, divided leaves or very thin incisions, a green coloration, and thread-like ribs. The flowers are pink or white compound umbels. This small herbaceous plant ranges in height from 10 to 50 centimeters; its long, slender, and white-colored roots (Sastry and Anandraj, 2013). It has been reported that irrigated crops can produce as much as 900 kgha-1 on average, while rainfed cumin crops can yield up to 400 kg/ha. (Kafi, 2006).

In the realm of plant nutrition, it is critical not only that each element be adequately accessible to the plant, but also that equilibrium be maintained and nutritional elements be observed. In recent years, the country's agriculture has suffered severe setbacks due to the excessive application of chemical fertilizers, particularly nitrogen and phosphorus, without regard for the nutrient balance in the plant. These include the depletion of soil essential elements, interference with the solubility and absorption of food components, contamination of underground and river water, and the introduction of toxic elements into the soil via fertilizer (Zahedi and Isma

Biological fertilizers have the potential to enhance the quality and quantity of medicinal plants and are a viable substitute for chemical fertilizers in sustainable agriculture. Cumin yields were enhanced both quantitatively and qualitatively through the application of biological fertilizers (Rezaei Chianeh et al., 2014). The study demonstrated that by minimizing environmental risks and ensuring the stability and health of the agricultural system, combined treatments utilizing bioorganic and chemical fertilizers can effectively address the nutritional requirements of plants to a significant degree. Additionally, these treatments promote the establishment of soil microorganisms for subsequent cycles (Movafaghian et al., 2014). Rai et al. (2018) found that the incorporation of bio-fertilizers in conjunction with minimal quantities of chemical fertilizers improved atmospheric conditions and human health. According to research (Azizi, 2017), the application of superabsorbents in conjunction with phosphate barvar2 biofertilizers and nitrogen has a beneficial impact on a variety of quantitative and qualitative characteristics of the fennel plant. Appropriate application of animal and biological fertilizers, in addition to their combination, can result in an increase in wheat yield and yield components that is comparable in magnitude to the yield increase induced by chemical fertilizers. Additionally, reducing the use of chemical fertilizers contributes to environmental health. Hence, for optimal results and environmental enhancement, it is recommended to utilize animal manures in conjunction with biological fertilizers (Lotfi Jalalabadi et al., 2013).

Utilizing biofertilizer Phosphate Barvar2 is comprised of beneficial phosphate-dissolving bacteria that facilitate soil acidification and the secretion of phosphatase enzymes, which liberate phosphate ions from phosphorous compounds for plant absorption (Tohidinia et al., 2014). Utilizing biofertilizer Auxin can be produced in greater quantities using phosphate Barvar2 than nitrogen fertilizers. Conversely, Nitroxin bio-fertilizer, comprising species of Azotobacter and Azospirillum, facilitates the synthesis of auxin and other hormones that promote plant growth in agriculture (Ansari et al., 2014). According to the findings of the study, supplementing chamomile with either animal manure in its unmodified form or with the mycorrhizal fungus can enhance the quality of dried flowers, increase the production of chamomile's secondary compounds and essential oils, and serve as a foundation for minimizing the application of chemical fertilizers while ensuring consumer food safety, economic viability, and environmental health (Kohan Mo and Agha Alikhani, 2014). Utilizing biofertilizer According to Azizi (2017), the application of phosphate Barvar2 and Nitroxin fertilizers on fennel plants results in favorable outcomes across several plant parameters, including harvest index, total dry matter, seed yield, plant height, essential oil percentage, and yield. Conversely, drought stress constitutes a significant impediment to the growth and development of agricultural plants, and one potential strategy for augmenting agricultural plant production is to enhance water utilization efficiency. The research findings indicate that black cumin, a low-water-use plant, maintains its phenol content even when subjected to drought stress conditions. Overall, the findings of this study suggest that drought has a beneficial impact on the antioxidant capacity of seeds derived from drought-resistant plants (Saeidnejad et al., 2013). Due to the irregular rainfall patterns in various regions of the country over the past few decades, agricultural products have been subjected to severe drought stress. Elevated temperatures and inadequate nutrition frequently exacerbate the consequences of drought stress. Drought resistance is enhanced in plants that have been adequately nourished and supplied with low-use elements (Mortazavi et al., 2015). Large quantities of water from precipitation or irrigation can be absorbed by superabsorbent polymers, which prevent the water from penetrating deeply and recirculate it to the plant in dry conditions. A material with exceptional absorbency can absorb a substantial volume of water and alleviate the strain associated with water scarcity. The water absorption capacity of these materials is approximately 400 times their weight. This enables them to readily supply the plant with stored water during periods of water scarcity, thereby substantially preventing stress and yield reduction (Azizi, 2017). According to research (Sajedinia et al., 2018), it was possible to increase the frequency of irrigation in tomato plants without negatively impacting fruit yield and quality by employing superabsorbent polymers.

In light of the growing interest in utilizing medicinal plants that have low water demands and the country's water constraints, the current study aimed to determine the impact of superabsorbent polymer and Nitroxin and Phosphate Barvar2 under conditions of drought stress on the cumin yield, both quantitatively and qualitatively.

2.Materials and methods

During the 2018 agricultural harvest, a split factorial experiment was conducted at the research farm of the Faculty of Agriculture, Lorestan University, situated in the vicinity of Khorram Abad (33° 26′ N latitude and 45° 27′ E longitude; height 1210 meters above sea level). The experiment followed a basic design of randomized complete blocks with three repetitions. March was chosen as the month to conduct the land preparation operation due to the favorable climatic conditions at that time. The ground was covered with a 1.5 3 square meter surface area subsequent to its leveling. Each plot comprises six rows of crops, spaced at 20 cm between rows and 2 cm between plants. Additionally, the separation between the plots and the blocks was assumed to be 1 meter for the former and 2.5 meters for the latter. Prior to sowing, soil samples were collected and subjected to physical and chemical analysis at various depths (between 0 and 25 centimeters and 25 and 50 centimeters) at the experimental site (Table 1).

Table 1. Physical and chemical characteristics of farm soil

Soil Texture	Sand	Silt	Clay	pH	EC	Mg	Ca	K	p	Organic carbon	Total nitrogen
		(%)			(dS/m)			p.p.m		(%)	
Loomy clay	24	44	32	8.5	0.47	2.9	3.3	410	9.4	0.55	0.98

Drought stress (W) was the primary determinant in this study; in the main plots, it was classified into two levels: no stress (W0) and stress (W1). The experimental secondary factors comprised superabsorbent polymer (S), Nitroxin (N), and Barvar2 (B). These bio-fertilizers were arranged in subplots according to two factorial levels: consumption and non-consumption. Both quantitative and qualitative characteristics were assessed in this study. Two square meters were taken in order to determine yield and yield components, two side rows were eliminated, and a half-meter was added to the beginning and end of each plot as a marginal effect.

To ascertain the quantity of essential oil present in the seeds, a 50-gram sample was extracted from each of the test plots. Following a three-hour grinding period, the essential oil was extracted through water distillation utilizing a Clevenger apparatus. The essential oil yield was determined using a percentage-based method (Zeinali et al., 2008). The yield of the substance in question was calculated by multiplying the seed yield by the essential oil yield (Tasdighi et al., 2015). The statistical software SPSS was utilized to analyze the sampling data. The graphs were created utilizing Excel 2013. The mean of the data was calculated utilizing Duncan's multiple range test.

3. Results and discussion

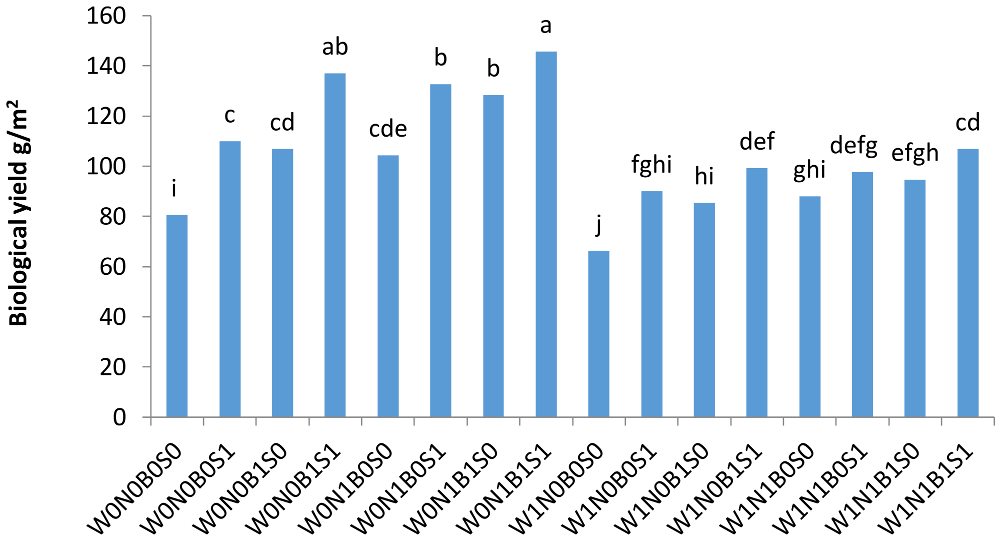
3.1. Biological yield

The findings of the study indicate that there is a statistically significant relationship ($P<0.01$) between the mutual effects of Nitroxin and Barvar2 bio-fertilizers and super absorbent polymers, and drought stress, with respect to biological yield (Table 2). The treatment involving the four mutual effects of Nitroxin, Barvar2, and superabsorbent polymer bio-fertilizers produced the highest biological yield (145.7 grams per square meter) in the absence of drought stress. Conversely, the treatment without Nitroxin, Barvar2, and superabsorbent polymer bio-fertilizers resulted in the lowest biological yield (66.33 grm^{-2}) under drought stress conditions (Figure 1). The findings of this study indicate that the application of superabsorbent polymers, both individually and in combination with biofertilizers, leads to a decrease in biological yield during periods of drought stress, as compared to the control treatment. As a result, taking into account recent droughts and humidity restrictions, the application of superabsorbents under conditions of drought stress enhances the plant's absorption of water and nutrients and improves the soil's physical properties. The findings of the current study align with those of previous research (Karami and Sepehri, 2013). Recent studies have demonstrated that under drought stress conditions, the application of Barvar2 bio-fertilizers in conjunction with 50% chemical fertilizers (urea, triple superphosphate, and potassium sulfate) not only enhances biological yield but also mitigates the adverse effects of dehydration stress while concurrently augmenting biological yield.

3.2. Grain yield

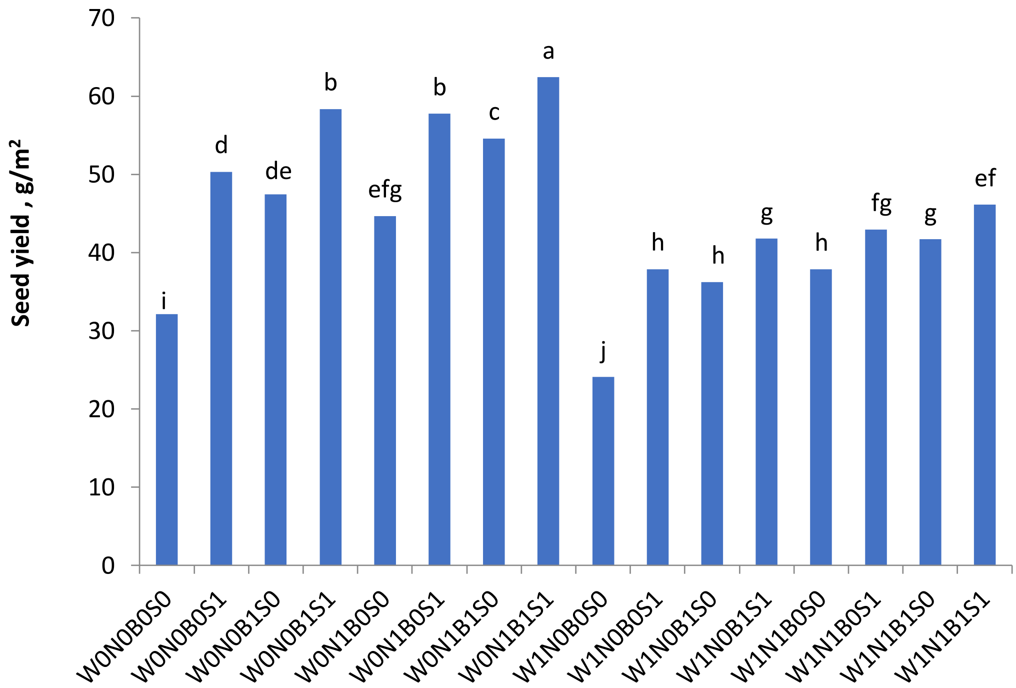
The results obtained from the variance analysis regarding the four interaction effects of drought stress, superabsorbent polymer, Nitroxin biofertilizers, and Barvar2 on grain yield do not exhibit statistical significance (Table 2). After conducting a comparison of the means of the four previously mentioned mutual effects, it was ascertained that the treatments labeled W0N1B1S1 and W1N0B0S1 generated the greatest grain yield (64.37 grm^{-2}) and the least (24.07 grm^{-2}), respectively (see Figure 2). According to the results obtained, it seems that the W1N0B0S1 treatment, which employed superabsorbent to maintain adequate moisture levels for the plant, did not effectively provide the plant with vital nutrients, resulting in a substantial reduction in grain yield. Based on the results obtained, it can be concluded that the decrease in grain yield during drought stress conditions in the W1N0B0S1 treatment can be attributed to the absence of Nitroxin Phosphate Barvar2 biofertilizers. This conclusion is reached despite the application of superabsorbent and the provision of moisture necessary for the plant. The augmented grain yield that is evident subsequent to biological fertilizer inoculation can be ascribed to the heightened nutrient accessibility for the plants. As a result, the increased availability of nutrients promotes the production of photosynthetic materials that are utilized by the seeds. Through the retention and storage of the super absorbent in the soil during periods of water scarcity, the plant was able to obtain the necessary water, resulting in an enhanced yield when compared to when the super absorbent was not employed. Superabsorbent polymers

(SAPs) have been found to be practical and highly effective in arid and semi-arid regions for the storage of water and specific nutrients (Jahan and Nassiri Mahallati, 2020). Zafarian et al. (2011) conducted an investigation to assess the effects of biofertilizers Nitroxin, Azotobacter, and Phosphate Barvar 2 on safflower seed yield and its constituents. The study's results validated that the optimal seed yield was achieved through the combined application of Azotobacter, Nitroxin, and Phosphate Barvar 2. This finding aligns with the results obtained in the current research.



W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 1. Comparison of the average quadruple effects of Nitroxin and fertile biofertilizers with superabsorbent under drought stress on the total dry matter of cumin



W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 2. Comparison of the mean quadruple interactions of Nitroxin and fertile biofertilizer application with superabsorbent under drought stress conditions on cumin grain yield

Table 2. Analysis of variance of the effect of nitroxin, phosphate-2 and superabsorbent polymers under drought stress conditions on cumin

		MS									
S. O. V	**df**	**Plant height**	**Number of umbrellas per plant**	**Number of seeds per umbel per plant**	**Thousand weights of seeds**	**Number of seeds per plant**	**Total dry matter**	**Seed yield**	**Harvest index**	**Percentage of essential oils**	**Essential oil yield**
R	2	22.490^{ns}	14.432^{ns}	4.163^{ns}	1.651^{ns}	504.596^{ns}	962.803*	513.004*	84.958^{ns}	5.240*	2.755*
A	1	43.225*	238.655**	33.117*	2.480*	5044.167**	8855.790**	1701.701**	1.619^{ns}	2.189*	0.239^{ns}
ERORR$_1$	2	1.352	0.935	1.926	0.155	457.933	32.476	18.617	5.529	0.222	0.031
B	1	40.498**	78.976**	10.954**	1.783**	18524.843**	2836.995**	857.350**	55.320**	1.744**	1.721**
AB	1	0.910*	13.157**	^{ns}0.199	0.083**	2424.368**	172.445**	22.963*	4.184^{ns}	0.194**	0.005^{ns}
C	1	1.837**	156.855**	21.561**	3.198**	36378.692**	3383.857**	912.286**	35.82**	0.263**	1.095**
AC	1	4.266**	14.008**	0.189^{ns}	0.129**	3321.178**	402.405**	105.851**	0.287^{ns}	^{ns}0.001	0.074^{ns}
BC	1	5.103**	15.019**	2.965**	0.628**	2118.559**	154.012**	90.256**	29.873**	0.220**	0.279**
ABC	1	0.531^{ns}	0.160^{ns}	0.160^{ns}	0.007^{ns}	23.144^{ns}	3.010^{ns}	2.746^{ns}	0.025^{ns}	0.012^{ns}	0.003^{ns}
D	1	18.563**	61.812^{ns}	11.535**	0.788**	15914.447**	5080.390**	1047.201**	7.979*	0.263**	1.041**
AD	1	0.115^{ns}	0.001^{ns}	0.001^{ns}	0.004^{ns}	1.006^{ns}	397.786**	54.060**	4.054^{ns}	0.023^{ns}	0.005^{ns}
BD	1	9.639**	8.526**	2.240**	0.413**	1567.168**	165.689**	16.333^{ns}	0.257^{ns}	0.004^{ns}	0.001^{ns}
ABD	1	0.239^{ns}	1.790**	0.102^{ns}	0.039**	288.463^{ns}	0.559^{ns}	20.306^{ns}	17.388**	0.000^{ns}	0.021^{ns}
CD	1	6.938**	8.526**	2.258**	0.245**	1837.316**	58.919^{ns}	31.493*	13.579**	0.146**	0.093*
ACD	1	1.304*	0.127^{ns}	1.303^{ns}	0.002^{ns}	0.002^{ns}	1.621^{ns}	4.404^{ns}	9.461**	0.064**	0.015^{ns}
BCD	1	2.063**	13.515**	3.791**	0.243**	3903.675**	0.069^{ns}	6.366^{ns}	10.935**	0.015^{ns}	0.000^{ns}
ABCD	1	0.105^{ns}	7.076**	0.173**	0.010^{ns}	1612.749**	107.461**	16.497^{ns}	0.349^{ns}	0.006^{ns}	0.001^{ns}
ERORR$_2$		0.195	0.286	0.090	0.004	88.767	16.853	4.905	1.433	0.007	0.015
	C. V	2.65	3.18	3.21	2.02	5.69	3.93	4.98	2.84	3.10	11.14

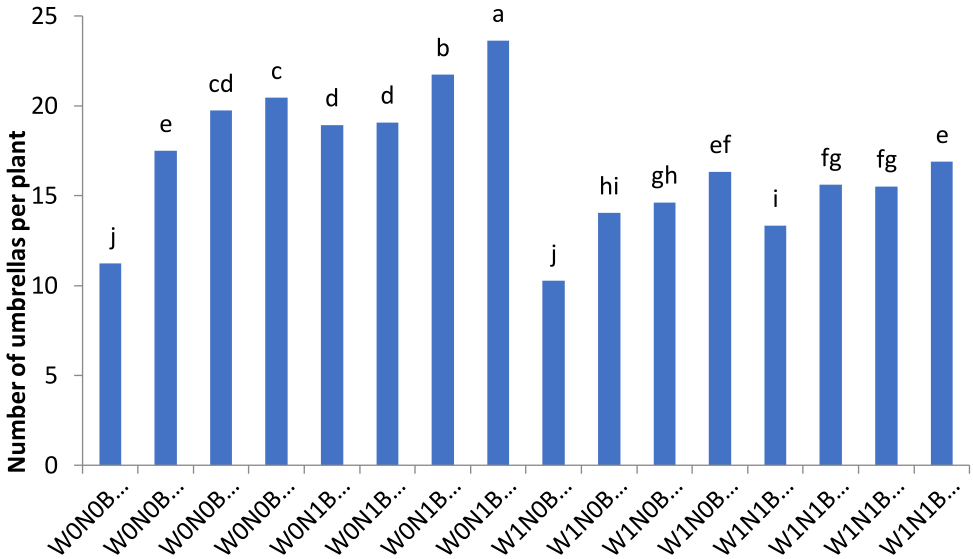
Significant at 1% level, * significant at 5% level and ns: Non-significant, R: repeat, A: drought stress, B: nitroxin, C: fertile phosphate, D: superabsorbent

3.3. Number of umbels per plant

The findings indicated that the interplay between bio-fertilizers, superabsorbent polymers, and drought stress significantly (P<0.01) impacted the umbel count of cumin plants (Table 2). Based on the average comparison results, the treatment containing super absorbent polymer, Nitroxin, Barvar2, and Barvar2 produced the greatest number of umbels per plant (23.63 umbels) in the absence of drought stress. Conversely, the treatment lacking biological fertilizers and super polymer yielded the fewest umbels (10.27). The adsorbent was acquired in conditions of drought stress (see Figure 3). Under conditions of drought stress, the number of umbels produced per plant is diminished among the treatments. In contrast to the control, the combined application of Barvar2 bio-fertilizers and Nitroxin with super absorbent resulted in the greatest number of umbels per plant. The superabsorbent polymer appears to mitigate the impacts of drought stress through its ability to retain moisture, create an optimal environment for bacterial activity in bio-fertilizers, and enhance the solubility of soil nutrients. Azizi (2017) demonstrated that the application of superabsorbents in conjunction with phosphate barvar2 biofertilizers and nitrogen had a beneficial impact on a variety of quantitative and qualitative characteristics of the fennel plant. These findings corroborate the results obtained in the present study.

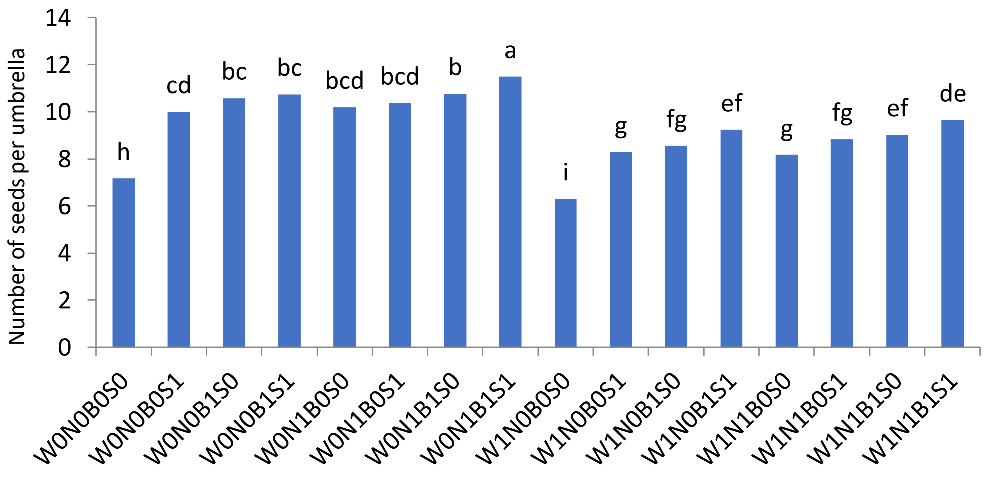
3.4. The number of seeds in the umbel

The findings of the investigation indicate that the interplay between Nitroxin biofertilizers, Phosphate Barvar2, superabsorbent polymer, and drought stress significantly impacts the quantity of seeds in the umbel (Table 2). In the absence of drought stress, the highest number of seeds in the umbel, as determined by the average comparison results, is 11.49, which is attributable to the mutual effects of superabsorbent polymer, Nitroxin biofertilizer, and Phosphate Barvar2. The umbel contains a minimum of 6.30 seeds, which can be attributed to the reciprocal effects of forgoing the application of bio-fertilizers and superabsorbent polymer under conditions of drought stress (see Figure 4). Drought stress consistently influences the number of seeds in the umbel, an attribute that is also genetically determined. Reducing the quantity of seeds contained within the umbrella will invariably result in a decline in the seed yield. The findings indicated that the application of superabsorbent polymer-containing biofertilizers Nitroxin and Barvar2 in drought-stressed treatments increased seed production and mitigated the negative effects of drought stress in comparison to the other treatments. This can be construed to mean that the plant maximizes umbel production under irrigation conditions by capitalizing on all available environmental factors, including adequate development of vegetative organs and suitable synthesis of photosynthetic materials. Sufficient seeds are acquired at this level as a consequence. Due to the reduction in production and storage of photosynthetic materials and the occurrence of drought stress, the quantity of umbels decreased, and consequently, so did the number of seeds contained within each umbel. Rahimi (2012) reported that an increase in drought stress led to a reduction in both the number of umbels per plant and the number of seeds per umbel of cumin. These findings align with the results obtained in the present research.



W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 3. Comparison of the mean of the four interactions of Nitroxin and fertile bio-fertilizers with superabsorbent under drought stress conditions on the number of umbrellas per cumin plant

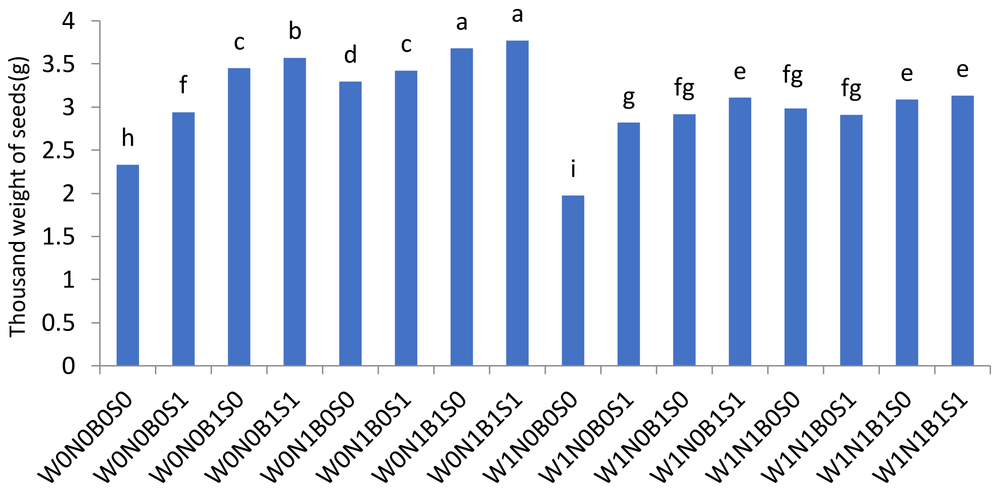


W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 4. Comparison of the mean of the four interactions of nitroxin and fertile biofertilizers with the use of superabsorbent under drought stress conditions on the number of seeds per cumin

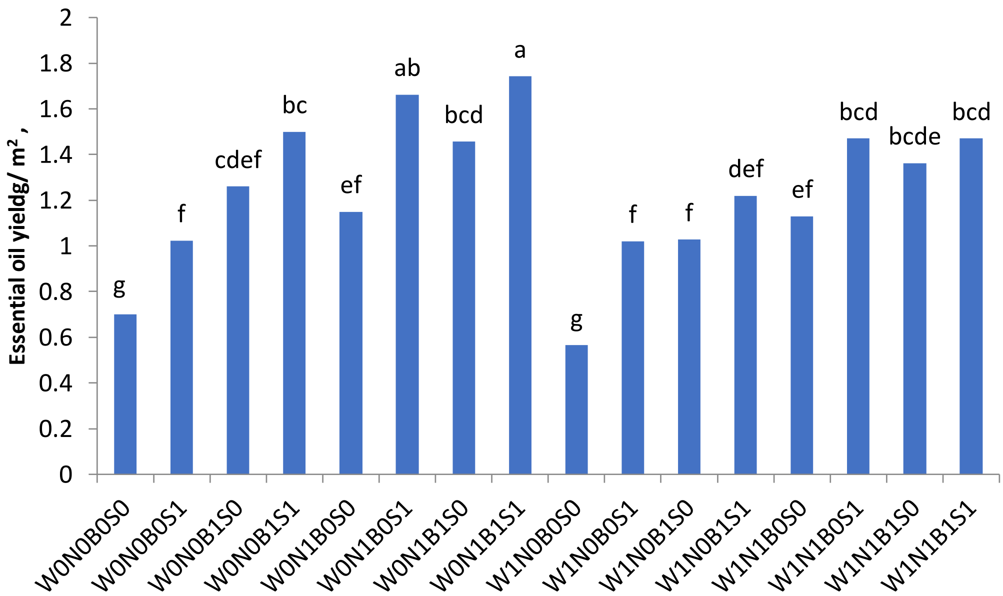
3.5. Weight of one thousand seeds

The findings indicate that the weight of one thousand seeds is not significantly impacted by the four interaction effects between superabsorbent polymers and Nitroxin and Phosphate Barvar2 bio-fertilizers under stress conditions (Table 2). To examine the impact of experimental treatments on the weight of one thousand seeds, the mean values of four interaction effects were compared. The findings indicated that the treatment involving the use of nitroxin, phosphate barvar2, and super absorbent polymer under drought stress conditions produced the heaviest weight of one thousand seeds (3.773 grams). Conversely, the treatment involving the absence of biological fertilizers and super absorbent polymer under drought stress conditions resulted in the lightest weight of one thousand seeds (1.973 grams) (Figure 5). Drought stress significantly impacted the weight of one thousand cumin seeds, resulting in a reduction in seed weight. In comparison to the other treatments subjected to drought stress, the combined application of Barvar2 with superabsorbent polymer and Nitroxin bio-fertilizers yielded a greater thousand seed weight. Drought stress appears to impede the transpiration of essential water and the transportation of nutrients to the seeds. Conversely, by diminishing the transfer of photosynthetic materials to the seeds, one thousand seeds undergo shriveling and their weight decreases. Utilizing animal manure and growth-stimulating bacteria increased the number of umbels per plant, the weight of one thousand seeds, and the seed yield of the medicinal coriander plant, according to the findings of Darzi et al. (2006). According to their statement, the co-administration of Azospirillium bacteria and animal manure likely facilitated nutrient uptake in the soil, thereby contributing to the weight augmentation of one thousand seeds.



W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 5. Comparison of the mean of the four interactions of nitroxin and fertile biofertilizers with superabsorbent under drought stress conditions on 1000-seed weight of cumin



W_0: No drought stress, N: Non- use Nitroxine, B_0 :Non- use Barvar$_2$, S_0 : Non- superabsorbent polymer w_1: drought stress, N_1: Use- Nitroxine, B_1: Use- Barvar$_2$, S_1 :Use- superabsorbent

Figure 6. Comparison of the mean of the four interactions of nitroxin and fertile biofertilizers with superabsorbent under drought stress conditions on cumin essential oil yield

3.6. Essential oil yield

The findings indicate that the four interaction effects between superabsorbent polymers and Nitroxin and Phosphate Barvar2 bio-fertilizers do not exert a statistically significant impact on the yield of essential oil when subjected to stress conditions (Table 2). The highest essential oil yield (1.743% per square meter) was observed in the treatment of Nitroxin and Barvar2 biofertilizers utilizing superabsorbent polymer under drought-free conditions, as determined by a comparison of the averages. The minimum value, which corresponds to 0.566 grams per square meter, is associated with the treatment of the quadruple mutual effects resulting from the absence of superabsorbent polymers and Nitroxin and Barvar2 biofertilizers under drought stress conditions (Figure 6). It appears that biofertilizer treatments have significantly created more favorable conditions for enhancing beneficial microbial activities in the soil, increased biological yield via the roots' favourable absorption of macro and micro mineral elements, and increased the yield of essential oils in comparison to the control treatment. Biofertilizers increase the yield of essential oil from fennel, according to some researchers (Mona et al., 2008).

4. Conclusion

The findings of the study indicate that the implementation of a superabsorbent polymer, phosphate Barvar2, and Nitroxin biofertilizers combined under drought stress conditions, particularly in the rainfed cultivation of cumin plants, improved nutritional conditions (by supplying moisture to the plant and increasing the solubility of nutrients). This combination effectively mitigates the detrimental impacts of drought stress conditions and yields favorable outcomes for both seeds and biological production. Hence, in accordance with the advancement of sustainable agriculture and the well-being of agricultural commodities, while also mitigating the detrimental environmental impacts of chemical fertilizers and optimizing water usage, it is advisable to encourage and cultivate cumin plant in conjunction with the utilization of biofertilizers and superabsorbent materials, given the recent drought scarcity and the minimal moisture requirements of cumin plants.

Acknowledgment

We express our gratitude to Lorestan University for granting us credit and furnishing the essential infrastructure to conduct this research.

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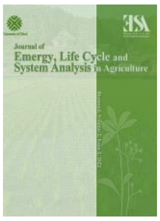
Journal homepage: jelsa.uoz.ac.ir



Changing carbon sequestration potentials based on different land uses; A review

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ARTICLE INFO

Article history:
Received: 6 January 2023
Accepted: 8 March 2023
Available online: 10 March 2023

Keywords:
Agriculture
Carbon sequestration
LULC
SOC

ABSTRACT

The organic carbon in soil is correlated with soil quality and is important for evaluating management practices and their related structural and functional consequences in land uses. Carbon input to different ecosystems varies with plant type, soil fertility, management practices, and climatic conditions. This review evaluates carbon sequestration potentials in various land uses, land use change effects on carbon sequestration, and ways to increase carbon sequestration in these land uses. According to various studies, protected forest ecosystems and cereal croplands had respectively the highest and lowest carbon sequestration rates compared to other ecosystems. In most of the reviewed cases, land use change reduces vegetation cover and prevents the maintenance of organic matter in the soil. Heavy soil destruction is based on the alteration of natural ecosystems into agroecosystems and urban land uses. In contrast, forests supply 20 to 100 times more carbon than croplands. Soil organic carbon content in agricultural lands is approximately 15-30% lower than in natural soils. Finally, it could be concluded that management practices and policies could strongly influence the carbon sequestration process. On the other hand, in all land uses, carbon sequestration potential can be increased by appropriate management activities. Thus, more attention to carbon sequestration for sustainability development and reasonable management is essential in landscape planning and policy support actions.

Highlights

- Carbon sequestration varies across ecosystems based on plant types, soil conditions, management, and climate.
- This review explores land use's impact on carbon storage and potential improvements.
- Land use changes that reduce vegetation or disturb soil typically reduce carbon storage.
- Converting natural ecosystems to agriculture or urban areas significantly reduces carbon storage.
- The article highlights the significance of carbon sequestration in sustainable development plans and policies.

1. Introduction

The atmospheric concentration of CO_2 increased by 31 percentage since 1750 (Sharma, 2005). Removing carbon from atmosphere and storing it in the terrestrial environment is one of the best options proposed to reduce greenhouse gas (GHGs) emissions (Albrecht and Kandji, 2003). One present approach for increasing global carbon storage and reducing CO_2 is carbon sequestration in soils. The carbon sequestration refers to the process of removing carbon from the atmosphere and depositing it in a reservoir while carbon storage refers to the quantity of carbon stored in a reservoir. The important variables in this approach are the capacity of soil carbon sequestration and some properties such as organic carbon amounts and bulk density (Olson, 2010). Soil organic carbon (SOC) sequestration was defined by Olson et al., (2013) as "the process of transferring CO_2 from the atmosphere into the soil through plants, plant residues, and other organic solids, which are stored or retained as a part of the humus". Recent studies have revealed that the dynamics of the SOC pool depends on the balance between input and output of carbon through different ways (Ding et al., 2012; Fan et al., 2014).

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https://doi.org/10.22034/jelsa.2024.419541.1054

SOC is primarily governed by land use/land cover (LULC), soil genoform and climate. However, land use per se has a significant influence on the soil carbon sequestration (Upadhyay et al., 2005; Zhang et al., 2012; Ross et al., 2016). According to various studies, recognizing relations between LULC and SOC provides essential data for estimating the LULC change effects on carbon pools in soils and it can be used for guidelines of mitigating anthropogenic GHGs emissions (Ross et al., 2016).

Upadhyay et al., (2005) demonstrated that the carbon sequestration process is a function of LULC changes and agricultural practices which are determined by socioeconomic criteria such as population. In developing countries, past land use changes, especially agricultural expansion and deforestation, have seriously affected the global warming process through emissions of GHGs (The Environmental Literacy Council, 2015). These are affecting the climate system, supply of forestry products, biodiversity, and soil degradation. The environmental degradation (e.g. from forests to rangelands or croplands) and global climate change became a significant concern globally (Wali et al., 1999). Land use change with improper management is one of the major reasons for creating greenhouse effects and global earth warming during recent decades (Fitzsimmons et al., 2004). The agricultural sector is responsible for nearly one-third of global warming and climate change (Tan and Lal, 2005).

It seems that carbon sequestration programs are win-win strategies (Lal, 2004) everywhere and all the time. In fact, carbon sequestration potential can be doubled by proper management activities (Askari et al., 2014). It restores eroded soils, enhances purifies water, plant biomass, land covers, organic matters, and reduces the enrichment level of carbon in the atmosphere (Lal, 2004).

Land use change often reduces vegetation cover and consequently prevents the preservation of soil organic matter. Restoring degraded land (a land that has lost some degree of its natural productivity) towards natural vegetation cover reduces these effects, and serves in favor of the accumulation of carbon by mediating of increasing carbon inputs to the soils as organic matter. These changes support to improved soil functions and fertility (Upadhyay et al., 2006).

Estimations of soil carbon sequestration show the different impacts for each of the LULC. Specifically, the difference of carbon sequestration relates to the type of management in land uses. Thus, it is influenced by locations, plant species type and management operations (Mortenson and Shuman, 2002). There are many processes and factors determining the rate and direction of change in soil carbon when soil management practices and vegetation are changed. These factors and processes comprise:

(1) rising the amounts of organic matter,
(2) altering the decomposability of organic matter that enhance SOC,
(3) situating organic matters in a deeper position
(4) improving physical protection by improving aggregation or organic mineral aggregates. These processes usually occur, when soils are converted from the annual plants cultivation to permanent vegetation (Conant et al., 2001).

In some countries such as Brazil, land-use changes have a powerful influence on carbon emission. The assessment of carbon sequestration and accumulation in plant and soils is needed to stabilize sufficient land cover to reduce soil erosion (Brown and Pearce, 1994; Bellassen et al., 2008). Post and Kwon (2000) concluded that vegetation types and LULC changes significantly influence carbon flux and accumulation, also soil respiration. Soil carbon sequestration diminishes with increasing depth of soil. Rice (2000) stated grazed rangelands have more carbon sequestration potential than non-grazed rangeland and soil carbon sequestration is decreasing with depth. For example, in the 0-20 cm soil depth, organic carbon content was higher than in 20-40 cm depth (Chibsa and Ta'a, 2009).

Carbon input and sequestration to ecosystems varies with vegetation type, management practice, soil fertility, and climatic condition. Basically, vegetation type or plant community refers to members of a group or aspect of plants that are often found growing in area together. They differ from the life forms, photosynthesis cycle, morphology and etc. Thus, vegetation types have different ability to carbon sequestration. Carbon sequestration in soils has the capacity to mitigate GHGs emissions, as well as to improve soil biological, physical, and chemical properties. Soils can act as a net source or sink of CO_2 and thus influence the process of global climate change (Godde et al., 2016). Kay (2000) and Celik (2005) confirmed a negative correlation between erosion and soil organic material. Consequently, Zhang et al. (2012) presented a range of carbon input from 1.6 to 2.1 Mg carbon $ha^{-1}yr^{-1}$ in without chemical fertilizer consumption condition to 2.6–5.1 Mg carbon $ha^{-1}yr^{-1}$ for chemical fertilization treatment alone among some rice-rice cultivation systems in southern China. Also, climatic condition can be effect on carbon sequestration rate. For example, carbon uptake in the forest ecosystem may increase or decrease marginally with a corresponding increase or decrease in precipitation, however with an increase in temperature, carbon uptake may decrease significantly showing that warming may be the main climate factor that impacts carbon storage in a tropical dry forest (Dai and Dupuy, 2015). Based on available scientific knowledge, these different carbon sequestration potentials were reviewed in this paper.

2. Research gaps and objectives

The literature review confirmed different LULC have unlike effects on the carbon sequestration. However, the detailed processes and relations have not yet been described comprehensively. For that reason, this review aims to evaluate carbon sequestration rates in various land uses and LULC change effects on soil carbon sequestration. The objectives of this paper are the outline of carbon sequestration potentials in four important land uses affected by current human activities. These include agriculture, rangeland, forest, and urban land uses. The ways of increasing the carbon sequestration in these land uses and effects of land use changes in carbon sequestration or increasing the rate of CO_2 in the atmosphere are discussed.

3. Research method

To address our research objectives, we conducted an extensive literature review to the evaluation of carbon sequestration potentials based on different land uses and ways increase the carbon sequestration. To refine the collection of searched literature that met our criteria, Scopus, Science Direct and Web of Science as the world's main citation databases were used. The search in web was set from the date of the first related article until the year 2019. Most of the publications are concentrated between 2002 and 2019. Several papers were identified by assessment of the bibliographies in the papers. The following keywords were used at each query: 1) carbon sequestration, 2) changing land use, 3) agricultural land use, 4) rangeland, 5) forest, and 6) urban areas. The consequence of this search is introduced in the Results and Discussion sections.

4. Results

4.1. Carbon sequestration in agricultural land uses

In agricultural ecosystems, a large part of carbon is accumulated in the soil. The input of carbon to these soils is recognized by the net primary production and it's residual on the field. Also, loss of carbon is recognized by decomposition and destruction of topsoil through erosion. The decomposition rates are related to environment temperature, soil chemical and physical conditions. In addition, carbon loss occurs from the following ways: low residues on the topsoil, use of the moldboard plow (MP), conventional cultivation practices, crop residue burning, and conventional agriculture system performance (Table 1). Generally, low crop yields, increase of soil carbon and high decomposition of organic matter rates can increase the loss of carbon from agroecosystems (Freibauer et al., 2004). Increased yields haven't produced higher input of carbon treatment because increasing yields were principally obtained through changes in the cropping system (Evans, 1993). While yield increases, the amount of plant residue is decreasing (Freibauer et al., 2004).

Agricultural soils can be a source or a sink for carbon, which is dependent on the actual SOC (Vleeshouwers and Verhagen, 2002) and appropriate management practices (such as conservation tillage, cover crop and cropping rotation) on croplands can decrease the amounts of enrichment of CO_2 in the atmosphere (Lal, 2004). The existing high content of SOC in agricultural lands can be related to fertilization operation, where related actions can enhance and improve soil structure (Carter, 2002). The results of Mortenson and Shuman (2002) showed major differences in the carbon sequestration in the current land uses especially in protected forests and cereal croplands. Usually, cropland soils are depleted in SOC as compared to soils under other regions. Agricultural land use shows the SOC losses of 30–40% compared to natural or semi-natural ecosystems (Don et al., 2011; Poeplau et al., 2011; Poeplau and Don, 2015). Previously, McGill et al., (1988) estimated that SOC of agriculture lands was approximately 15 to 30% lower than soils of natural ecosystems.

Many reports have recommended no-tillage (NT) as a practice to mitigate GHGs emissions through soil carbon sequestration (Ogle et al., 2012; West and Marland, 2002; Johnson et al., 2007). In croplands, NT method has been suggested to replace chisel and moldboard plow (MP) systems as a way to sequester SOC (Luo et al., 2010; Ogle et al., 2012). Baker et al. (2007) and Luo et al. (2010) for example suggested to farmers, altering from MP systems to NT as having great potential for SOC sequestration. Challenges still continue to understand the complete impact of NT adoption on soil organic carbon pools (Ogle et al., 2012). Gonçalves et al., (2019) were used as a database for long term soil management based on the conservation agriculture principles include minimum tillage, maintain crop residues and diversity. Their results demonstrated that SOC continuously increased after conservation management performance adoption in 1985 until 2015 and the carbon sequestration potential for sub-tropical croplands was 2.5 Pg carbon at 0-20 cm and 11.7 Pg carbon at 0-100 cm. Principally, conservation agriculture is a farming system that maintains a permanent soil cover to assure its protection and avoids soil tillage. It reduces land degradation and increase biodiversity and water and nutrient use efficiency (FAO, 2016). In another study, Zhang et al. (2013) analyzed the soil carbon sequestration based on the DNDC model and resulted in that NT system can be the main advance in green technology in North China. Alam et al., (2019) concluded that rice fields under non-puddled system and with increased crop residue retention are an effective GHGs mitigation alternative in "Northwest Bangladesh".

Sewage sludge amendments or organic manure, incorporation of straw and intensification through ley rotations have been suggested as those approaches for increasing carbon inputs in agricultural land uses are (Smith et al., 1997). Recently, the production of winter cover crops was introduced by Mazzoncini et al., (2011). Principally, cover crops termed catch crops or intercrops are those plants replaced bare fallow in winter and are plowed under as green manure before the sowing of the main plant. These crops have a considerably high SOC than the main crop (Poeplau and Don, 2015).

In agricultural systems, the plants with C4 carbon fixation pathway remarkably lead to the greater carbon accumulation and consequently increased the carbon supply in the soil. Srinivasarao et al., (2016) conducted a study with two levels of CO_2 of 550 and 700 m-mol mol^{-1} on some C3 and C4 crops under rainfed conditions, during 2005–2010. Observations revealed that the carbon pool and carbon management indicators decreased at 700 m-mol mol^{-1} levels of CO_2. Their results indicated that the higher root biomass of C4 plants contributed to the higher carbon input and stock in the soil. In another study, Yan et al., (2013) investigated the carbon input and SOC stabilization in paddy and upland soils under different fertilization practices. Their results indicated that the carbon sequestration efficiency was better in paddy soil than in upland soil, which may be attributed to greater physical and chemical stabilizations but lower microbial activity in paddy fields. As a conclusion, we can increase the carbon sequestration in agricultural land use by some methods and systems include NT system, sewage sludge amendments, organic manure, ley-farming rotation, cover crops, cropping higher biomass plants (C4 plants), agrobiodiversity, organic farming systems, conservation agriculture systems, agroforestry, perennial crop cultivation, crop residue mulch and intercropping system (Table 1). Agrobiodiversity is the sub-set of general biodiversity directly developed and managed by humans. It refers to the biodiversity of agroecosystems along with species of crops and farm animals, and the genetic variance within populations, varieties and races (Kazemi et al., 2018). According to the International Federation of Organic Agriculture Movements (IFOAM, 2004), organic farming is an agricultural system that promotes environmentally, socially and economic sound production of food and fibers, and excludes the use of synthetically compounded pesticides, fertilizers, livestock feed, growth regulators and genetically modified organisms. Organic farming considerably increases the carbon storage in the soil than the other current agricultural systems. Also, agroforestry is the collective term for land use systems and technologies in which woody perennials (such as trees and shrubs) and agricultural crops or animals are used deliberately on same parcel of land in some form of spatial and temporal arrangement (FAO, 2020). This integrated system has the capacity to carbon sequestration.

Table 1. Ways to reduce / increase of carbon sequestration in different land uses.

Land use	Loss of carbon sequestration by…	Increase of carbon sequestration by …
Agriculture	Topsoil erosion	No-tillage system
	High decomposition of organic matter	Sewage sludge amendments
	Low crop residue	Organic manure
	Moldboard plow (MP) systems	Ley- farming rotation
	Conventional cultivation practices	Cover crops
	Conventional agriculture system	Cropping higher biomass plants (C4 plants)
	Crop residue burning	Agrobiodiversity
		Organic farming
		Conservation agriculture
		Agroforestry
		Intercropping systems
		Perennial crops cultivation
		Crop residue mulch
Rangeland	Traditional management	Exclosure
	Over-grazing	Converting marginal agricultural lands to pastures
	Low precipitation	High precipitation
	Convert to farms	Chemical fertilizers
		Irrigation management
		Sowing legumes and grasses or other species adapted to the environment
		Improvement of soil fauna
		Grazing management
		Sustainable grazing
		Sowing improved species
		Direct inputs of water, fertilizer or organic matter
		Restore degraded lands
Forest	Deforestation	Protected forests
	Fuel-wood utilization	Growth of wood biomass species
	Loss of soil	Conserve forest soils
	Changes in land use	Tax for CO_2 emissions
Urban	Urbanization	Urban design
		Urban forests
		Urban green spaces
		Green roofs
		Street trees
		Urban agriculture
		Urban Parks
		Cultivation of conifers species, turfs, and home lawns

4.2. Carbon sequestration in rangeland land use

Rangelands include the biggest and various resources of the land surface (Reeder and Schuman, 2002; Lund, 2007). Because of the rangelands extent, a little variation in soil carbon contents of rangelands would have a great effect on GHGs and carbon sequestration (Follett et al., 2001). The conventional management of these ecosystems had resulted in physiognomic and floristic changes, losses of SOC, and desertification (Golluscio et al., 1998; Lal, 2002). Thus, providing political and financial incentives to organize rangelands sustainability as carbon sinks offer major carbon sequestration situation.

Principally, the major differences between rangelands and pastures are the kind of vegetation and level of management that each land area receives. Basically, rangelands are those lands on which the native vegetation is predominantly grasses, grass-like plants, forbs, or shrubs suitable for grazing or browsing use. They include natural grassland, savanna, many wetlands, some deserts, tundra, and certain forb and shrub communities. But, pastures are those lands that are primarily used for production of adapted, domesticated forage plants for livestock (EPA, 2020)

Typically, in rangelands, the vegetations have a huge influence on SOC and a large amount of SOC is form in the topsoil as a result of the influence and presence of biotic processes (Conant et al., 2001). If the vegetation mass is improved, the amount of carbon within the plant-soil system increases (Mahdavi et al., 2011). The results from Askari et al. (2014) demonstrate that the highest amount of organic carbon was observed in rangelands in comparison to other land uses. Yazdanshenas et al., (2018) reported that carbon storage depends on type of vegetation and soil surface cover in rangelands of Isfahan province (Iran).

Rangelands continuously have been grazed by animals, and as such, those rangelands exhibited a little amount of SOC. For instance, Joneidi Jafari (2009) concluded that grazing management has a significant influence on the capacity of soil carbon sequestration. Subsequently, the livestock density and deforestation led to an approximately 12 percentage decrease in soil carbon in Iran.

Based on the results of Askari et al., (2014), the amount of SOC in the first 20 cm of the topsoil of rangeland is the highest and consequently, carbon sequestration is the highest. The lowest amount of SOC and carbon sequestration observed in converted rangeland to farm. As a result, no significant difference was seen among land uses and different depths for carbon sequestration except for soil surface layer of rangeland (Table 2).

Table 2. The mean amount of carbon sequestration in different land uses in two soil depths (Askari et al., 2014).

Land use type	Soil layer depth(cm)	Organic carbon percent	Carbon sequestration (ton ha^{-1})
Agriculture	0-20	1.35	29.16b
	20-40	0.9	23.98b
Rangeland	0-20	1.9	50.92a
	20-40	1.08	25.04b
Olive planting	0-20	1.25	27.48b
	20-40	1.37	31.51b
Converted rangeland to farm	0-20	0.82	20.04b
	20-40	0.66	14.99b

The results of another study (Niknahad Gharmakher et al., 2015) revealed significant differences between SOC in different soil depths under different management systems (Table 3). They calculated that the carbon sequestration on the grazing and exclosure systems were about 52.45 and 71.78 ton carbon ha^{-1}, respectively. Feyisa et al., (2017) addressed the effects of enclosure management (15–37 years old) on carbon sequestration in East African rangelands. They reported that enclosure system had a significant role in carbon sequestration and, SOC was higher in this system than open grazing systems.

Restoring degraded rangelands is important to carbon sequestering. In addition, shifting marginal agricultural lands to pastures can sequester carbon. Similar to agricultural lands, management alternatives for improving rangelands include use of fertilizers, grazing management, irrigation management, sowing grasses and legumes, planting stress-resistant species, and enhancement of soil fauna (those organisms that inhabit the soil) (Follett et al., 2001; Lal, 2004; Niknahad Gharmakher, 2015). These activities can enhance productivity in rangelands while promoting carbon sequestration. Overall, the rate of carbon sequestration in the grasslands is reduced by inappropriate management, overgrazing, converted rangeland to farms, and low annual precipitation (Table1).

Table 3. The mean of soil carbon rate on exclosure and grazing area in two soil depths. (Niknahad Gharmakher et al., 2015).

Region	Soil layer depth(ton ha^{-1})	Organic carbon percent	Soil Carbon organic (ton/ha)	Total soil Carbon organic (ton ha^{-1})
Exclosure	0-10	6.08	42.12a	71.78a
	10-20	3.655	29.66b	
Grazing area	0-10	4.06	26.87a	52.45b
	10-20	3.38	25.58a	

4.3. Carbon sequestration in forest land use

A number of pools and fluxes characterize the carbon cycle in forest ecosystems. Pools are locations of carbon in the forest, forest floor, and soil. Each pool contains a quantity of carbon that is referred to as the stock. Carbon transfers between pools happen through different processes (fluxes), including photosynthesis, combustion, and respiration (Byrne and Black, 2003).

Forest management can extremely influence the carbon budgets and fluxes. Forests can have a key influence on climate change through the carbon emission or sequestration. In forest ecosystems, carbon is captured in the tree biomass and also in soils (Sedjo and Sohngen, 2012). Joneidi Jafari (2009) indicated that in forest areas including species from Aceraceae and Rosaceae families, and lack of invasive species in lower stands, carbon sequestration can be increased by around 23 percentages in the forest soil of Iran. In another project, Mortazavi Jahromi (2006) has revealed that the tree biomass is straightly associated to carbon sequestration and usually, protected forests in Iran have the highest amounts of carbon sequestration than other studied land uses.

Forests accumulate 20–100 times more carbon per unit area than agricultural lands and therefore have a serious responsibility in reducing GHGs, and thus increasing the SOC (Brown and Pearce, 1994). In research of Jafari and Mesri (2015) in Iran, the amounts of carbon sequestration for seven land uses were estimated (Table 4). This research showed that each ecosystem had a special impact on the carbon sequestration amount. Based on this research, the highest and lowest of carbon sequestration amount were obtained in the protected forests and cereal agroecosystems, respectively. Also, the protected forest had 2-5 times more carbon sequestration than other studied land uses (Jafari and Mesri, 2015). Chu et al., (2019) were assessed the forest carbon sequestration in the “Three North Shelterbelt Program” region (China), using the

InVEST model during 1990–2015. Their results showed that carbon sequestration reduced by 13.37 Pg carbon with a reduced rate of 1.92% in 1990–2015 and shrubs were more appropriate than trees. In another study, carbon sequestration amount is calculated at 274,571 tons for experimental forest of "National Taiwan University, in Nantou County" (Taiwan) (Chang et al., 2017).

With the literature, we demonstrate carbon sequestration varies in forest ecosystems. This land use plays an important role in the carbon budgets and fluxes, thus, a comprehensible understanding of this role is necessary to increase the SOC content and consequently carbon sequestration rate. According to various studies, land use changes, deforestation, soil erosion and wood consumption can reduce the carbon sequestration potential in the forest ecosystems (Table1).

Table 4. Variations of carbon sequestration in different land uses (Jafari and Mesri, 2015).

Land sue	Organic carbon percent	Bulk density (gr.cm^2)	Carbon sequestration (ton.ha^{-1}) (depth 0-30 cm)
Protected forest	8.57a	10a	257100a
Open forest	2.75b	10a	82500b
Walnut –apple garden	3.60b	7a	75600b
Walnut garden	3.80b	8a	91200b
Rangeland	4.67b	8a	112080b
Frijol farmland	3.54b	7a	74340b
Cereal cropland	4.22b	4b	50640b

4.4. Carbon sequestration in urban land use

Urbanization severely changes the ecosystems functions and structure, environmental equilibrium (a state of dynamic equilibrium within a community of organisms), disrupts cycling of carbon, other elements, and water. Lal and Augustin (2012) predicted that in 2050, about 70 percentages of the people will live in cities on a global scale. The number of megacities reached to 37 in 2017. Yet, urban regions have a huge carbon sink capacity in soils and biota. Effective management and judicious planning can increase SOC in these ecosystems and offset some of the anthropogenic emissions. In urban land use, main components with regards to carbon sequestration include turfs, green roofs, home lawns, urban forests, park and recreational/sports facilities and urban agriculture (Table 1). A lawn is a piece of residential, commercial or industrial land on which grass grows. Basically, turf is the term used by horticulturists referring to grass that is mowed and maintained with the same uses as a lawn. Grass used in a landscape customarily is referred to as a lawn while grass used on a baseball field or golf course is referred to as turf. Turf is valuable in the landscape for environmental contributions such as protecting soil from erosion, capturing runoff water, reducing dust and heat irradiation (Peffley, 2016).

Urban regions release a high amount of the GHGs (Svirejeva-Hopkins et al., 2004) and supply somewhere about 40 - 85% of total GHGs emissions (Satterthwaite, 2008). At the global scale, the study of ten cities shows how a balance of technical factors (urban design and shape, power generation, and waste processing) and geophysical factors (climate, gateway status and access to resources) indicate the GHGs attributable to these cities (Kennedy et al., 2009). The effects of development of urban on climate change are intensified by reduce of carbon contents (Hutyra et al., 2011a). In addition, soils have a low carbon sequestration in the urban land use (Pouyat et al., 2006; Sallustio et al., 2015).

A united approach is required for soil carbon management in the urban land use. The main factors such as the managers and users, local professionals, NGOs and local government affect on the carbon management in urban regions (Lorenz and Lal, 2015).

Urban forests accumulate carbon. They influence on air temperature and also change carbon releases from many urban sources (Nowak, 1993) such as transport, services, and goods production and household consumption (Abdollahi et al., 2000; Wilby and Perry, 2006; Gill et al., 2007). Some researches propose that urban trees may be an important carbon sinks in the carbon cycle (Velasco et al., 2016; Nowak et al., 2013). For example, the total tree carbon content and carbon sequestration in urban regions of the U.S. were estimated at 643 and 25.6 million tons per year, respectively. The entire urban tree carbon storage and sequestration were calculated as 7.69 and 0.28 kg C m^2 of tree cover per year, respectively (Nowak et al., 2013). Velasco et al. (2016) were analyzed the CO_2 flux in Mexico City and Singapore. Their results suggested that vegetation in sub-tropical regions either acts as an emission sink or source of carbon and the biogenic influence to the total CO_2 flux is 1.4% in Mexico City and 4.4% in Singapore. Capability of CO_2 sequestration and economic value of four parks in Rome (Italy) were analyzed by Gratani et al., (2016). Results indicated that tree-lined streets/avenues presented the highest amounts of carbon sequestration and the economic value of this sequestration was $ 23,537/ha.

Nowadays, many indices apply for estimation of carbon sequestration potential. For instance, Scharenbroch (2012) used growth rates, life spans, maximum tree sizes, and tolerances to urban stress for tree species. Hostetler and Escobedo (2016) recommended that green space with mowed, pruned shrubs and trees, and irrigated and fertilized lawns are better for the performance of carbon sequestration programs.

The soil carbon stocks of urban green spaces were calculated in three cities with rapid development in South Korean. Based on results, the soil carbon stock was 105.6 for Seoul, 26.4 for Daejeon and 43.6 for Daegu (Yoon et al., 2016). In this respect, Abbasnejad and Khajeddin (2012) evaluated the urban reforestation effect on carbon sequestration by Quick Bird satellite imageries. Their survey shows that the reforestation on a barren area increased sequestration amounts of carbon.

Nowadays, the effects of urban expansion patterns on GHGs and carbon sequestration are issues of the scientific societies all around the world. Typically, urban land use plays an important function in the GHGs emissions, thus, a clear understanding of this problem can improve the potential of carbon sequestration in urban. In final, we can increase the carbon sequestration in this land use by urban forests, urban green spaces, green roofs, street trees, urban design, urban agriculture, cultivation of conifers species, turfs and home lawns and urban parks (Table 1).

5. Discussion

At the global scale, LULC change is widely identified as a net storage of GHGs. To address this issue, associations between SOC, LULC classes, and in the first step LULC change must be investigated at the local scale (Ross et al., 2016). The first step toward soil destruction could be the conversion of natural areas into agricultural areas (Luciuk et al., 2000). In this regard, we can refer to the experiences of Xun et al., (2010) in China. They considered the LULC change effects on SOC, the nutrient in the semi-arid regions and carbon decomposition. Their results indicated that changing from cultivated land to shrub land or pastureland caused increasing soil carbon sequestration and improvement of soil nutrient fixation.

The loss of SOC usually observed during agriculture may be reversed by changing such system to permanent pasture (Jones et al., 2016). Many researchers have confirmed that the introduction of pastures for grazing after farming may recover soil structure due to enhance in SOC and roots activity and act as a buffer to SOC loss after harvesting (Elliott, 1986; Conant et al., 2001; Guo and Gifford, 2002).

The literature review showed that the LULC has a significant effect on SOC (Jones et al. 2016). The degree of the impact of LULC conversion differs and depending on the type of activity undertaken post-conversion and the ecosystem's resistance to change (Schipper et al., 2010; Seybold et al., 1999). This effect is exclusively high in intensive agricultural systems (Jastrow et al., 1996). Globally, carbon sequestration showed a negative relationship with initial carbon stocks in soil, and the effects of climatic variables on soil carbon sequestration are different between the LULC conversion types (Deng et al., 2016).

Based on available scientific knowledge, the correlation between biodiversity and SOC has been acknowledged with the coincidence of change with biomass value and carbon content. According to this, all practices that increase biodiversity in all land uses, can improve ecosystems potential to sequester carbon (Kazemi et al., 2018; Hajjar et al., 2008). Considering the carbon sequestration rate in the world, carbon sequestration potential can be increased by appropriate management activities in all land uses.

6. Conclusion and Outlook

This paper aimed to detect changes in carbon sequestration potentials based on different land uses. The results of the literature review show that almost land use changes have considerably reduced soil carbon in the past. Soil carbon stocks significantly increased after conversions from croplands to grassland but declined after change from grassland to cropland, forest to cropland, and forest to urban.

Based on results, a number of opinions need to be emphasized. First, the carbon sequestration rate is related to the carbon input rate in ecosystems. Carbon sequestration rates vary by climate, soil properties, topography, human-related activities, and management history. Some studies shown that the effects of climatic elements on carbon sequestration were closely associated to the land use change type. Also, in all land uses, current carbon sequestration potential can be increased by appropriate management activities.

Second, similar findings were reported by many researchers that indicated organic carbon sequestration in natural land uses is more than converted ecosystems. In addition, soils in urban areas have very low carbon rates. Soils under agriculture are depleted in carbon as compared to soils under natural areas and farming leads to SOC<30–40% than natural ecosystems.

Third, according to various studies, the protected forests and croplands under cereal production had the highest and lowest carbon sequestration compared to other land uses, respectively. Thus, more attention to carbon sequestration for sustainability development and reasonable management is essential in these land uses.

Fourth, management option can enhance productivity while promoting soil carbon sequestration in rangelands, forests, croplands, and urban areas. Accordingly, we recommend the following management practices to increase carbon sequestration in agroecosystems: application of conservation tillage, no-tillage system, cover crops, residue mulch, use of compost, green manure and manure in field’s fertilization, agrobiodiversity and other sustainable systems of water and soil. It is also suggested that the following options be developed in urban ecosystems: conserved urban green space, cultivation of conifers species, turfs and home lawns, developing urban agriculture, green roofs, parks, urban forests, and other effective management practices can enhance carbon pool in urban. Similar to urban land uses, management alternatives for improving rangelands include the use of grazing management plan, irrigation, and fertilizers management, sowing improved legumes and grasses and improvement of soil fauna. Moreover, according to various studies protected forests, growth of wood biomass species, conserve forest soils, tax for CO_2 emission increase the carbon sequestration potential in the forests.

Finally, it could be concluded that today, the soil carbon sequestration is a successful and win-win strategy in the all countries and ecosystems. Thus, this paper proposes that supplementary researches are required in order to introduce carbon sequestration ways in different land uses. Also, more attention to carbon sequestration for sustainability development and reasonable management is essential in landscape planning and policy support actions.

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Journal of Emergy, Life Cycle and System Analysis in Agriculture

Journal homepage: jelsa.uoz.ac.ir

Investigation of organic and chemical fertilizer on the essential oil and seed yield of Moldavian balm (*Dracocephalum moldavica* L.)

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ARTICLE INFO

Article history:
Received: 6 January 2023
Accepted: 18 February 2023
Available online: 1 March 2023

Keywords:
Azola
Dracocephalum moldavica L.
Essential oil
Nitrogen
Seed yield.

ABSTRACT

In order to examine the impact of biofertilizer on the essential oil and seed yield of Moldavian balm, a factorial experiment with a randomized complete block design and three replications were designed at the Khoy Agricultural Research Center in the West Azerbaijan Province. Five fertilization levels were applied: 100% urea (70 kg N ha−1), 75% urea (52.5 kg N ha−1) + 25% azocompost (3.85 ton ha^{-1}), 50% urea (35 kg N ha−1) + 50% azocompost (7.77 ton ha^{-1}), 25% urea (17.5 kg N ha−1) + 75% azocompost (11.55 ton ha^{-1}), and 100% azocompost (1555 ton ha^{-1}) on two Moldavian balm landrace (Urmia and the modern cultivar SZK-1). The results of this study indicated that a 100% urea fertilizer regime produced the highest dry herbage yield. The highest seed yield was achieved with a 100% urea fertilizer application of approximately 1,122 kg ha^{-1}. Inoculating seeds with Azotobacter and Azospirillum bacteria resulted in the highest harvest index, with a value of 22.4 percent. The Urmia landrace genotype had the highest yield of essential oil (20.5 kg ha^{-1}). Based on the findings, the 50% urea + 50% azocompost fertilizer treatment had the greatest effect on the majority of the investigated characteristics.

Highlights

- Five fertilization levels were examined on essential oil and seed yield to two Moldavian balm landraces.
- 100% urea fertilizer at 1,122 kg ha^{-1} yielded the most seeds.
- Azotobacter and Azospirillum bacteria inoculated seeds yielded the highest harvest index (22.4 percent).
- The 50% urea + 50% azocompost fertilizer treatment had the greatest impact on most of the characteristics.

1. Introduction

Since May 1978, the World Health Organization (WHO) has been studying medicinal plants. This study prompted the initial identification of 20,000 species of medicinal plants and a more detailed investigation of a short list of 200. Many of these plants have their origins in the world's tropical forests, and their present use is largely rooted in traditional medicines, which play a major role in maintaining the health and welfare of both rural and city dwellers in developing countries (Mahajan *et al.*, 2005). These days, around one third of human -needed medicines have herbal origins, and scientists, physicians, and pharmacologists all try to persuade people toward changing the other two thirds to medicinal plants too. Moldavian balm (*Dracocephalum moldavica* L., syn. Moldavian balm) is a perennial herb belonging to the Lamiaceae (Labiatae) family (El-Baky & El-Baroty, 2007). This plant is native to central Asia and has been naturalized in eastern and central Europe (Griffiths, 1994). In Iran, it is predominantly found in the north of the country, especially in the Alborz Mountains, where it is known as "Badarshoo." It is frequently consumed as a food additive (e.g., in yogurt) or as an infusion for its organoleptic properties. As a herbal drug, it is used in stomach and liver disorders, headache and congestion (Rechinger, 1986). Extracts of the plant are also used for their antitumor properties (Chachoyan & Oganesyan, 1996). Essential oil extracts from Moldavian balm have been reported to possess antibacterial,

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https://doi.org/10.22034/jelsa.2024.380025.1049

antimicrobial, and antioxidant activities (Kalinkina et al., 1991; Dastmalchi et al., 2007).

The indiscriminate use of nitrogen fertilizer in intensive agriculture has increased crop performance but also harmed ecosystems. Integrated nutrient management approaches advocate the controlled use of nitrogen fertilizer. Integrated nutrient supply involves the application of mineral fertilizers, bio-fertilizers such as those derived from *Azolla* spp., green manure crops, and bacterial inoculations (Richard & Woodbury, 1992; Epstein, 1997). The compost must be added to conventional NPK fertilizer to improve soil structure, making the soil easier to cultivate, encouraging root development, providing plant nutrients, and enabling their increased uptake by plants. Moreover, compost aids water absorption and retention by the soil, reducing erosion and run-off and thereby protecting surface waters from sedimentation; it also helps bind agricultural chemicals, keeping them out of waterways and protecting ground water from contamination (Leamaster et al., 1998). Members of the floating fern genus *Azolla* belong to the family Azollaceae. They host a symbiotic blue -green algae (*Anabaena azollae*), which can fix and assimilate atmospheric nitrogen. In Asia, *Azolla* spp. are used primarily to provide nitrogen nutrition to crops, such as rice. However, *Azolla* spp. can also accumulate other mineral nutrients, such as phosphorus and potassium, which become available to other plants when *Azolla* decomposes. Moreover, *Azolla* spp. are used as green manure, a water purifier, a biological herbicide, and as animal feed (Arora & Singh, 2003). Compost has already been established as a recommended fertilizer for improving the productivity of several medicinal and aromatic plants, such as mint, palmarosa, Moldavian balm, marjoram, *Acorus calamus* L., amaryllis, peppermint, *Tagetes erecta* L., chamomile, and basil (Rao, 2001; Kandeel et al., 2002; Naguib, 2003; Hussein et al., 2006; Gharib et al., 2008); However, little information is available about the effects of the combined application of azocompost and nitrogen fertilizer on the quality and quantity of essential oils in Moldavian balm plants. Thus, the objective of the study described herein was to determine the effects of different sources of nitrogen on the content and composition of essential oils in two genotypes of Moldavian balm in Iran.

2. Materials and methods

Field experiments were carried out during 2010 at the field research station of the Khoy Agricultural Research Center in West Azerbaijan Province (38º 35' N, 44º 52' E, and 1,040 m above sea level). Climate data for the growing seasons is provided in Table 1. The climate in this region is semi-arid, with warm and dry summers, a mean annual rainfall of 286.6 mm, and a mean annual temperature of 12 ºC. The soil is classified as a clay loam, with 38% clay, 22% silt, and 40% sand. The soil was air-dried and crushed before its pH, electrical conductivity (EC), and saturation percentage were evaluated. Next, we determined total organic carbon (using the Walkley and Black method, which involves sulfuric acid), total nitrogen (using the Kjeldahl method), available phosphorus (using the Olsen procedure), available potassium after extraction with ammonium acetate, and levels of the micronutrients iron, zinc, copper, and manganese after extraction with diethylene triamine pentaacetic acid (DTPA) (Tandon, 1995). Details of the properties of field soil are shown in Table 2. The experiment was laid out as a factorial in a randomized complete block design with three replications. They comprised a factorial combination of two genotypes (G1, the landrace genotype Urmia, and G2, the modern cultivar SZK-1), inoculation with bacteria (B1: Seed inoculation with Azotobacter and *Azospirillum* bacteria, and B2: No inoculation), and five fertilization regimes: F1, 100% urea (70 kg N ha^{-1}); F2, 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}); F3, 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}); F4, 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}), and F5, 100% azocompost (15.55 ton ha^{-1}). The physicochemical properties of the organic compost are shown in Table 3.

Table 1. Monthly temperature and precipitation during the growing season

Month	Average temperature (°C)			Total precipitation (mm)
	Minimum	Maximum	Mean	-
April	3.58	15.25	9.41	34.63
May	8.32	23.16	15.74	9.55
June	12.93	27.35	20.14	52.93
July	17.12	33.58	25.35	11.01
August	16.52	32.31	24.41	4.03

Table 2. Physicochemical characteristics of field soil

Soil Characteristics	Values
EC (dS m^{-1})	1.24
pH	7.7
Organic carbon (%)	0.81
Total N (%)	0.075
Available P (mg kg^{-1})	4
Available K (mg kg^{-1})	150
Fe (mg kg^{-1})	8.4
Zn (mg kg^{-1})	1.14
Cu (mg kg^{-1})	2.6
Mn (mg kg^{-1})	7.4

The composted manure and half of the total urea applied were broadcast by hand and incorporated immediately into the soil using a rototiller three days before planting. The remaining half of the urea was applied as a top dressing when the Moldavian balm seedlings were at the six-leaf stage. The plots were 3 m long and consisted of six rows, which were spaced 0.375 m apart. A 2-m alley was maintained between all plots to eliminate any influence of lateral water movement. Seeds were planted by hand on April 9, 2010, at a rate of 1 g of seed m^{-1} of row, and then were thinned at the four-leaf stage to achieve a density of approximately 133,333 seeds ha^{-1} of field. Weeds were controlled by hand weeding using a hoe and/or a rototiller whenever necessary. Throughout the growing season, sufficient water to support optimal plant growth was supplied. The plants were harvested once flowering was complete. We used an all-glass Clevenger-type apparatus to conduct 2.5 h of hydro-distillation on dried aerial parts (50 g) of *Dracocephalum moldavica* L., which were collected after flowering was complete. This method for the extraction of oils is recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983). The analysis of variance (ANOVA) of the data from each attribute was computed using SAS version 9.1 (SAS Institute, 1988). Microsoft office Excel (2007) was used for figures drawing

3. Results and Discussion

The analysis of variance for dry herbage yield, essential oil yield, seed yield, and harvest index (HI) is summarized in Table 4. The results showed differences among genotypes were significant for essential oil yield and seed yield, and bacteria had a significant effect on seed yield and harvest index ($P<0.05$) (Table 4). Analysis of variance also showed that fertilizers had a significant effect on dry herbage yield, essential oil yield, and seed yield, and the interaction between bacteria treatments and fertilizers was just significant for essential oil yield (Table 4). The F1 (39003 kg ha^{-1}) and F5 (3083 kg ha^{-1}) regimes produced The highest and lowest dry herbage yields for each genotype was found on the F1 (3903 Kg ha^{-1}) and F5 (3083 Kg ha^{-1}) regime, respectively (Figure 1). Nitrogen is one of the most important nutrients for crop production and is essential for ensuring optimal dry matter production, leaf surface area, and rates of photosynthesis (Rao, 2001). Also, Heidarzadeh et al. (2021) showed that the use of chemical fertilizer had the highest biomass in *Dracocephalum kotschyi* Boiss. It can be due to the easy absorption and availability of chemical fertilizers (Heidarzadeh et al., 2021). Nitrogen deficiency decreases both vegetative and reproductive phonological development, yield components, and total yield in most plants. Available nitrogen in organic manure is released gradually into the soil, which results in a reduction in dry matter production relative to nitrogen fertilizers such as urea.

Table 4. Mean square values from analysis of variance (ANOVA) of dry herbage yield, essential oil yield, seed yield and harvest index under effect of genotype, fertilizer and bacteria.

S.O.V	df	Dry herbage yield	Essential oil yield	Seed yield	Harvest index
Rep.	2	334157.44ns	16.354ns	5718.156ns	8.63ns
Genotype	1	85.94ns	112.882*	57817.29**	23.69ns
Bacteria	1	313237.87ns	0.018ns	60872.01*	54.74*
Fertilizer	4	1316638.79*	104.54**	230131.82**	9.71ns
Genotype × bacteria	1	603237.68ns	28.649ns	711.143ns	6.94ns
Genotype × fertilizer	4	184820.07ns	32.735ns	4839.184ns	5.65ns
Bacteria × fertilizer	4	260457.47ns	64.004*	6344.001ns	5.78ns
Genotype × bacteria × fertilizer	4	281506.99ns	14.325ns	3400.1ns	3.07ns
Error	38	420913.39	20.602	10668.033	13.65
CV		18.33	23.68	10.9	17.26

* = p < 0.05, ** = p < 0.01, ns = non-significant

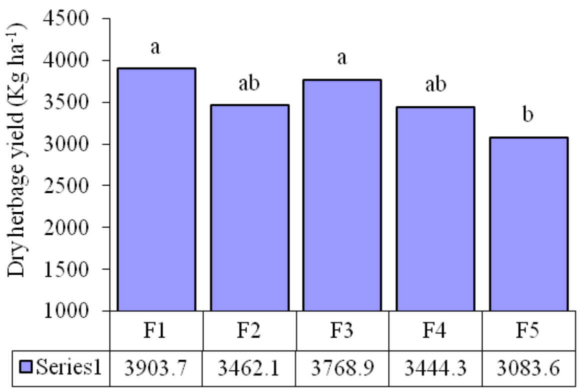

Figure 1. Effect of fertilizer treatments on dry herbage yield of Moldavian balm

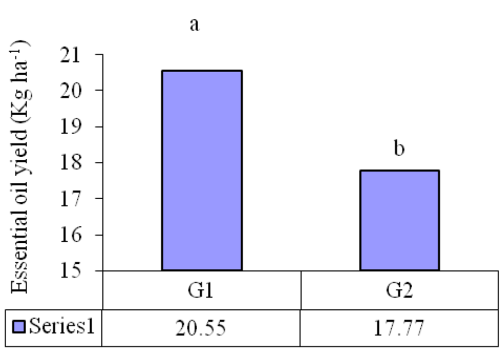

Figure 2. Effect of genotype on essential oil yield of Moldavian balm

The comparison between genotypes (G1, the landrace genotype Urmia, and G2, the modern cultivar SZK-1), showed that G1 (20.5 Kg ha^{-1}) had the highest amounts of essential oil yield and had a significant difference with G2 (17.7 Kg ha^{-1}) (Figure 2). The result of this study showed that there is no significant difference between the F1 and F3 fertilizer regimes for essential oil yield, but the highest essential oil yield was obtained with the F3 fertilizer regime (Figure 3). The lowest amount of essential oil yield was observed in the F5 fertilizer regime (Figure 3). Increases in the percentage oil content following the application of nitrogen and/or compost were observed in basil (Sifola & Barbieri, 2006), *Nigella sativa* L. (Ashraf et al., 2006), *Oenothera biennis* L. (Sekeroglu & Ozguven, 2006), Moldavian balm (Hussein et al., 2006), marjoram (Gharib et al., 2008), and chamomile (Naguib, 2003). Based on the comparison of interactions between fertilizer regimes and bacterial treatment, there is no significant difference among F1B1, F3B1, F4B1, F1B2, F2B2, and F3B2 treatments, but the highest amount of essential oil yield was measured in the F3B2 treatment (23.33 Kg ha^{-1}). But Delfieh et al. (2022) showed that there was no positive effect of biofertilizers (Azotobacter and Azospirillum) on fennel. The F5B1 treatment had the minimum amount of essential oil yield (Figure 4). There is a significant difference between G1 and G2 for seed yield, and the highest seed yield was obtained for the G2 genotype (980.9 kg ha^{-1}) (Figure 5).

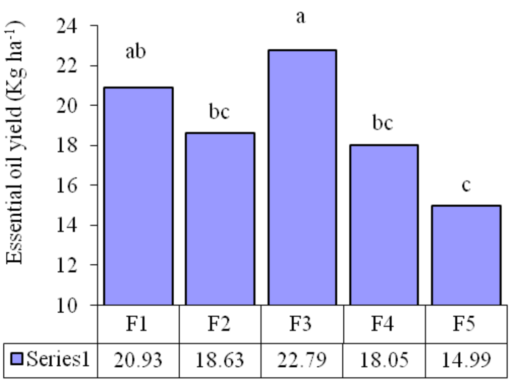

Figure 3. Effect of fertilizer treatments on essential oil yield of Moldavian balm

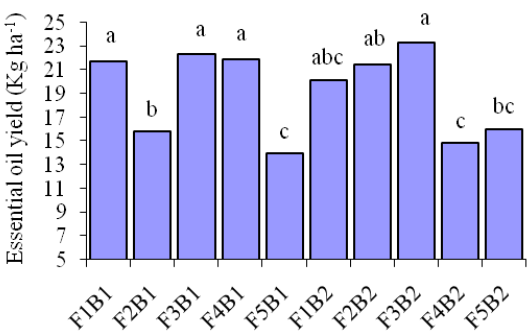

Figure 4. Effect of interaction of fertilizer×Bacteria treatments on essential oil yield of Moldavian balm

The results of Figure 6 showed that the application of the B1 treatment caused an increase in seed yield. The result of the comparison of fertilizer treatments for seed yield of both genotypes showed that the F1 fertilizer had a significant difference from other fertilizer treatments (Figure 7), and the highest seed yield was obtained in this treatment with an amount of about 1122 kg ha^{-1}. We observed that the harvest index (HV) of Dracocephalum moldavica L. can be affected by bacterial treatments. Our results showed that the highest HV was obtained in the B1 treatment, with a value of 22.4 percent.

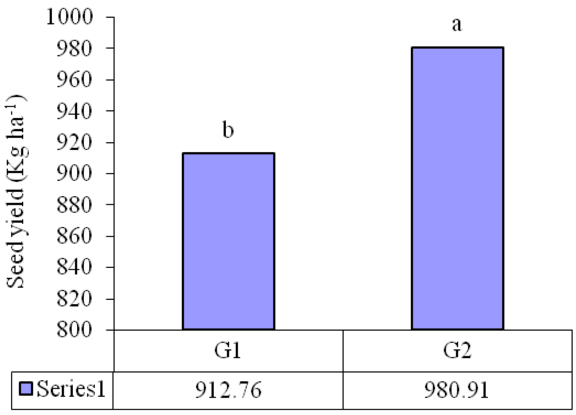

Figure 5. Effect of genotype on seed yield of Moldavian balm

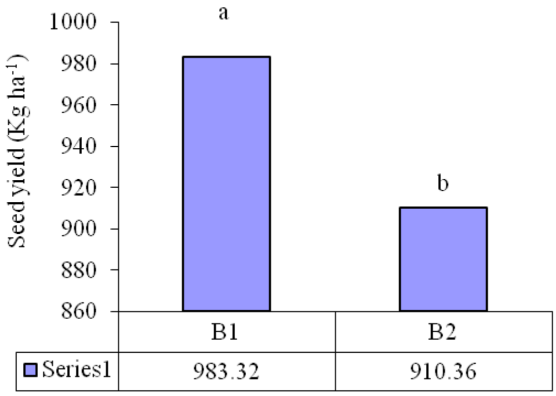

Figure 6. Effect of Bacteria treatment on seed yield of Moldavian balm

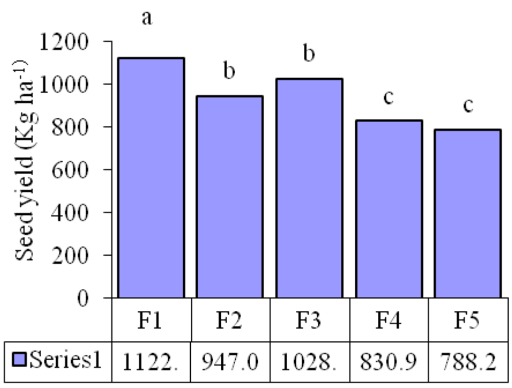

Figure 7. Effect of fertilizer treatments on seed yield of Moldavian balm

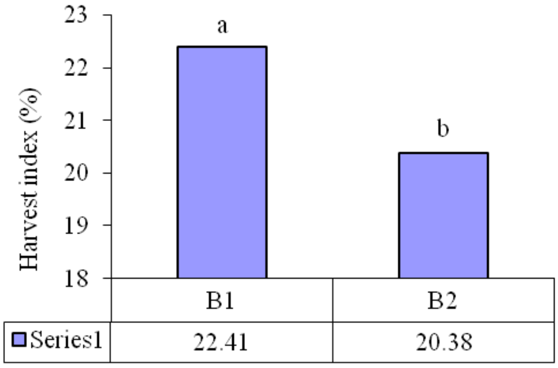

Figure 8. Effect of bacteria treatment on harvest index of Moldavian balm

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Guide for Authors

Preface

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Short Communications: It is appropriate for recording the results of small-scale research or providing information on novel models or hypotheses, innovative methodologies, procedures, or apparatus. Research papers of 2500-3500 words in length, with tables, illustrations and references.

Structure of Articles

Text should be written in a succinct and cohesive manner, with an emphasis on significant points, conclusions, breakthroughs, or discoveries, as well as their broader relevance. All running text should be saved as a Word document. All writings should be written using Times New Roman font. Both American and British English format are accepted, but not their mixture. The font size for title is 14 point and for the main text is 12 point. The subtitles should be written in Bold and font size of 12 point. Figures and tables can be put within the text or at the bottom. Figures should have a high enough resolution to allow for refereeing.

The original research articles should contain the following sections:

Title

The title should be clear, intelligible to experts in different disciplines, and represent the substance of the article. There may be no abbreviations in the title. The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Name(s) of the author (s) not visible in the manuscript (Double Blind Reviews).

Abstract

The title's information does not need to be duplicated in the abstract. The abstract should not be more than 350 words long. It must include the study's goal, methods, findings, and conclusions. Abbreviations should be used sparingly and explained when first used. The abstract is presented separately from the article in a single paragraph after the title page in the manuscript file.

Keywords

Provide a maximum of six keywords appear immediately after the abstract with alphabetical order. Keywords should cover the most precise phrases in the article and should not be the same as the terms used in the title.

Introduction

The introduction should provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study. It should define the problem addressed and why it is important, with a brief review of the key literature and conclude with a brief statement of the overall aim of the work.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn. Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements

Acknowledgements of persons, grants, money, and so forth should be included before the reference list in a distinct section.

References

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). In the text, papers with more than two authors should be cited by the last name of the first author, followed by et al., (in italics), space, and the year of publication (example: Jones et al., 2020). If the cited manuscript has two authors, the citation should include both last names, space, and the publication year (example: Smith and Ebrahim, 2018).
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Format for Periodical

Author, A.A., Author, B.B., Author, C.C., (Year of publication). Title of article. *Title of Periodical*, *volume number* (issue number), pages.

Chen, H., Yu, C., Li, C., Xin, Q., Huang, X., Zhang, J., Yue, Y., Huang, G., Li, X., Wang, W., (2016). Modeling the impacts of water and fertilizer management on the ecosystem service of rice rotated cropping systems in China. *Agriculture, Ecosystems & Environment*, *219*, 49-57.

Format for Books

Author, A.A., Year of publication. Title of work: Capital letter also for subtitle. Location: Publisher.

Strunk Jr., W., White, E.B., (2000). The Elements of Style, fourth ed. Longman, New York.

Chapter in an Edited Book

Author, A.A., Author, B.B., Year of publication. Title of chapter, in: Title of book (Eds.). Publisher, Location, pages of chapter.

Mettam, G.R., Adams, L.B., (2009). How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Tables and Figures

Please submit tables and figures as editable text and not as images. All figures and tables should be embedded while correctly positioned.

Tables

Please avoid using vertical lines in tables. Please use "Table" in both text and captions. Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. The table caption appears above the table using Times New Roman font with font size of 10 points in bold. Use no border for the tables. Footnotes to tables should appear beneath the tables and should be designated by a lower-case superscript letter, †, or z, y, x, etc.

Figures

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used (preferably in the caption). The caption should allow the reader to understand the main elements of what is being shown without needing to refer to then main text. The figure caption appears below the figure and written using Times New Roman font with font size of 10 points in bold. Use no border for the figures. The font size within the figure should not smaller than 8 point and bigger than 10 point. Try to present the figures in gray scale instead of color illustration. Use (Figure) at the end of the sentence and captions and use Figure in text.

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Present simple formulae in the line of normal text where possible. In principle, variables are to be presented in italics. Number consecutively any equations that have to be displayed separately from the text in the right margin (if referred to explicitly in the text).

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